# 4
# Blood Technologies;

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## PART 1: TECHNOLOGIES FOR BLOOD COLLECTION AND PROCESSING

### Introduction

Modern blood bank technology began less than 100 years ago, when several technological obstacles were overcome. First, it was necessary to arrest the blood clotting mechanisms which normally convert liquid blood into a gel in a matter of minutes. Second, physicians needed tools by which blood could be collected from one individual and infused into another with minimum risk to donor and recipient. Third, there was a lack of understanding of the immune system and the potential for incompatibility between donor red blood cells and red blood cell antibodies produced by recipients.

The coagulation problem was solved by the addition of citrate into freshly collected blood (344); citrate compounds are still the basis for stored blood anticoagulants today. Preservatives such as glucose were soon added to extend the shelf life of stored red cells from several days to several weeks.

Devices used in collecting, storing, and administering blood have ranged from quill and tubing contraptions to syringes (used currently for newborn exchange transfusions), to reusable vacuum glass bottles, still the standard in parts of the world today. In the United States, glass bottles have been replaced by flexible plastic containers with attached satellite bags to hold separate blood components.

Landsteiner's description of ABO blood groups in the early **1900s and other** researchers' elucidation of antigen/antibody relationships led to simple laboratory tests for compatibility between donors and recipients, but 15 percent of transfusion recipients remained at risk until the Rhesus or Rh blood types were discovered (331,342). From that time to the present, determination of the donor and recipient ABO and Rh blood types has been the most important procedure in preventing potentially fatal hemolytic transfusion reactions.

In 1945, a new test was described (Coombs) which made possible the recognition of previously undetectable antigens and led to the identification of more than 400 new red blood cell types over the subsequent 30 years (284). However, probably fewer than 30 of these types are of clinical concern in routine transfusion practice (220).

As the demand for blood increased, the larger blood banks began to consider adaptations of clinical chemistry analyzers which could perform multiple tests on a single blood sample to remove some of the tedium and manual labor from mass blood-typing. These systems have evolved into computerized testing devices which deliver samples, interpret results, and flag any spurious findings for further investigation.

In the beginning, donor blood collection and testing were conducted in the same setting as blood transfusion; each hospital would screen donors on the basis of specific patient needs, and donors were often a patient's family and friends. World War II resulted in the creation of donor centers across the United States to collect large amounts of plasma for use in treating combat casualties, and intensive research into improving the storage life and function of red blood cells. When the war ended, community blood centers began to appear under the auspices of American Red Cross chapters, community service clubs, and local medical societies. These centers acted as staging areas from which mobile operations could be sent for blood collections, after which the blood was returned to the center for processing and subsequent distribution to hospitals.

Blood collection and processing in a community blood center can function as an assembly-line operation because of the need for repeating the same battery of tests on a number of donor

samples, ranging from 200 per day in medium-size centers to over 1,000 per day in large centers. On the other hand, transfusion services repeat the entire workflow upon receipt of physician orders which, except in cases of standing orders for elective surgeries, are unpredictable and often urgent. Therefore, batch or assembly-line processing is not as feasible, except for verification of donors' blood types and elective surgery workups.

Blood is inherently variable, with each collected unit representing a unique "lot. " Because it is impossible to test each unit for effectiveness before transfusion, the use of scientifically proven protocols and regular checks on representative products are important.

The use of computers for donor resources and inventory management at the community blood center, and standard policies for surgical blood needs at the transfusion service have improved blood utilization, decreased outdating, reduced charges to the patient, and improved management of donor resources. Computer management of discrete functions has been accomplished at many U.S. blood centers, but often lack coordination among donor recruitment, collection, laboratory, inventory, and financial management departments. One comprehensive information system has been designed as a joint venture between two community blood centers (Blood Center of Southeastern Wisconsin and the South Florida Blood Service) and Arthur Andersen & Co. The goals of all such systems are to minimize manual documentation and subjective analysis, and provide useful statistics for operational management and planning.

A number of automated data management systems throughout the world utilize uniform bar coding technology for the identification of donor units and samples for production and inventory management. This uniformity, which has the advantage of standardized machine-readable as well as simplified eye-readable features, is the result of a special task force of the American Blood Commission, whose efforts were funded by the National Heart, Lung, and Blood Institute and endorsed by the International Society for Blood Transfusion.

Figure 9 shows an example of this labeling format. The labeling scheme has been endorsed by FDA (182) and appears on 60 to 70 percent of products distributed by U.S. blood banks. However, not all blood centers who label their products with bar codes have the equipment to make use of the machine-readable information, and few transfusion services have the capability. Nevertheless, eye-readable uniformity and simplicity are universally appreciated aspects of the commonality label, given a history of multiple color-coded label schemes and superfluous information on earlier blood product label formats. The uniform label has allowed developers of automated testing systems and management information systems to design equipment and software around a single machine-readable format for use in the United States and several countries in Europe and the Far East.

As yet untapped, but applicable, technologies include: recruiting by computerized telecommunications linkages, donor and physician education via cable health networks, and robotics laboratories to perform certain repetitive tasks (209). The feasibility of these alternatives is unproven, but studies are under way.

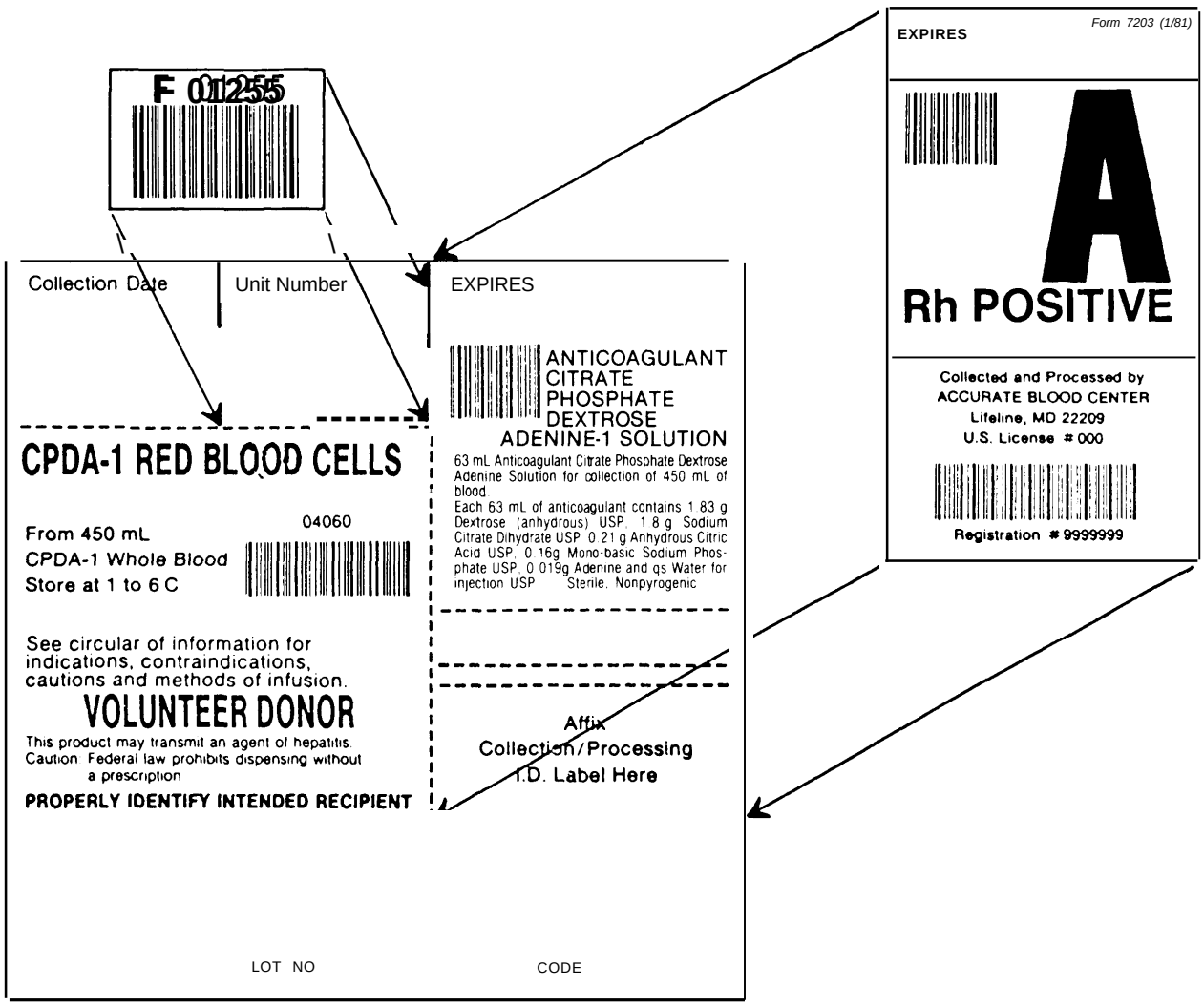
## Blood Collection

Technologies involved with collection of blood from donors address three basic functions: 1) accurate identification of donors, 2) determination of donor suitability, and 3) collection of blood.

Donor identification is accomplished in most blood banks by the use of a cardboard or plastic card embossed with name, address, permanent identification number, and blood type. Most blood banks also have rosters of "special" donors against which the donor's identification is compared. "Special" donors include individuals whose blood is extraordinarily rare (for special needs), as well as those whose blood is unacceptable for transfusion due to laboratory findings on previous donations.

Donor suitability is determined by an assessment of current health status (temperature, blood pressure, screening test for anemia, etc. ) and an

**Figure 9.— Uniform Labeling Format for Blood Products**




SOURCE: E Rosslter, "Technologies for Blood Collection, " 1984

interview covering past and present illnesses, immunizations, hospital procedures, or other conditions (including travel outside the United States, lifestyle, and exposure to other individuals) which could make donating blood unsafe for the donor or transfusion unsafe for the recipient. While some of the donor acceptability criteria are straightforward, most are subject to varying degrees of interpretation at the time of screening, In the absence of factual information to support all the decisions that affect donor suitability, intuition is often used **(598).** There are also concerns among blood bank physicians about the adequacy of current donor criteria when used for frequent, long-term donors (562).

Pre-donation cell counts or hemoglobin measurements generally assure that donating will not be unduly risky for the donor and that an adequate number or volume of the desired component can be collected. Findings of diminished platelet effectiveness in individuals taking aspirin

has led to deferral of such donors in platelet-apheresis programs (565). (See ch. 2, pt. 3, for a discussion of the risks to donors. )

One measure of the safety of the collection process is the frequency of moderate to severe reactions in which donors faint or lose consciousness during or after donation. A rate of 0.3 percent or less is regarded as acceptable (44).

Collection of whole blood is accomplished by the use of pre-sterilized collection sets which consist of a needle, tubing and container pre-filled with anticoagulant/preservative solution. The main collection bag may have one or more smaller, satellite bags attached to it by tubing. After venipuncture, blood flows by gravity into the main container, mixes with the anticoagulant/preservative and remains there until an internal closure is opened to the satellite containers for component preparation. Table 26 lists the usual types of components that can be made from each container configuration.

Underutilization of the multiple bag set can result when changes in blood orders or incidents during the collection process necessitate preparation of fewer or different components than the ones for which the configuration was designed. For example, a bad (traumatic) venipuncture results in decreased platelet and cryoprecipitate yields; therefore, if a triple pack is assigned to a donor who subsequently has a traumatic venipuncture in the collecting process, only red blood cells and plasma may be prepared. The empty, extra satellite container, which represents a cost of $4.52 (based on differences in the price of double and triple packs with a 5-day platelet storage container in 1984) is lost. The extent of this problem has been estimated by some to affect 7 to 10 percent of collected units (472).

**Table 26.—Standard Blood Collection Container Systems**

| Bag configuration | Components prepared[a] | Cost per pack[b] |
|---|---|---|
| Single pack | Whole blood | $3.18 |
| Double pack | Red blood cells<br>Plasma | $6,52 |
| Triple pack | Red blood cells<br>Cryoprecipitate or platelets<br>Plasma | $11 .04' |
| Ouadruple pack | Red blood cells<br>Platelets<br>Cryoprecipitate<br>Plasma | $14.55[c] |
| Quintuple pack | Pediatric doses of red blood cells and plasma | $17.78 |

[a]Products listed do not include modified whole blood end resulting byproducts, or components prepared by breaking original (sterile) seals.
[b]List prices fo,CPDA-1 systems from Fenwal Division, Baxter-Travenoi, Deerfield, iL.
[c]Price is for 5-day platelet product; 3-day platelet or cryoprecipitate container price is reduced by $1.44.

SOURCE: E. Rossiter, "Technologies for Blood Collection," 1984.

Successful adaptation of sterile docking devices to routine component production operations could eliminate the need for multiple bag configurations; all blood could be collected into single packs (at $3.18 each in 1984) and those units needed for component production could be coupled with separate transfer packs (at $2.25 each in 1984) via sterile docking technology (projected cost of $0.50 per component) (3)0 Savings of $0.50 to $1.00 per component would be possible. Further savings could be realized on less expensive storage containers for products designated for further manufacturing (e.g., plasma fractionation).

During the last 10 years, a new technology originating in the United States called "apheresis" has become popular for the collection of large amounts of a specific component from a single donor. (The therapeutic use of this technology was reviewed in a July 1983 OTA case study, The ***Safety, Efficacy, and Cost Effectiveness of Therapeutic Apheresis. )***

Plasmapheresis can be accomplished by manual techniques, which involve collection containers and techniques similar to those for whole blood collection. After centrifugation and separation of the plasma, the red cells, white cells, and platelets are reinfused into the donor. The entire process is then repeated, resulting in two final plasma products and involving a second reinfusion of the donor's residual cells. The procedure takes approximately 90 minutes. Plasmapheresis is a relatively uncommon procedure in community blood centers and transfusion services; for example, less than 0.1 percent of the total plasma collections in the Red Cross in 1983 were by plasmapheresis (48).

Plasma may be collected as a byproduct of automated cytapheresis (cells) procedures for platelets or white blood cells. Current automated

technologies are not cost effective compared to manual methods for routine plasmapheresis (2), but are useful nonetheless in collecting large amounts of antibody-rich plasma from specially immunized donors for reagent use or further manufacturing into injectable products, such as hyperimmune anti-Rh(D) for prevention of hemolytic disease in the newborn.

Membrane filtration technology under development may provide a cost-effective alternative to automated plasmapheresis. One developer projects a 20- to 35-minute procedure in which operator intervention is minimized by microprocessor control.

All automated apheresis devices work on the principle of centrifuging whole blood to enable the separation of components, transfer of the desired component to a storage container, and reinfusion of the remaining components. The first apheresis devices were largely mechanical processors, but later machines are microprocessor-controlled with multiple modes for different component schemes. Operator experience and technique have been shown to substantially affect equipment performance.

Collection techniques for plateletapheresis are fairly new. Effectiveness and safety data show some variation in end-products, mainly in platelet yield and undesirable contamination by extraneous white blood cells and red blood cells **(198).** Current plateletapheresis technologies are summarized in table **27.** The costs of all systems are comparable **(1984** prices); hardware is approximately $30,000 and collection container systems $65-85, except for the 5-day container system.

Platelets collected by apheresis (single-donor platelets) have been proven to be as safe and effective as platelets prepared from whole blood (random-donor platelets) (301, 501), but there is controversy surrounding the extent to which plateletapheresis products should be used in lieu of platelets from whole blood collections. Critics of "routine" plateletapheresis cite economic reasons (pheresis platelets costs ranged from $200 to $350 per dose; manually collected platelets, from $125 to $200 in 1984), the higher prevalence of donor complications, and lack of clinical data to support widespread and indiscriminate use of plateletapheresis technology (483). Nevertheless, few blood bankers doubt that apheresis technologies could someday replace manual platelet collections.

The 24-hour shelf life of apheresis platelets (compared to the 3- or 5-day shelf life of platelets separated from whole blood collections, because the collection system in apheresis is opened in the separation and reinfusion of the donor's cells) has been a major problem in inventory management with these products. But one company, Fenwal, is now marketing a closed system for the production of apheresis platelets with a 5-day shelf life.

**Table 27.—Plateletapheresis Technologies**[a]

| Technology | Manufactured model | Year developed | Product dating period | Comments |
|---|---|---|---|---|
| Intermittent-flow centrifuge | Haemonetics 30 | 1973 | *24* hrs. | First system approved by FDA. Requires extra step to remove extraneous cell contamination. |
| | Haemonetics V-50 | 1983 | 24 hrs. | Produces platelet-rich plasma. |
| Continuous-flow centrifuge | Fenwal CS 3000 | 1979 | *24* hrs. | Fewer extraneous red blood ceils. Lower extracorporeal volume. |
| | | 1983 | 5 days | Sterile docking device technology and a different type of storage container add $40 to collection container costs. |
| Dual stage continuous-flow centrifuge | IBM 2997 | 1977 | **24** hrs. | Manufacturer plans no further development of product line.[b] |

[a]These devices can also be used to collect white blood cells.
[b]Letter to customers from IBM, Jan. 24, 1984.

SOURCE: OTA, 1983, Poindexter, 1984, and Fenwal Division of Travenol Laboratories, Inc

Leukapheresis (for collection of white blood cells) can also be performed (independent of or concomitant with platelet collection) with the same technology; costs and techniques are similar to those for plateletapheresis. Although the safety, effectiveness and efficiency of leukapheresis technologies, and leukocytes as a transfusion product have been under study for over a decade, use of the technology remains controversial (90,405,468). Clinical data to support the use of white blood cell transfusions are extremely difficult to obtain because of the morbidity of the recipients; white blood cells have generally been used for individuals with overwhelming infections unresponsive to antibiotics.

## Blood Component Preparation

The proportion of whole blood donations that are separated into components varies among blood centers from around 50 to more than 99 percent, reflecting local medical preferences and practices. The minimum yield from one unit of whole blood is one unit of red cells and one unit of plasma. Additional components which are commonly separated are platelets and cryoprecipitate.

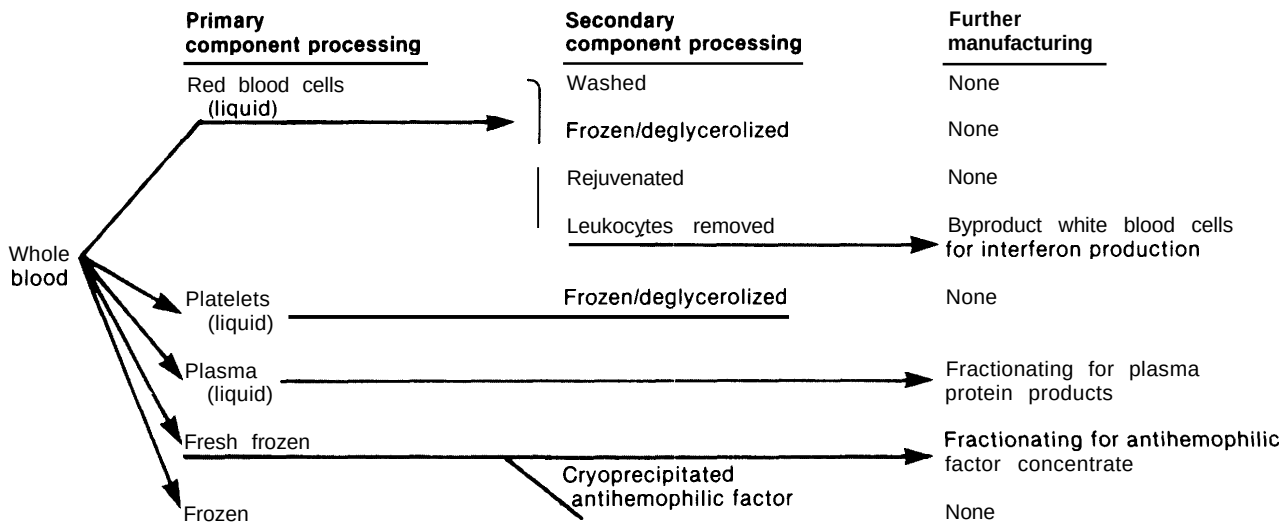
The plasma and cellular components of collected whole blood will separate by gravity over a period of about 24 hours. The most dense cells, red blood cells, settle out to fill the bottom half of a container. Less dense white cells settle in a thin, visible layer on top of the red cell mass. Platelets are distributed among the white blood cells, with some platelets remaining suspended in the plasma layer.

This separation process can be accomplished in a matter of minutes using high-speed centrifuges. The flexibility of the plastic collection container allows removal of the top layer into a sterile attached satellite bag.

The original collection container configuration dictates the number and type of components which can be prepared from whole blood (see table 26). Through a scheme of primary and secondary processing steps, a variety of end-products can be produced, either for direct transfusion or for further manufacturing into other products (fig. 10). Secondary processing adds to product cost and is generally limited to special applications.

On average, 80 percent of whole blood collections are processed into two or more components, most commonly red blood cells and frozen or liquid plasma. Platelets are also prepared from over 30 percent of collections and have become the "driving force" behind scheduling and planning of component production. Platelet shelf life is the

**Figure 10.—Derivation of Blood Components**




SOURCE: Rossiter, E., "Technologies for Blood Collection," 1984.

shortest of all whole blood components, except for granulocytes; the product must be prepared within several hours of blood collection to preserve platelet function and survival. Because over 60 percent of blood collection operations are offsite periodic shuttles back to the blood center are necessary throughout the day. Special collection operations are scheduled over weekends and holidays to avoid shortages.

The development in 1981 of new plastics for storing platelets increased shelf life from 3 to 5 days and has alleviated some of the production constraints. Initial studies show improved efficiency by an increase in product availability and decrease in outdating (414b). Longer shelf life has also allowed a reduction in the number of shuttles and weekend operations, further improving component production efficiency. Notable increases in platelet demand and utilization were experienced in 1983 in the Red Cross, where 75 percent of the platelet production was in the 5-day plastic containers **(48,351).**

The extended shelf life of platelets is possible because of two different new plastics which allow better exchange of oxygen and carbon dioxide through the walls of the platelet storage container, thereby preventing the buildup of acid (as metabolic processes continue in the stored product) that is toxic to platelets. The new plastics have an additional advantage over previous formulations in that they lack DEHP, a common plasticizer used in construction, home furnishings, clothing, etc., which is known to leach into blood during storage and therefore has caused concern over potential toxicity (562).

The storage life of liquid-stored red blood cells and whole blood was extended from 21 to 35 days by the addition of adenine to the basic preservative solution in 1978. The improved efficiencies from this change have resulted in **50** percent reduction in outdating, now at approximately 5 percent **(48).** Further extension of red blood cell shelf life to 49 days is now possible. Red blood cells are first suspended in CPD anticoagulant (one of the standard preservatives), then an additive nutrient solution (AS-1, or ADSOL™, from the Fenwal Division of Travenol Laboratories) is added after other components are separated. It is not known whether the additive system approach (still in the introductory phase in 1984) will allow appreciable further reduction in red blood cell outdating, but there are other advantages to additive systems recognized from years of use in Sweden and Australia **(267,345).** They include increased plasma recovery (the additive solution replaces the buffering function of plasma during storage), better red cell survival in the recipient, and transfusion viscosity similar to that of whole blood,

Potential disadvantages include the additional cost of the collection container (approximately 13 percent more), extra manipulation during component preparation, and the potential need to remove excess solution in patients subject to circulatory overload **(392).** Nevertheless, the additive solution approach represents a technology designed to make possible specific preservative solutions tailor-made for each blood component (315).

Future component storage research is aimed at further increases in platelet shelf life and the use of ascorbate (vitamin C) to improve maintenance of certain red blood cell functions.

Recent advances and commercial successes in extending the shelf life of platelets are likely to spawn further improvements in dating periods. Use of additive solution systems, if widely accepted in the blood banking community, will facilitate research into component-specific preservatives. There also is interest in automating component production by the adaptation of robotics systems used in the U.S. automobile industry. Such a system has been designed, and its feasibility and potential impact are ***under*** study (209).

## Testing and Labeling of Blood From Donors

With every donation, blood samples are tested for selected red blood cell antigens and antibodies and certain transmissible diseases (table 28). Test methods, their scientific bases, and potential clinical relevance vary greatly.

**Table 28.—Testing of Blood Donor Samples**

| Test | Test detects | Reason performed | Clinical significance |
|---|---|---|---|
| ***Red cell antigens and antibodies:*** | | | |
| ABO group | A and/or B antigens on red blood cells. Presence of antibodies to A or B in plasma. | Assure ABO compatibility of donor and recipient; prevent hemolytic transfusion reaction. | Hemolytic transfusion reaction could occur in 97% of recipients due to natural antibodies to A and B red cell antigens. |
| Rh type | D antigens on red blood cells. | a. Prevent immunization of an Rh negative recipient. | a. 15% U.S. population is Rh negative; one-half of these would be immunized by an Rh positive transfusion. |
| $D^u$ | *Weak variant of D antigen.* | b. Prevent hemolytic transfusion in a recipient already immunized. | b. 1% of Rh negative patients already immunized to D from transfusion or pregnancy. |
| Antibody screen | Unexpected antibodies (other than A or B). | a. Prevent passive transfer of antibody to recipient.<br>b. Prevent antigen/antibody reaction in recipient.<br>c. Identify rare donors or those with reagent-grade plasma. | a. Confusing results in subsequent blood bank testing.<br>b. Rarely notable.<br>c. Less than 0.5% of donor population has any antibody; reagent-grade or rare types much rarer. |
| ***Screens for transmissible disease:*** | | | |
| Syphilis screening (STS) | Reagin, an antibody-like substance present in people who have syphilis and a number of other acute and chronic conditions. | a. Originally, prevention of the transmission of syphilis by transfusion.<br>b. Control of syphilis in donor population.<br>c. Detection of biological false positive donors. | a. No longer a problem.<br>b. Of doubtful effectiveness.<br>c. Unclear; accounts for 70-90% positive STS tests. |
| Serum or Type B hepatitis | Hepatitis B surface antigen (HBsAg). | Prevention of transmission of type B or serum hepatitis. | 7-10% of blood recipients at risk for some type of hepatitis; HBsAg test has significantly reduced posttransfusion type B, but has had no effect on other types—1-3% mortality for hepatitis B. |

SOURCE' U.S. Department of Health and Human Services, FDA Panel, 1979

Red blood cell tests determine the donor's ABO and Rh type. Mild incompatibility can result in an ineffective transfusion due to reduced red blood cell survival. Major ABO group incompatibility can result in fatal hemolytic transfusion reactions. Because of this danger, the donor ABO and Rh type are confirmed by the transfusion service, and ABO compatibility may be verified by cross-hatching.

Standard tests for red cell antigens and antibodies are based on the principle of hemagglutination; i.e., the clumping of red blood cells that can occur as a result of specific antigen-antibody reactions. When a red cell antibody comes into contact with its target antigen on the surface of red blood cells, it combines with the antigen, forming antibody "bridges" between cells, which lead to visible clumps. Some antibodies (e.g., A and B) are very effective in bridge formation; others need antiglobulin (Coombs) reagent to form visible antibody bridges.

Table 29 demonstrates the serological basis for determining the ABO group of an individual. Because A and B antibodies are naturally produced by individuals who lack one or both of the antigens, it is possible to determine the ABO group by testing for either antigens or antibodies. Common practice is to perform both tests and check for agreement.

Rh type is determined by testing for D antigen on red cells; there is no naturally occurring an-

**Table 29.—Determination of ABO Blood Group**

| | Antigens on donor's red blood cells | | Antibodies in donor plasma | |
|---|---|---|---|---|
| Donor blood group | A | B | A | B |
| 0 | No | No | Yes | ***Yes*** |
| A | Yes | No | No | Yes |
| B | No | Yes | Yes | No |
| AB | Yes | Yes | No | No |

SOURCE E. Rossiter, "Technologies for Blood Collection, " 1984.

tibody to D as with A and B antigens. Some blood banks perform two different tests for D antigen to provide an internal check. Blood banks also perform additional, more sensitive antiglobulin (Coombs) testing on samples giving negative results for D antigen. This technique will detect weak or suppressed D antigen, but its clinical significance is unclear.

ABO and Rh types are genetically determined and do not change. For this reason, some European blood centers do not repeat ABO and Rh determinations on donors who have been previously tested (sometimes multiple testing will be conducted before subsequent testing is discontinued). This practice would significantly reduce the testing workload in the United States, because approximately 80 percent of blood donors have donated before (48). However, Federal regulations currently require ABO and Rh testing on every donation. On the other hand, some blood banks in Canada and Europe do more elaborate Rh typing than is done in the United States, testing for C and/or E antigens (of the same family as D). This practice has been discontinued in most U.S. blood banks with no increased risk to recipients **(562).**

ABO and Rh testing can be performed by manual or automated methods. Table 30 summarizes the different manual techniques used for detecting red cell antigens and antibodies. All manual techniques involve mixing of sera-containing antibodies and red blood cell suspensions, and visual examination for clumping. Tube and microplate techniques allow centrifugation of the test mixture for easier reading of the test reaction. The predominant manual test technique is the tube method, although use of microplates (actually a miniaturization of the tube technique) has been increasing since the late 1970s. In 1984, within the Red Cross alone, 14 centers used manual microplate techniques as their primary method, and another 7 used microplates as a backup to automated methods.

The Technicon AutoAnalyzer, introduced in 1967, was the first attempt to automate routine donor testing. It used continuous flow technology already proven in clinical chemistry analysis, It automatically samples and performs ABO, Rh, and STS (serological test for syphilis) tests, but relies on the operator to identify samples, read reactions, and interpret and record results. Nevertheless, the AutoAnalyzer was widely used (still about 200,000 worldwide), easy to repair and maintain, and, at about $30,000, within the price range of a number of medium to large blood centers; the largest of them often had two. The AutoAnalyzer is no longer produced but is still used in a number of centers.

Table 31 lists the fully automated testing technologies used in the United States in 1984. All identify samples via bar-coded label, sample automatically, perform tests, read results by a photometer, and interpret results via dedicated computer. All systems also have some capability to perform antibody screening and STS, but are not generally used for these purposes in the United States. Costs per donor sample range from $0.83 to $1.23 v. $0.53 for the AutoAnalyzer (208). These systems are much more difficult to maintain and repair. Overall costs of the fully automated

**Table 30.—Comparison of Manual Technologies for Red Cell Serological Testing of Blood Donors**

| Type | Advantages | Disadvantages |
|---|---|---|
| Slide/tile testing . . . . . | Simple, fast, inexpensive. | Less sensitive. Not adaptable to automation. Difficult to use in batch testing. Coombs testing impossible without supplemental use of tubes. |
| Tube testing . . . . . | Allows the use of more sensitive techniques than slide. Adaptable to batch testing up to approximately 200 samples. | Requires labeling and manipulation of tubes. Uses larger amount of reagent and sample than slide or microplate. |
| Microplate. . . . . | More sensitive than tube or slide. Uses less sample, reagent and space. Easily adaptable to large batch testing. | More dexterity required for sampling and plate handling. May require new reaction interpretation techniques. |

SOURCE E Rossiter, "Technologies for Blood Collect ion," 1984

**Table 31.—Automated ABO/Rh Test Technologies in the United States, 1983**

| System name | Manufacturer | Principle | Number of centers | Price (x1,000) |
|---|---|---|---|---|
| AutoGrouper | Technicon[a] | Continuous flow analysis | 11 | $184 |
| MiniGroupamatic | Kontron[b] | Discrete analysis | 5 | $115 |
| G-2000 | Kontron | Discrete analysis | 8 | $260 |
| G-360 | Kontron | Discrete analysis | 8 | $460 |

[a]Tarrytown, NY
[b]Everett, MA

SOURCE:L Friedman, "Status ot Automated ABO/Rh Testing in the US" ARC internal Report, 1983

systems are beyond the grasp of most community blood centers (only 32 of 213 U.S. community blood centers were using them in 1984).

The testing of blood donor samples for unexpected antibodies involves the use of reagent red bloodcells selected on the basis of antigenic content. The test was originally designed to detect antibodies in recipient plasma that might cause difficulty in crosshatching, but it has become a standard test for every sample entering the blood bank. Tube or microplate testing is necessary to detect clinically significant antibodies that react only with Coombs reagent. Microplate techniques are more efficient and more sensitive for this purpose (487).

Although traditional screening techniques have included multi-phase testing with various enhancers and potentiators, there is little justification for elaborate procedures on donor samples. Regular blood donors who have not been transfused or pregnant since the last negative antibody screen could be excluded from subsequent testing, but the effort required to selectively exclude these donors may negate the benefits of decreased testing.

A new and abundant source of antibody production was developed in the 1970s and is being applied commercially in the 1980s. Monoclinal antibody technology (see ch. 6, pt. 2) allows large-scale production of antibodies with selected specificity and potency (575). Production of antibodies in this manner should eventually become less expensive and have the added advantage of reducing variability in test results that is inherent in using naturally produced human or animal serum. Monoclinal antibodies also can be easily labeled with indicator substances (radioactive, fluorescent, etc.) (436). Only a few monoclinal reagents have been approved for blood bank products, but many are under development.

Recent increases in the use of microplates for manual ABO/Rh testing have led to renewed interest in automating that technique. Initial developmental work from at least three companies indicates that "ELISA" readers, photometers currently used for reading enzyme-linked immunosorbent assays (an immunologic test) can be adapted for reading hemagglutination reactions. Coupled with dedicated computers for data analysis and interpretation, market projections indicate that these devices could be a more economical alternative for automated ABO/Rh testing (table 32). The automated microplate system prototypes require more manipulation of test samples than the current automated technologies, but the techniques for manual and automated microplate testing are the same, allowing easy conversion from one technology to the other.

Still another technology under development for several years by Gamma Biological, Inc. (Houston, TX) involves modified microplate technology (Microtear™), which offers visual or automated interpretation of hemagglutination by the "streaming" of red blood cells after centrifugation.

All of the testing technologies described thus far are liquid-phase tests; i.e., they involve the mixing of sera-containing antibodies with red blood cells in suspension, and the qualitative reading of the presence or absence of agglutination. A solid-phase immunoadsorbance technique has been patented by Rosenfield and Kochwa (U.S. patent # 4,275,053, June 23, 1981), and similar developmental work has been presented by Plapp and associates (1983), which offer quantitative evaluation of antigen-antibody interactions. Greatly increased sensitivity results from

**Table 32.—Traditional Automated Technologies v. Automated Microplate Systems (AMS)**

| | Tradition ala | AMS[b] |
|---|---|---|
| Machine Costs | $115K-$420K | $25K-$60K |
| Cost per sample ABO/Rh | $0.83-$1.23 | $0.68 |
| Operator intervention | Minimal | Centrifugation, resuspension, and loading of microplates required. |
| Backup technology | Second machine, manual tube, or microplate. | Standard manual microplate technology is inherent alternative. |

[a]Figures are based on actual operations.
[b]Developers include Dynatech Laboratories, Alexandria, VA; Flow Laboratories, McLean, VA; and Kontron International, France. Figures are based on estimates from developers

SOURCE Friedman, personal communication, 1983

affixing the reaction components to a solid surface (usually a microplate well), but the utility of this technique in routine donor processing has not been proven.

Except for the semiautomated AutoAnalyzer techniques developed in the late 1960s, screening technologies for syphilis have been unchanged for the past 15 to 20 years. The tests are not specific; approximately 90 percent of positive reactions are due to causes other than syphilis. Questions have been raised about the health status of false positive reactors; some scientists believe the test may be indicative of pre-disease states, the infectivity of which is unknown **(562).** However, the overwhelming opinion of FDA expert panels, AABB, and ARC is that the STS test need not be required for donor samples. Federal regulations and most State health departments currently require STS testing, in spite of the lack of scientific evidence to support its continued use.

Hepatitis testing technologies have undergone rapid evolution since the discovery of Australia antigen (80). The antigen so discovered was a marker of hepatitis B virus and led to development of a succession of increasingly more sensitive tests for hepatitis B surface antigen (HBsAg). Like other tests on donor blood, it is repeated on every donation, regardless of the donation frequency and history, and once a donor has had a confirmed positive test, he or she is permanently disqualified as a donor. No confirmation of the (negative) test is performed by the transfusion service.

Current "third generation" technologies for HBsAg testing are compared in table 33. Enzyme immunoassay (EIA) are of equal sensitivity as radioimmunoassay (RIA) techniques, but are increasingly preferred over RIA due to cumbersome monitoring, licensing and waste disposal requirements for the radioisotopes used in RIA testing. Both EIA and RIA have automatic reading of reactions and interpretation of results. Abbott Laboratories, the leading company in the field of HBsAg testing, is developing a more complete automated system which identifies samples by bar code and can interface with other blood center computers.

Once all testing is completed on donor samples, blood components with unsatisfactory test results must be disposed of and satisfactory units labeled according to ABO, Rh and a statement of satisfactory test results (e.g., for HBsAg), and released for distribution. This is a most crucial procedure, since the mistaken release of a unit reactive to HBsAg would go undetected, and blood labeled incorrectly for ABO group could be transfused into an incompatible recipient.

Labeling and release of blood components are tedious and laborious tasks. They are usually performed in batches by a team of two or more persons, with one individual applying adhesive labels and the other checking laboratory and donor records. To minimize human error and facilitate the process, computers storing laboratory and donor suitability data can be used to print appropriate labels or to verify that a correct bar-coded label has been applied, and that all testing on a blood component has been satisfactory. Such systems, fully integrated with automated testing sys-

**Table 33.—Current Technologies for HBsAG[a] Testing**

| | Agglutination test type | | Immunoassay test type | |
|---|---|---|---|---|
| | Latex | Red blood cell | Radio-plastic | Enzyme-plastic |
| Test surface | Slide/tile | Micro plate | Bead or tube | Bead or tube |
| Sensitivity | Good | Good | Excellent | Excellent |
| Specificity | Fair | Good | Excellent | Excellent |
| Interpretation of results | Subjective | Subjective | Objective | Objective |
| Positive results indicated by | Clumping of particles | Clumping of red blood cells | "Radioactive"[b] bead | Colored solution |
| Automated reading | No | Under development | Yes | Yes |

[a]Hepatitis B surface antigen.
[b]Higher than levels of background radioactivity.

SOURCE: Huestis, Bove, and Busch: *Practical Blood Transfusion*, 1981.

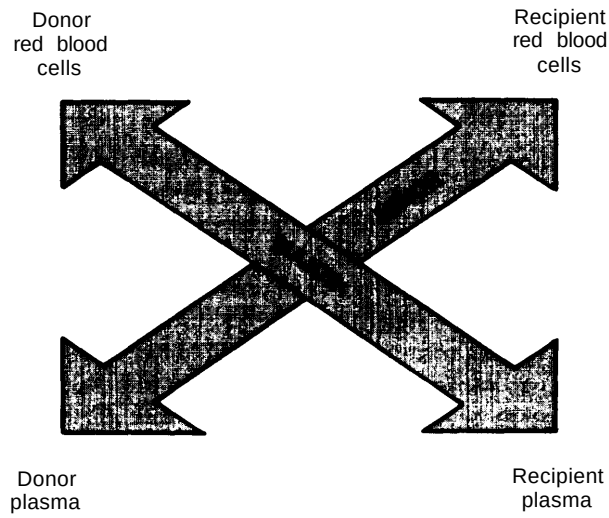
terns, are operational in probably not more than 10 community blood centers, and no onsite printers approved by the American Blood Commission are being used, but they are the ultimate goal of any facility introducing computers into the laboratory.

## Pre-Transfusion Testing Techniques

The purpose of test procedures in the transfusion service is to minimize risk of major serologic incompatibility. The ABO/Rh type of the donor unit is rechecked, the ABO/Rh type of the recipient determined, and a crosshatch performed between the recipient sample and selected donor samples. Though not required by Federal regulations, AABB accredited transfusion services must also perform a recipient antibody screen. Recipient antibody screening yields much the same information as the crosshatch, and there has been increasing questioning of the need for both tests since the antibody screen was required for accreditation by AABB in 1970. The next revision of the AABB standards is likely to allow some options in choosing between the tests.

The first crosshatch was a simple mixing of two blood samples and observing for hemagglutination. The test eventually evolved into two distinct procedures —mixing of recipient plasma with donor red blood cells, and mixing of donor plasma with recipient red blood cells (fig. 11). The former is now regarded as the "major" crosshatch, because it is of prime importance in predicting the likelihood of a hemolytic transfusion reaction (recipient's antibodies would destroy the infused red cells). The latter, "minor" crosshatch, is no longer required by Federal or voluntary standards.

**Figure 11.—The Crosshatch**




SOURCE: Rossiter, E., "Technologies for Blood Collection," 1984.

In spite of the diversity of approaches taken in testing, decisions about when to crosshatch and how many units to hold available have become increasingly standardized. Eighty-three percent of responders to a recent survey (588) used a type-and-screen (T&S) procedure in which preliminary testing (ABO, Rh, and antibody screen) is performed on low-risk surgical patients, and blood is made available (though not crosshatched). The T&S policy is implemented after each blood bank reviews its own transfusion statistics across "surgery-related" groups and determines which elective surgical procedures seldom result in transfusion. In the rare instances in which blood is needed during or after these surgeries, blood will be released for transfusion with an abbreviated crosshatch.

Studies of the safety of T&S practices indicate that they are both safe (408b) and economical (203). T&S and similar policies which establish a standard number of crosshatched units for surgeries that usually require transfusion, improve blood resources management through lower inventory needs, improved utilization, and decreased outdating.

### Conclusions

Technologies developed and put into use during the last 10 years have made blood products more effective, and have increased their safety. The biggest improvements have been in efficiency, with increased availability and better utilization of products.

Trends for the next 5 to 10 years in the use of technologies will be continued increases in automation and computerization, and further improvements in blood preservation. These changes are a natural result of technological changes made since the late 1970s and reflect the dynamic climate in which U.S. blood banks operate.

## PART 2: PLASMA FRACTIONATION TECHNOLOGIES

### Introduction

Plasma fractionation began in this country during World War II. At the onset of the war, liquid human plasma was in use in the battlefields of Europe, but there was a need for a blood substitute that was easy to transport and which could be administered on the battlefield under widely varying climatic conditions.

At the request of the Medical Advisory Committee of the Red Cross and the National Research Council's Committee on Blood Transfusions, Edwin J. Cohn, head of the Department of Physical Chemistry at Harvard University, agreed to undertake an investigation to determine whether or not the plasma of animals could be made safe for transfusion. By mid-1940), Cohn notified the Red Cross of the development of a system which would yield not only albumin but other protein components of plasma as well, but said he felt that dependence should be placed *on* the use of human plasma rather than that of animals. The Red Cross then opened a blood donor facility at Peter Bent Brigham Hospital in Boston to provide plasma to support Cohn's work. In April 1941, the first human serum albumin produced by Cohn was released for clinical trials, and by July 1941, pilot production facilities had been expanded.

In December 1941, clinical studies had not yet been concluded, but human derivatives from Harvard were rushed to Pearl Harbor for use in treatment of injured servicemen. By 1942, the Armed Forces had authorized regular use of normal serum albumin (NSA) and issued production contracts to several pharmaceutical firms. Training of technical personnel from these firms was conducted by Cohn and his staff. Cutter began to supply the product in late 1942, and by 1943 seven U.S. companies were involved in fractionation and supply of human plasma products. Following World War II, these companies continued to process plasma to meet the demand for various plasma proteins for civilian use.

During the beginning of plasma fractionation in the 1940s, albumin production and sale provided the economic support for the industry. Later in the 1950s, the need for gamma globulin for prophylaxis against poliomyelitis, infectious hepatitis, and childhood viral diseases provided financial security for the indusy. Subsequently, prevalence of these diseases was markedly decreased, and at about the same time methods for recovering Factor VIII were greatly improved, leading to increased treatment of hemophiliacs in the United States and abroad. As a result, sales of Factor VIII have been a significant source of revenues from the mid-1960s to the present.

### Plasma Collection

Three types of plasma are used to produce plasma derivatives; liquid single-donor plasma, fresh-frozen, single-donor plasma, and "source" plasma, which is collected by plasmapheresis. Liq-

uid and fresh-frozen, single-donor plasma are primarily obtained through volunteer agencies as "recovered" plasma from whole blood donations. "Recovered" plasma is either fresh frozen within 6 to 24 hours of collection or is salvaged from outdated whole blood at the time the red cells are discarded. Only fresh-frozen plasma is suitable for Factor VIII fractionation. Increased use of components has resulted in an increasing proportion of recovered plasma being frozen within a few hours of collection to protect the labile coagulation factors. Single units of fresh-frozen plasma are then shipped directly to the fractionators.

In plasmapheresis, 500 ml of whole blood is drawn into a sterile container in which there is anticoagulant solution (either ACD Formula A, CPD, or Trisodium Citrate). The container is then detached and the blood centrifuged in order to separate the red cells from the plasma. During this time the phlebotomy needle is kept open with an infusion of isotonic salt (NaCl) solution. The red cells are then reinfused into the donor, and the process is repeated a second time. The 0.5 to 0.6 liters of collected plasma are then frozen. The average elapsed time for such plasmapheresis is 90 minutes. Each donor is permitted to undergo a double-bleeding of this type twice per week.

At least three systems for the automation of plasmapheresis are currently undergoing research and development: membrane filtration, continuous flow centrifugation, and intermittent flow (batch) centrifugation. As yet, none is able to compete economically with the traditional manual technique. Although the manual system is labor-intensive, the total cost of a double pack of plasma is in the range of **$22 to $25 (277),** including $7 to $10 for the donor fee, $8 for the blood bag set and saline solution, $2 for donor screening and testing, and $5 for labor. In the case of automated centrifugal systems, the disposable plastic bowls alone cost more than this; Haemonetics $55, Fenwal $60, and IBM $65. However, these costs could plummet if simplified to be used for source plasma alone. The state of the art of the automated centrifugal system has been developed far beyond the needs for plasma collection because the same basic hardware (with costs of approximately $20,000) and disposable plastic bowls can be used to prepare platelet concentrates, buffy coats (leukocytes), and washed red cells.

On a theoretical basis, filtration through membranes of appropriate pore size should be the simplest way to separate liquid plasma from the cells in whole blood. Earlier development of this technology has been hampered by the fact that filtration does not simultaneously yield cellular components such as platelets to help amortize the development costs. However, its potential value in therapeutic plasmapheresis may catalyze its evaluation in the collection of normal (source) plasma. Membrane filtration at low temperatures has also been attempted experimentally to isolate Factor VIII-rich cryoprecipitate. Large-scale application of this new technology has not been attempted.

The estimated time required for a centrifugal plasmapheresis separation is 30 to 40 minutes, and for continuous flow membrane filtration, **50 to 60 minutes.** When donor preparation time is added in, the overall plasmapheresis time is slightly longer than 1 hour. This compares with approximately 90 minutes for the traditional manual system (2). The interrupted flow centrifugal system also requires only a single venipuncture, as opposed to the two needles or large, double lumen needle necessary for continuous flow. The principal advantages of automated plasmapheresis are: 1) the donor is always attached to his/her own red cells or whole blood, thus preventing accidental administration of mismatched cells; and 2) the potential population of plasma donors may be able to be expanded significantly throughout the world. Automation also raises the possibility of decentralized plasmapheresis, and, by shortening the overall procedure time, also enhances the possibility of inclusion of volunteer donors for source plasma. The remaining unanswered question is whether or not increased volumes of plastic disposable will lower costs sufficiently to compete with a manual system in which labor costs are only 20 percent of final production costs.

There are two types of plasmapheresis donors: standard donors and specialized donors. High-titer antibody preparations (hyperimmune globulins) are made from the plasma of specialized

donors. Successful passive immunization is greatly improved by use of gamma globulin fractions high in specific antibody content against the individual diseases. The hyperimmunization of selected (specialized) donors through repeated stimulation of their immune systems with specific antigens (noninfectious products made from the inactivated protein of the underlying virus or bacteria) can provide a pool of high-titer antisera. This technique has provided specific globulins for the management of such diverse disorders as rabies, herpes zoster, and hepatitis B.

In a similar manner, individuals who inadvertently have been immunized through prior blood transfusions or pregnancy, or who elect to become donors of blood group antibody sera, can have their titers restimulated over a period of years and maintain their role as specialized plasmapheresis donors for the production of anti-A, anti-B, or anti-Rh immune globulin for blood group typing, or, in the case of anti-Rh, for the prevention of Rh hemolytic disease of the newborn (erythroblastosis fetalis).

Some overlap occurs between standard and specialized plasmapheresis donors. Immunization against tetanus, for example, is so commonly practiced and easily accomplished that it is common for standard donors to receive occasional booster doses, making possible the recovery of a high titer of tetanus antibodies from routine gamma globulin fractionation.

## Fractionation Methods and Products

The principal method used for the isolation of the different plasma proteins continues to be the cold-ethanol precipitation technique of Cohn and collaborators (127). This method employs a four-variable system of temperature, ionic strength, ethanol concentration, and pH to precipitate:

- Fraction I—chiefly Factor VIII and fibrinogen,
- Fraction II—the gamma globulins,
- Fractions III and IV—other coagulation proteins and trace components such as ceruloplasmin and iron-binding globulin, and
- Fraction V—the albumins.

Fraction VI, the residue remaining, is currently discarded due to lack of known therapeutic usefulness.

Method XII, the final technique evolved by the Cohn group (128), gave promise of considerable simplification by using metallic precipitation of the globulins with zinc diglycinate, but this method was never commercially adopted due to preliminary evidence of contamination of the albumin fraction with hepatitis virus. It also was impractical in its requirement for decalcified plasma (through ion-exchange treatment) rather than the usual citrated plasma.

Due to a shortage of industrial sources of alcohol in England following World War II, the principal fractionation facilities which were constructed at the Lister Institute were modified to permit use of ether rather than ethanol. The basic principle of the four-variable Cohn system was nonetheless used, and this method was continued for a period of approximately 20 years, following which the ethanol system was again employed. Nevertheless, many minor modifications of the basic alcohol precipitation technique have been introduced successfully with improvement in yield, cost effectiveness, and stability of individual products.

### Factor VIII

In the original alcohol fractionation method, Factor VIII precipitated with fibrinogen in Fraction 1-.. The yield of Factor VIII by this method was similar to that developed by cryoprecipitation, but contamination of the final product with other proteins was considerably higher with the original alcohol fractionation method. These concentrates were used to treat hemophilia during the 1940s and 1950s, and were marketed primarily for their fibrinogen content. After the development of the cryoprecipitate technique by Pool, et al. (442), Fraction I-o was abandoned as too crude a preparation to use for its Factor VIII content. The fibrinogen, which the recipient received as a side effect of the Factor VIII replacement, led to problems in red cell crosshatching. Contamination with hepatitis virus was also so prevalent as

to no longer justify its commercial preparation, and approval of Fraction I-0 (fibrinogen) as a plasma product was withdrawn by FDA in the late 1970s.

"Cryoprecipitate" was discovered by Pool and associates (442,443) to trap a significant portion of Factor VIII, and thus provided a simple physical step to harvest it. Currently, cryoprecipitate is either prepared as a single unit in a plastic bag during the routine collection and processing of a single unit of plasma in a blood bank, or the plasma is frozen and shipped to the fractionation industry.

The concentration of Factor VIII in cryoprecipitates varies widely, depending on such factors as the size of the ice crystals which form and the local salt concentrations left behind when water crystallizes into ice (419). Federal regulations require that the average Factor VIII potency of a single unit of cryoprecipitate be greater than 80 units as determined on four representative containers each month (21 CFR pt. 640.56). (The Factor VIII content of any single unit that is being used therapeutically cannot, of course, be measured. ) For example, the average content of a bag of cryoprecipitate as determined by one Red Cross blood bank was assayed as containing: 1) 120 units of Factor VIII (range of 105 to 130), 2) 46.6 mgm of fibronectin, 3) 326 mgm of fibrinogen, and 4) present but not quantified amounts of von Willebrand factor (420).

With commercial preparations of Factor VIII concentrates, a higher concentration of Factor VIII can be achieved with less volume of infusion needed to administer it, and an exact Factor VIII content of each preparation can be assayed rather than assumed. Moreover, the stability and packaging of the concentrates is more suitable for home therapy.

When large pools of frozen material are processed, thawing is carefully controlled to improve yield and stability. The best yields of Factor VIII from cryoprecipitate are approximately 25 percent of the starting material. (As mentioned in ch. 3, heat-treated Factor VIII concentrates are now available. )

### Gamma Globulins

Essentially all gamma globulins are recovered from Cohn Fraction II using the classic cold-ethanol technique. Immune serum globulin (ISG) is dispensed as a 16 percent protein solution for intramuscular injection in amounts up to 10 ml, or as a solution for intravenous injection (Intravenous gamma globulin, or IVGG).

### Albumin

The production of albumin from fraction V-o utilizes pH, ionic strength, and ethanol concentration to precipitate a highly purified concentrate of this principal oncotic agent of plasma. The powdered albumin is then resolubilized as either a 5 percent solution, with the same oncotic pressure as normal plasma, or as a 25 percent concentrate of salt-poor albumin. The 25 percent concentrate is marketed as a hyperoncotic concentrate which draws water from the extravascular tissues to restore blood volume and circulatory competence in a recipient suffering from hypovolemic shock.

An alternative method of albumin production is the elimination of the subfractionation steps which precipitate Fractions IV and V and reconstitution of these pooled fractions as an albumin solution. This material, plasma protein fraction (PPF), contains 83 to 90 percent albumin, no more than 17 percent of the total protein as alpha or beta globulin, and no more than 1 percent as gamma globulin. Currently, after the preceding fractions are removed, 30 percent of all fractionated plasma goes into PPF production and 70 percent into albumin production.

Albumin (and gamma globulin) can also be prepared from human placentas. Such processing is different from plasma because of the large amount of connective tissue, free supernatant hemoglobin, and other extraneous material which must be removed by filtration, adsorption, and centrifugation, but the final products are of high purity. The average yield of albumin by this technique is 5 gin / kg of placenta.