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Improved methods for frozen storage of glycerolized red blood cells (RBCs) are needed both to meet military and civilian needs in periods of high demand. Existing deglycerolization systems are labor intensive and considered "open" systems resulting in a maximum storage of 24 hours following washing. The washing of blood cells with flat sheet membrane filters has been proven effective by the Advanced Haemotechnologies. AHT's thawed blood processing system development in year one of the Phase II proposal has focused on optimizing the disposable operation and configuration as well as developing hardware. Suggested key operational parameters include the total volume concentration of blood in the filter and shear induced hemolysis of the blood during washing. Advances in each of the areas were made for washing fresh and frozen thawed red blood cells. Successful completion of the Phase II development will lead to a closed, sterile washing system for thawed blood and should allow for extended storage thawed RBC products.

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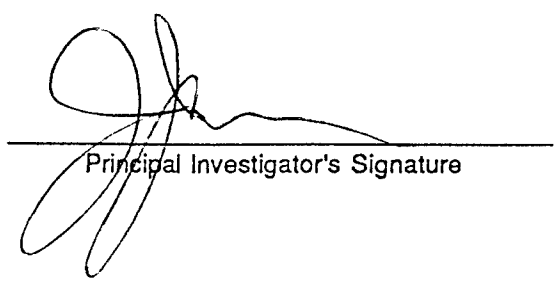

Principal Investigator's Signature

4/12/95

Date

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Introduction

The ability to maintain an adequate supply of all types of blood for military and civilian needs in times of high demand must be addressed either by increasing the collection of whole blood units or by making better use of collected blood components. In response to the latter, significant progress has been made in the technology of freezing, thawing and washing red blood cells (RBCs).

The advantages of frozen/thawed (FT) storage of RBCs include the removal of plasma, platelets, white blood cells (WBCs), anticoagulants and the PVC bag plasticizer (DHEP). Cryopreservation also allows for the long-term storage of rare red cell types and selected red cells lacking antigens which commonly sensitize recipients (1). Some reports have indicated that washing of frozen/thawed RBCs (FTRBCs) leads to a reduction in the level of CMV virus (2) through the removal of plasma and white blood cells. In addition, cryopreservation is used for autologous storage when larger volumes of blood are requested for covering planned surgical procedures. Finally, a blood banking system which combines liquid storage and frozen storage of rejuvenated O-positive and O-negative cells would ensure adequate blood supply in times of high demand or emergency. The major disadvantages of frozen RBC storage include limited post-wash storage and higher processing costs, including disposables, hardware and labor costs associated with storage and washing.

This report summarizes the status of the development of a novel, closed sterile system for washing FTRBC using membrane based technology developed by Advanced Haemotechnologies (AHT).

Frozen Storage of RBCs: In the development of FT technology for RBCs, several cryoprotectants have been evaluated for the freezing of RBCs including glycerol, hydroxyethyl starch and polyvinylpyrolidone.

Glycerol is generally accepted as the best cryoprotectant for freezing RBCs. Several factors are known to affect the degree of FT hemolysis of glycerol frozen RBCs, including: red blood cell and glycerol concentration; conditions for mixing blood with glycerol; composition of the freezing container; the freezing rate and storage temperature; and the rate of thawing and thawing temperature (3). Two approaches to freezing RBCs in glycerol have been developed: high glycerol (~40%) with frozen storage at -80°C; and low glycerol (~20%), requiring storage in LN_2 vapor.

The use of 20% glycerol to FTRBCs has yielded good results with respect to RBC recovery and 24 hour in-vivo survival (1,4). This method allows for simpler and faster washing procedures to remove the glycerol. However, the major drawback of this approach is the necessity of maintaining -130°C throughout storage. Studies have shown that increased hemolysis occurs affecting recovery of RBCs if the temperature is elevated above -130°C even for short periods during storage (4). This presents logistical problems with the storage of low glycerol frozen blood in LN_2 vapor, especially during transport.

Freezing RBCs in 40% glycerol simplifies the requirements for freezing and storage, but requires additional washing to effectively remove the glycerol. Cells treated with 40% glycerol are frozen in -80°C freezers in standard PVC bags at a rate of ~1°C/min and stored at this temperature. Transportation of frozen units is simplified through the use of mechanical refrigeration and dry ice to maintain -80°C. Currently, the FDA has approved frozen storage for up to 10 years. Valeri et. al. have reported that FTRBCs can be thawed and washed after storage for up to 21 years at -80°C and remain safe and therapeutically effective (5).

The intracellular environment of glycerolized cells is hypertonic relative to plasma and the first solution used must be somewhat hypertonic. This allows the glycerol to begin diffusing out of the red cell while the intracellular environment remains hypertonic. After equilibration of the thawed RBCs with a hypertonic solution, the next step is washing with solutions progressively less hypertonic and final suspension in an isotonic electrolyte solution containing glucose. Several approaches to washing thawed cells, previously frozen in 40% glycerol, have been attempted (6,7,8,9). Early methods for deglycerolization required more than 4 L of wash solutions yielding varying recoveries (1). As deglycerolization methods developed, a number of devices, protocols, and wash solutions were evaluated over the past 40 years (6,8,9). With the exception of the agglomeration method developed by Huggins (6,10), where low ionic strength diluents were used to cause reversible agglutination and sedimentation of cells, most methods have relied on centrifuge-based technologies. Today two centrifuge devices for deglycerolization are commonly used: the Cobe 2991 or 2992 and the Haemonetics 115 cell washers. The deglycerolization procedure with both of these devices employs initial dilutions of the thawed blood with 12% sodium chloride followed by 0.9% sodium chloride-0.2% glucose (NaCl-glucose) solutions (1). After dilution, the cells are washed with 1.5 to 2 L of NaCl-glucose with either the Cobe or Haemonetics cell washers. The Cobe cell washer can be operated in manual or automated modes, using a series of batch washing/centrifugation/dilution steps. The Haemonetics cell washer employs a bowl configuration in which the diluted thawed unit is washed continuously with NaCl-glucose. Both the Cobe bag and the Haemonetics bowl have rotary seals making the devices "open systems". Currently, a 24-hour shelf life is imposed by the FDA on previously frozen-thawed, deglycerolized red cells, as well as on non-frozen red cells washed with these existing commercially available cell washers because the rotary seals in these systems are not closed and sterility can be broached during processing (11). With both devices, the washing procedure requires approximately 30 minutes and requires operator intervention during washing.

A major issue regarding frozen blood supply is the length of time within which RBCs must be used after thawing(12). Sterile docking devices are now available for joining plastic tubing without violating sterility so that the hazard of contamination need no longer be a factor limiting subsequent storage if a washing device with a closed sterile system is available. The AHT Thawed Blood Processing System (TBPS) system is designed to meet this criteria by providing a closed sterile disposable for the washing of RBCs.

The U.S. Department of Defense plans to maintain a stockpile of 300,000 cryopreserved units of RBCs by the year 2004 (4). In order to have these units readily available for use at the battle field site, advances in the washing technology are required, especially with respect to blood sterility and automation. The device proposed for development by AHT will meet both of these requirements. Specifically, this projected device offers several advantages over current centrifuge based technology including: automated dilution and washing protocols; compact, portable hardware; and a sterile, closed washing system allowing for extended storage following deglycerolization.

History of TBPS Development

Cell separation utilizing microporous membrane filters to separate plasma from cells is termed plasma filtration. The first commercialized applications have been therapeutic plasmapheresis and donor plasma collection. Cross flow filtration as opposed to "dead end" filtration has been almost universally used. In recent years, high shear rate designs such as the Baxter Autopheresis - C^{R} rotating cylinder plasma collection system have proven effective and cost competitive compared to centrifuge systems.

In 1990, L. S. Gordon obtained a patent for "Apparatus and method for the autotransfusion of blood" using a two stage, flat sheet plasma filter. Advanced Haemotechnologies developed a system which made use of

membrane based cell separation for intraoperative autotransfusion, the PlateletPlus™Blood Processing System.

Extensive testing performed during the development of the PlateletPlus™ System, a prototype, which demonstrated the suitability of plasma filtration for blood cell washing and concentration in the intraoperative situation. The PlateletPlus™ system advanced the science of cell washing to a new level by combining the three steps of conventional machines (fill, wash and empty) into one continuous process.

The requirements for cell washing for deglycerolization are somewhat different. The PlateletPlus™ System for intraoperative autotransfusion incorporates 2 filter stages to achieve a reduction in supernatant concentration of 75%. It also reduced the supernatant volume by approximately 70%, for a total reduction of 92%. Deglycerolization requires a reduction of supernatant concentration of 98%. However, the total blood volume processed is much lower than typically seen in intraoperative autotransfusion. This suggested that a single stage batch processing system would be more appropriate for deglycerolization. AHT has developed the Thawed Blood Processing System (TBPS) for filtration of frozen-thawed cells during deglycerolization. For this application the filter disposable has been modified into a two membrane, single rotor batch processing device in which a full unit of thawed blood is pumped into the filter housing, concentrated and washed with saline/glucose.

AHT completed the elements of the Phase I proposal with the development of a device which meets the objectives of the Phase I proposal. Specifically, an output hematocrit of approximately 40%, residual glycerol levels of $\leq$ 1% and a unit wash time of less than 25 minutes.

The conclusions of the Phase I experiments were as follows:

- Membrane selection studies indicated that the optimal membrane was a Pall 1.2μ LP membranes. The Pall LP membrane showed the best saline and plasma flow rates. Evaluation in autotransfusion devices indicated the Pall LP membrane was least susceptible to membrane occlusion during washing of blood bank units.

- Evaluation of the AHT autotransfusion device for washing calcium-doped RBCs indicated that with such continuous flow device sufficient 40:1 washout could not be obtained. Calcium-doping was used in all early stage evaluation of prototypes to allow for a readily accessible indication of washout at low cost. The use of a recalculating circuit employing an autotransfusion filter has been designed and may be evaluated in Phase II.

- Evaluation of the Batch Unit Processors (BUP) demonstrated that sufficient calcium washout could be achieved. The BUP devices washed approximately a third of a unit. Of the basic configurations evaluated, the BUP I, with a single rotor gave superior results compared to the BUP II, with two rotors. The primary difference between the performance of the two configurations was a lower operating pressure with the BUP I. Based on past experience with the AHT device, a lower operating pressure typically leads to a reduction in any shear-induced hemolysis.

- Processing units of calcium-doped RBCs was performed with Whole Unit Processors (WUP) devices. The single rotor WUP I was superior to WUP I devices with surface modification with respect to transmembrane pressure and calcium washout. All WUP devices required approximately 1500 ml of wash volume to achieve greater than 40:1 calcium washout. This wash volume is essentially equivalent to the current Haemonetics 115 device.

- Washing frozen/thawed RBCs with the WUP I and WUP II/B/H devices yielded glycerol washouts of less than 1% and an average hematocrit of approximately 40% in a washing time of less than 20 min.

The TBPS is a fixed volume disposable filter with an internal chamber capable of holding a unit of red blood cells. The TBPS main filter body is comprised of a hollow, disk shaped chamber with 1.2μ Pall Loprodyne micro-pore filtering membranes forming the upper and lower surfaces of chamber. Inside the chamber is a disk rotor. The rotor is mechanically connected to a cylindrical magnet assembly that magnetically "locks" with the rotating driving magnet coupler on the Drive Unit Console. This coupling provides a closed sterile system and eliminates the need for rotating seals and the associated risks of leaks.

The spinning internal rotor creates mixing forces in the filter that aid in the cell washing process. The efficiency of the membrane is maintained by gently spinning rotors that continuously sweep past the filter membrane. The spinning rotors also impart angular momentum to the blood. Since blood flow is constrained by the casings, pressure is generated which contributes to the transmembrane pressure necessary to effect filtration. This provides a very simple method to control transmembrane pressure without complex servo-control circuits.

Phase II Proposal Goals and Summary

The Phase II Program was defined in seven experimental series. These series are summarized as follows:

1. Design of the filter
   - Disposable volume selection
   - Wash solution flowrate and rotor speed
   - Options for washing second units
2. Optimization of Operation
   - Disposable volume selection
   - Wash solution flowrate and rotor speed
   - Dilution sequence of thawed blood
   - Minimization of operator manipulation with TBPS
3. Preliminary Comparative Evaluation of TBPS vs Haemonetics 115
4. Sterility Evaluation
   - Filter sterility
   - Washed blood sterility
   - Biocompatability
5. Evaluation of washing or dilution with metabolic additive solutions
6. Baboon survival study
7. Design - Construction of prototype hardware

The goal of this Phase II program is to achieve a device consisting of a disposible sterile filter washing device and console designed to wash frozen-thawed blood to the following specifications:

Glycerol washout to less than 1%
Supernatant hgb less than 150 mg/dL post washing and wash recovery of 85%
Absence of significant potassium leaks in RBC's
Hematocrit of 80% or 60% (following wash with additive solutions)
Sterile blood product

Survival of RBC's at AABB standard
Stability during storage in additive solutions for up to 5 days

The focus of the Phase II development in year one has been on Experimental Series 1, 2 and 7. AHT's approach to finalizing the TBPS filter disposable has been to perform evaluation of operational parameters utilizing pooled fresh blood units. Results with fresh blood studies were then confirmed by washed thawed-frozen blood. The major disposable issues addressed in year one have been operational conditions including target hematocrit of washed blood in the filter, the saline and blood flow rate and the rotor speed. Disposable design issues addressed have included filter rotor type and filter volume. The results obtained for various operational conditions for washed fresh and thawed frozen blood are discussed later in this report.

The console hardware is being designed and prototyped by evaluation of the function and integration of the required components. A motor is used to drive the rotor inside the filter through the use of a magnetic coupling drive. Solenoid controlled pinch valves automate the control of the flow of all solutions with a pump device delivering the thawed blood along with all solutions used for dilution and processing. A shaker is used to mix the thawed unit during dilution. A pressure monitoring device is used for constant measurement and control of pressure within the filter during processing. Man/machine interfaces will include visual screen, keyboard, printer, bar code reader, and audible alarms for user-friendliness, effectiveness, and efficiency.

Experiemental Methods

Standard Preparation of Blood Samples for Evaluation:

Packed Red Blood Cells: Packed RBCs are purchased from St. Lukes Episcopal Hospital or Gulf Coast Regional Blood Center. Fresh packed RBCs units are used to define optimal washing conditions with each device configuration. Prior to use, each unit are filtered through a microaggregate filter to remove any clots. Blood units (300g of packed RBCs) are diluted to a hematocrit of 20-25%, the hematocrit of frozen/thawed cells following dilution prior to washing.

Thawing of Frozen Thawed RBC: The first step in the deglycerolization process is diluted with 12% sodium chloride and 0.9% sodium chloride-0.2% saline according to Valeri's method (5). 50 ml of 12% saline is added to the thawed unit with mixing on a shaker. Following this addition, the shaker is turned off and the red cells allowed to equilibrate for 2 minutes. The same procedures are followed to second (100 ml) and third (150 ml) additions of 0.9% sodium chloride-0.2% glucose to the thawed red cells. The diluted thawed cells are washed with 1.5 L of wash solution in a current revision of the TBPS disposable filter.

Washing of Thawed or Fresh Cells: The TBPS manual prototype console allows for control of blood and wash solution flowrates, rotor speed, and the monitoring of total membrane pressure. A brief description of the procedure for washing cells with the TBPS console follows.

The filter is primed with 0.9% saline/0.2% glucose at 200 ml/min with the rotor off. For the remainder of the washing procedure the pump speed and rotor speed are maintained at values listed for each experiement in the results tables. The typical range for rotor speed is 600-1200 rpm and for flowrate between 100-150 ml/min. After priming, the blood is pumped in. When all the blood is concentrated in the filter, the saline/glucose wash is initiated for up to 2000 ml. After the wash is completed, the blood is pumped out of the filter into a satelite bag for storage.

Both fresh blood and frozen thawed cells are washed at St. Lukes Episcopal Hospital and the NBRL using TBPS disposables and current revision consoles according to instructions provided by AHT and adapted from the Naval SOP. Samples collected during the thawing and washing process are analyzed by St. Lukes Episcopal Hospital (SLEH), Memorial Hospital Woodlands, or Surgimedics where appropriate.

Methods of Analysis

CBC: CBC is determined on washed Frozen Thawed Red Blood Cells (FTRBC) to determine red cell number recovery, total hemoglobin, hematocrit, MCV, MCH and MCHC, and WBC and platelet counts. CBC analysis is either performed at Memorial Woodlands Hospital or St. Lukes Episcopal Hospital on a Coulter Counter or comparable analyzer.

Glycerol washout: Glycerol washout is determined by increased refractive value. It has been shown that if the residual glycerol concentration is above the desired 1%, then the measurement on a hand-held refractometer, will be in excess of 30.

Marker washout: For preliminary studies where fresh blood is used for evaluation, calcium is used as a marker for washing efficiency. The correlation between calcium washout and glycerol washout has been demonstrated. Calcium chloride is added to a unit of diluted packed RBC's prior to washing to give a concentration of 30-40mg/dL. The washed product is then analyzed for calcium colorimetrically on a Kodak Ektachem at Memorial Hospital Woodlands..

Potassium will be measured at NBRL by flame photometry.

Calculations for Spreadsheets: Several values reported in this report's tables were derived from measured parameters. The calculations used for these are as follows:

Target hct = (blood volume in filter * pre-wash blood hct)/fixed volume of filter:
hct recovery = (post hct/Target hct/100)
Calcium washout = (sup vol out * [post Ca]/(sup. vol in * [pre Ca]) - 1 * (-100)
Post wash hgb Recovery % = (post cellular hgb/pre total hgb) X 100

Supernatant (Plasma) Hemoglobin: Supernatant hemoglobin is determined by the colorimetric method of Lijana and Williams and Standefer and Vanderjagt, using 3, 3', 5, 5' - tetramethylbenzidine as chromogen. (Sigma Procedure No. 527)

Results and Discussion

Disposable Volume and Outlet Hematocrit

The TBPS is a fixed volume filter, therefore, the concentration of blood, i.e., the final hematocrit for washed blood will be dependent on both the volume of red cells washed and the capacity of the filter. An additional theoretical limitation is the viscosity of the blood and the effect of increased blood viscosity on shear induced from the spinning rotor in the filter.

Two filter designs have been evaluated to determine the maximum hematocrit which can be achieved in the filter disposables. The filters used were developed in Phase I, specifically the WUP I (518 pin) and BUP I (214 plain). Based on an internal volume measured to be 518 mls and 214 mls for the WUP I and BUP I, respectively, the grams of red cells placed in the device were adjusted to evaluate the maximum hematocrit of each device. Examples of such calculations are shown in Table 1 for each device.

Table 1a: Sample Calculation of Blood Volume for BUP I

Internal Volume of Filter (ml)	Target Final hct in Filter (%)	Volume of RBCs (ml)	Initial hct of RBC for Washing (%)	Volume of RBC to be Washed (ml)
214	40	85.6	20	428
214	50	107	20	535
214	60	128.4	20	642
214	70	149.8	20	749

Table 1b: Sample Calculation of Blood Volume for WUP				
Internal Volume of Filter (ml)	Target Final hct in Filter (%)	Volume of RBCs (ml)	Initial hct of RBC for Washing (%)	Volume of RBC to be Washed (ml)
518	40	207.2	20	1036
518	50	259	20	1295
518	60	310.8	20	1554
518	70	362.6	20	1813

The proposed uses for the BUP and WUP were to wash one or two units, respectively. During the course of year one the approach of processing two units simultaneously in one filter has been deemed by the Navy as less desirable than two single units washed with the same filter in series. Results for two unit washing by each approach are discussed later in this report.

The BUP filter has a volume of 214 mls. Table 2 shows calculations of the RBC capacity of this filter at hematocrits up to 70%. The range of frozen unit volumes is significant, however, the typical range is between 200-240 grams per unit (Table 2). These unit weights convert to a cell volume of 173 to 208 per unit. Given this range it is likely that the 214 plain filter will need to be increased somewhat in volume to accommodate larger units of frozen/thawed blood. However, for the development efforts in year one, only one additional volume was evaluated, a 320 ml filter. Results for this filter are discussed and contrasted to the 214 ml filter later in this report. As additional data is collected, especially with frozen thawed blood, studies with filters with increased volume will be undertaken.

Table 2: Sample Calculation of Blood Volume for BUP I

Internal Volume of Filter (ml)	Target Final hct in Filter (%)	Volume of RBCs (ml)	Initial hct of RBC for Washing (%)	Volume of RBC Suspension to be Washed (ml)
214	40	92	20	458
214	50	114	20	572
214	60	137	20	687
214	70	160	20	801
280	40	120	20	599
280	50	150	20	749
280	60	180	20	899
280	70	210	20	1049
320	40	137	20	685
320	50	171	20	856
320	60	205	20	1027
320	70	240	20	1198

Net Weight of RBC Unit Before Freezing (hct=75%)	Net volume of RBC Unit Before Freezing (hct=75%)
150	140
200	187
220	206
240	224
300	280
330	308

214 Plain Filter Results: The 214 Plain (BUPI) design holds 214 ml of volume. Preliminary tests were performed using Calcium-doped blood blank red blood cells to establish operational conditions and dependent variables. The operating conditions for each trial are listed in the results tables. Variables presented are the blood volume used, saline wash volume used, average flow rate, rotor speed, target hematocrit (theoretical hematocrit of washed blood product), a recovery calculated based on hct, calcium washout and pre and post-wash sup. hgb levels.

Evaluation of the Effect of Increasing Target Hematocrit on performance of the 214 plain filter was performed. The results are summarized in Table 3 for tests within four ranges of target hematocrit, 55%, 55-65%, 66-80% and 70-80%. Testing was performed with fresh calcium-dropped RBCs. Results in Table 3 indicate that the recovery based on hematocrit > 95% on average when the target hematocrit was between 50 and 65% (See Groups A and B). The apparent high recovery (>100%) for the Group A is an unexpected result. Such results are only seen in early experiments performed in the first quarter and there is no obvious explanation as to why these low hct tests would show such high recoveries. However, when the target hematocrit exceeded 65%, the final hematocrit was an average appreciably less than the target (Group C and D). These results suggest that the maximum hematocrit achievable with this filter configuration is appromxiately 60-65%. There is an apparent relationship, between elevated pressures, increased hemolysis, and achieving post wash hematocrits less than target hematocrits. This relationship supports the need to evaluate modifications in the filter volume and rotor to address these issues.

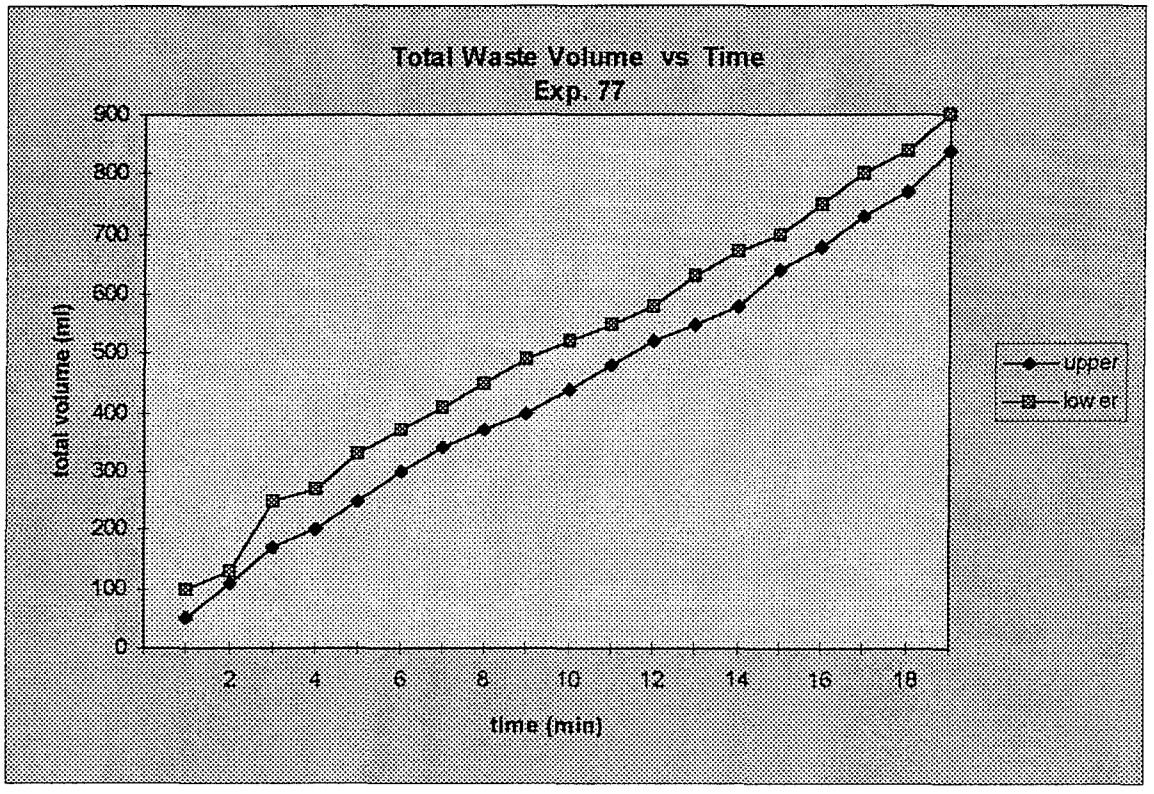
Efficiency of marker washouts for fresh blood cells doped with $CaCl_2$ as a washout marker was determined. Results for washout for these cells washed with the 214 plain rotor are summarized in Table 4. The typical glycerol level following thawing frozen blood is approximately 40% and must be reduced to less than 1%. This equates to a washout percentage of 97.5%. For the Ca^{+2} doped RBCs washed with the 214 plain filter, 33 of 37 had washout percentages in excess of 97.5%. This result clearly indicates the TBPS in this configuration can remove $CaCl_2$ and predicts sufficient removal of glycerol frozen thawed blood under like conditions.

The Role of Transmembrane Pressure was also reviewed for all 214 plain filter tests. Table 5 lists the test results divided into groups separated as a function of maximum transmembrane pressure. Examining this data, there is a dramatic decrease in the hct recovery for tests where the pressure exceeded 300 mmHg. This decreased recovery does not correspond to an appreciable difference in post supernatant hgb levels. However, a correlation does exist with respect to the target hematocrit. The average target hematocrit for Group B is more than 10% greater than those filters which operated at pressures less than 300 mmHg. This result suggests that there is insufficient mixing by the plain rotor in the 214 plain filter to prevent partial occlusion of the filter during washing, leading to increased pressure. These results supported the transition to modified rotors to improve mixing.

214 Plain Evaluation of the Waste Flow Rate from each membrane within the TBPS was performed in two trials to determine if there was different waste flow rates through the upper and lower casing membranes. The goal being to determine if a differential in occlusion of the lower or upper membrane existed. The processing conditions and performance results for these trials are listed in Table 8. The target hematocrit for these runs was set at 85-86 percent to exaggerate the effects of any membrane occlusion. As expected for higher target hematocrit runs the pressure was high and the final hematocrit only 52-55%.

The total flow as a function of processing time for each test is shown in Figure 1. This figure illustrates clearly that there is no divergence over time of the waste volume flow. The irregular behavior at the beginning of each run seems to be a result of displacement of air that was not completely removed during the priming. It is also interesting to note that although the area of the lower casing is less than that of the upper casing by approximately 9%, this seems to have no bearing on the waste flow rate. This is likely because the rotor sits closer to the lower membrane, leading to more effective mixing at the lower membrane surface thus compensating for the smaller area. This hypothesis supports the concept that improving the mixing at the membrane surface will improve waste flow rate and accordingly reduce pressures in the filter.

Figure 1: BUP Evaluation of Waste Volume Measurement, Upper and Lower



The effect of rotor speed on the performance of the 214 plain filter was evaluated by monitoring the transmembrane pressure within the filter and the waste supernatant hgb as a function of rotor speed. Two experiments were performed in which the 214 plain filter was filled with blood and washed with saline at 100 ml/min. at 1200 rpm initially followed by decreases to 1100, 1000, 900, 800, 700, 600 and back to 1200 rpm in series.

Results for the effect of rotor speed on cell washing with the 214 plain rotor filter are shown in Figure 2. These results indicate that as the rotor speed is decreased, the pressure increases, with a maximum pressure reached at approximately 700 rpm. This result further supported the conclusion that mixing would be critical to improving filter performance by reducing occlusion of the filter membranes.

All results with the 214 plain rotor suggest that the target hematocrit and associated partial occlusion of the membrane leading to pressure increases are the dominant dependent variables with this filter type. An additional parameter evaluated as the blood and saline flow rate but did not appear to affect performance.

214 Plain Evaluation of the Effect of Adding Blood and Saline Simultaneously

A potential optimization point is the hematocrit of the blood when entering the TBPS. The possible advantages of lowering the inlet hematocrit are reducing the viscosity for the cells as they are initially washed and increasing the overall change in hematocrit which contributes to washout. One means of decreasing the initial hematocrit was to mix the blood and saline simultaneously inline as they were pumped into the filter.

We performed two sets of trials to assess whether with the 214 Plain TBPS design this manipulation would affect the performance of the device. Results in Table 6 suggest improved post supernatant hgb levels but no other apparent improvement. The benefit of this modification will be evaluated further in later testing.

214 Plain Preliminary Studies Washing Frozen-Thawed RBCs

Experimental Series I studies with Frozen thawed blood have been completed. Nine units of frozen blood were thawed, diluted and washed with the 214 Plain TBPS filter using standard conditions of 100 ml/min flow rate and a rotor speed of 1200 rpm. Processing conditions and NBRL testing results are listed in Table 7. These results indicate that target hematocrits of up to 60% can be achieved when the pressure in the filter is kept below 110 mmHg. In four of the tests the pressure was above 200 mmHg. Three of the four of these likely showed elevated pressures due to overloading the filter with cells as evidenced by theoretical target hematocrits of greater than 80%. Steps were taken to prevent such overloading in future tests. The exception to this set was the fourth sample with high pressure which achieved its target hematocrit despite the elevated pressure. Supernatant hemoglobin levels post washing were elevated in the high pressure tests compared to the tests which operated at less than 100 mmHg.

Results suggest that all nine units had acceptable freeze/thaw recovery, validating that the procedures used are consistent with the NBRL SOP. Red cell recovery was calculated based on the amount of hemoglobin lost to the waste and on the measurement of cellular hemoglobin in pre and post wash samples. In all tests the waste hemoglobin calculation yielded higher recoveries than the pre/post analysis. A likely explanation for this discrepancy is that not all the cells were removed from the filter in some of the test. Waste recovery results indicate that washing with the TBPS device can yield acceptable recoveries of greater than

80%. In order to improve the pre/post recovery additional steps were taken to improve the method of removing cells from the filter in future tests.

The washout of glycerol, determined by the osmolarity of the supernatant was very good for all nine units. This indicates that for the volume of blood being processed in these tests that 1000 mls of wash solution was sufficient. Two indicators of cellular damage post washing are the supernatant hgb and the intracellular potassium levels. For units washed at pressures below 150mmHg the supernatant hgb levels were well below the AABB limit of 500mg/dL. However, to improve post wash hgb levels, additional saline wash volume may be required. Intracellular potassium was measured for three units. The measured level averaged 4.1 vs a normal of 6-7mEq/RBC. A reduction in potassium is an indication of membrane stress. This result suggests that in the 214 Plain configuration, the TBPS potentially caused stress to the RBC membrane, likely due to shear, resulting in potassium leakage. This observation supported the requirement to modify the TBPS rotor in order to reduce the shear. This we believed could be accomplished by improving mixing while reducing the rotor speed.

Filter Rotor Modification Evaluation

Rotor Design Options

In order to increase the final hematocrit inside the filter without observing high pressure, the membrane had to more effectively cleared. This means the rotor had to be modified to create more turbulence (but not more shear, since shear itself leads to hemolysis).

With the 214 plain filter, there was apparently inadequate mixing within the filter because the current rotor behaves essentially as a flat, smooth plate inside the filter. One or more simple structural modifications to the rotor should improve mixing within the filter, thus allowing greater target hematocrits while reducing internal pressure.

Previous testing results with the BUP I have suggested that the upper limit for the outlet hematocrit was 60%- 65% likely as a result of inadequate clearing of cells off the membrane which yielded over pressure in the filter. Given these results, we performed preliminary evaluation of several rotor design configurations.

In order to determine the effectiveness of different rotor configurations for mixing within the filter the vortex, pressure, and fluid velocity were observed. The fluid flow and pressure at the inlet line were evaluated for several rotor configurations: plain rotor, rotor with 4 radial bars, rotor with 4 straight bars at an angle to the radius, and rotor with 4 ridges with kinks in each bar to approximate a smooth windmill design. It was found that the rotors with straight ridges caused the greatest fluid velocity for a given rotor speed. However, the curved shape of the bars provided centripetal force that served to decrease the centrifugal pressure on the outer rim of the filter and increase the pressure in the low pressure area in the center of the filter, thus equalizing the pressure in the filter. Further testing was performed with a 12 ridge windmill configuration which demonstrated that this design appeared to optimize mixing.

During the evaluation of new rotors, it was determined that the bond strength of the membrane to the upper and lower casing plastic was insufficient to handle the forces and pressures generated. To solve this we evaluated several support media and selected one which provided efficient bond strength.

Based on these preliminary results several rotor configurations were evaluated for washing blood this quarter. Each different rotor design was tested with the 214 plain filter as a control. Initial studies with modified rotors were performed with calcium-doped fresh RBCs.

The initial modified rotor studies evaluated the effect of three modified rotors on performance of the TBPS filter. The filters evaluated included:

a. 214 plain - the original flat rotor
b. 214 3R - a flat rotor with three vertical fins positioned on each side of the rotor, perpendicular to the rotor edge at 0, 120, and 240 degrees
c. 214 3R 2H - a 214 3R rotor with two holes drilled in the rotor to allow for mixing between each side of the rotor
d. 214 12R 6H - a flat rotor with 12 ridges on each side of the rotor, arranged in a curved pattern to improve mixing and reduce shear, and with 6 holes in the rotor

Review of the results (listed in Table 8) and comparison of the results obtained with each type of filter rotor design indicate that modifications to the rotor could improve performance for RBC washing. All tests we performed with high target hematocrits to maximize any effects from membrane occlusion. 214 plain filter results for similar hematocrits are listed for comparison. Results with the 214 3R rotor in the 214 filter compared to the 214 plain filter suggested that the addition of three ridges on each side of the rotor did not improve the target hematocrit or operating pressure during washing of higher hematocrit samples. However, results for the 214 3R 2H disposable, in the one filter evaluated, suggested that the presence of the holes increased the output hematocrit and decreased the operating transmembrane pressure. This combined effect of ridges on the rotor surface and the holes in the rotor lead to the design of the lead prototype filter, the 214 12R 6H, a 214 ml filter with a rotor with 12 ridges on each side of the rotor and 6 holes drilled in the rotor.

The 214 12R 6H rotor was designed to maximize mixing and allow for lower rotor speeds and shear. As seen in preliminary testing, the 214 12R 6H disposable showed a further increase in the output hematocrit and operating pressure at an elevated target hematocrit. While the target hematocrit was not achieved in on average (Table 8), note that the operating pressure was lower compared to other modified rotors with fewer ridges or holes.

Previously, with the 214 plain rotor disposable, increasing the flowrate frequently lead to an increase in the pressure within the filter and an apparent increase in hemolysis. In two preliminary experiments, 102 and 104, (see Table 9) we varied the flowrate from 100 up to 200 ml/min during the washing of calcium-doped RBCs with the 214 12R 6H and did not observe an effect on the transmembrane pressure. This suggested that the variables which affected the original 214 plain rotor disposable may not be as dependent with the modified rotor disposable.

Given this we designed an experiment in which we evaluated the effect of rotor speed vs. the transmembrane pressure and waste plasma hemoglobin concentration at two set flow rates, 100 and 150 ml/min for washing fresh RBCs with the 214 12R 6H rotor disposable. We initially set a flowrate of 900rpm for all the modified rotor disposables. The protocol for this experiment was to pump RBCs sufficient for a 60% hematocrit into the disposable, begin washing with the rotor at 900rpm, after equilibration the rotor speed was changed to 1000, 1100, 1200, 900, 800, 700, 600, 500 and 900 rpm for 3 mins at each rotor speed in series. The transmembrane pressure was monitored each minute and waste supernatant samples collected immediately before changes in rotor speed following equilibration at each rotor speed.

Figure 3 represents results for washing RBCs comparing the effect of rotor speed vs. the transmembrane pressure and waste plasma hemoglobin with fixed flowrates of 100 and 150 ml/min, respectively. The lines presented represent the average of four experiments performed for each flowrate according to the above described protocol. It is impressive to note the similarity in the pressure vs rotor speed curves for each test, at a flowrate of 100 ml/min. (Figure 4). Reviewing Figure 3 several observations can be made:

- The general shape of the pressure and waste hgb as a function of rotor speed is similar and distinctly different from that observed with the 214 plain rotor filter (Figure 2).
- The pressure increased from 900-1200rpm, decreased back to starting pressure upon return to 900rpm, dropped to a low value of 600-800 rpm, and increased significantly as the rotor speed was decreased to 300-500 rpm. It should be noted that once high pressures were achieved from low rotor speed, upon increasing the rotor speed to 900rpm the pressure did not return to the baseline level. This is probably due to cellular occlusion of the membrane from the inadequate mixing at low rotor speeds.
- The waste hgb levels decreased dramatically independent of flowrate when the rotor speed was decreased from 900 to 800rpm, with a minimum level of hemolysis at 500rpm.
- Comparing the pressure and waste hgb curves there is an apparent tradeoff between obtaining sufficient mixing to minimize pressure and limiting hemolysis from shear.
- This rotor design allows for washing at flowrates up to 150 ml/min at reduced rotor speeds, transmembrane pressure and hemolysis.
- The waste hgb levels were higher for RBCs washed with a 100 ml/min flowrate vs the 150 ml/min flowrate. This is likely an artifact from the increased wash volume at the higher flowrate resulting in a lower residual hgb in the filter at the point of equilibration of pressure and start of the experiment. In future experiments this artifact will be eliminated by washing cells until the hgb level normalizes at the initial rotor speed, this will allow for more accurate measurement of changes in waste hgb as a function of rotor speed.

These results indicate that the rotor modifications in the 214 12R 6H rotor filter did improve performance compared to the original 214 plain rotor filter.

These experiments were repeated with the rotor speed adjusted following an equilibrium period at the beginning of washing to obtain a baseline waste hgb level. Results for this experiment are shown in Figure 5 The results are very similar with or without the equilibrium period suggesting that the dramatic decrease in supernatant hgb at between 900-800 rpm was real and not an artifact due to lack of equilibrium (Compare Figures 3 and 5).

214 12R 6H Results

Evaluation of the 214 12R 6H filter performance was made relative to operational parameters. Results of these parameters are discussed below.

The effect of target hematocrit on performance is listed in Table 10. These results are similar to the 214 plain rotor in that as the target hematocrit increased the hematocrit recovery decreased. This result does not clearly correlate to the other operational parameters, such as flowrate and rotor speed. However, for all samples with target hematocrits greater than 70%, the transmembrane pressure was increased relative to test with hematocrits less than 70%. The rise in pressure, with higher hematocrits did not increase affect post-wash supernatant hgb levels on average.

In addition to the decreased hematocrit, during testing we observed a larger than expected post wash volumes under certain conditions. It was determined that a 20-30 ml increase in the volume of the filter

could be observed as a function of transmembrane pressure, resulting in variable actual vs. target hematocrits. To reduce this volume change we evaluated using a stainless steel clam shell to encase the filter. A summary of the results with fresh blood testing with and without the clam shell are listed in Table 11.

There was a dramatic increase in the transmembrane pressure when the clam shell was used, set B compared to set A, with each run at a 100 ml/min flowrate. These results suggest that the addition of the clam shell does prevent swelling of the filter and leads to increased pressure. The target hematocrits vs. actual were equivalent for the 100 ml/min flowrate samples on average (Sets A & B independent of the clam shell). In contrast, post-wash hemoglobin increased three fold with cells washed at 100 ml/min with the clam shell vs. without (Sets A vs. B).

The dependence of increased pressure on performance is not only limited to tests performed with the clam shell (Table 12). These results which include samples processed with and without the clam shell further support a relationship between higher (>90%) target hematocrits, elevation in post-wash supernatant hgb levels, and increased transmembrane pressure. Two of many possible interpretations is that:
a. Hemolysis leads to occlusion and increases the pressure which increases filter volume thereby decreasing actual hematocrits relative to target or
b. Pressure from blood occluding the filter at higher target hemotocrit causes hemolysis and the higher pressure leads to increased filter volume thereby decreasing actual hematocrits relative to target hematocrits. We believe the former to be the most likely explanation of our results. The benefits of controlling filter volume at the expense of increased pressures will be investigated further.

Pressure within the filter is also a function of flowrate, the higher the flowrate of solution into the filter, the higher the pressure. We have determined flowrates of 100 ml/ml show lower supernatant hgb levels (Table 13).

The efficiency of the 214 12R 6H with respect to marker washout was also assessed. Table 14 tests a summary of calcium washout results for calcium-doped fresh red blood cells washed with the 214 12R 6H filter. The washout in all tests was greater than 99%, a signficant improvement over the acceptable washout for the 214 plain rotor (Table 4). This result further confirms the benefits of rotor modification performance.

214 Modified Rotors Part II

While the 12R 6H rotor has shown improved results vs. the plain rotor we continued our evaluation of alternative rotors. The rotors tested in the 214 ml filter disposable included:

A) 214 plain - the original flat rotor
B) 214 plain 6H- the original flat rotor with six holes drilled in the rotor to allow for mixing between each side of the rotor
C) 214 12R - a flat rotor with 12 tubing fins positioned on each side of the rotor
D) 214 6B - a flat rotor with 6 rigid plastic blades positioned on each side of the rotor
E) 214 6B 6H - a flat rotor with 6 rigid plastic blades on each side of the rotor with six holes drilled in the rotor to allow for mixing between each side of the rotor
F) 214 12B 6H - a flat rotor with 12 rigid plastic blades on each side of the rotor with six holes drilled in the rotor to allow for mixing between each side of the rotor

Each of these filters were compared to 214 12R 6H rotors in using matched pooled blood units each washed with different filters with various rotor configurations. Table 15 lists results for 6 sets of these data, sets A-F. Observations which can be made from these results include:

- Flat rotors (Sets A, B) yield lower actual vs. target recoveries than rotors with ridges or blades (sets C-F).
- Filters with flat rotors (Sets A, B) lead to lower post wash supernatant hgb levels vs. filters with rotors with ridges or blades (sets C- F).
- Filters constructed with blades vs. ridges (Sets E and F) caused increased hemolysis as evidenced by elevated post-wash hgb levels. These results indicate that a smoother ridge vs. a blade configuration will be required to increase the mixing ability of the rotor.

All experiments were performed with the clam shell configuration, therefore pressure was elevated in each configuration.

Another component of the rotor type evaluation was to monitor the waste supernatant during the washing for hgb levels. Figure 6 shows a plot of supernatant hgb, monitored at the waste line vs. the washing time. The results shown here indicate several interesting phenomenon. First there is a sharp peak in the supernatant hgb with all rotor types tested except the 6B rotor which shows a broad peak. This elevation in supernatant hgb may be due to hemolysis of the initial cells pumped into the filter and diluted into the saline used for priming the filter. Priming with the dilute blood is to be evaluated to determine if this supernatant hgb peak can be reduced. Second, the post-wash supernatant samples have significantly higher hgb levels for two of the configurations compared to the others and much higher than the hgb levels of the final waste line sample. This suggests that the membrane may be somehow selectively retaining hgb and passing saline during washing. This is supported also by the relative lack of difficulty removing calcium or glycerol compared to hgb with this device. These results are being investigated further.

Based on these results we evaluated the 214 12R and the 214 6R 6H rotor configurations in addition to the 214 12R 6H filter for washing frozen-thawed cells.

214 12R 6H Frozen Thawed Blood Results

Twenty-two frozen-thawed units were processed with 214 ml filters with several rotor designs. Table 16 is a compilation of all tests performed and the data obtained from the NBRL laboratory testing for the 214 ml filters. For all these filters independent of rotor type and operation conditions the average recoveries were 71.9 and 66.9%, respectively by waste hgb and pre/post hgb recovery calculations. The post-wash supernatant hgb for all samples averaged 694mg/dl. While these results are not sufficient to meet the desired objectives of the program, we believe with continued development, we will be able to meet the objectives. The following tables evaluate the results based on the effect of operating conditions; wash volume, estimated by the total waste volume; transmembrane pressure and rotor design configuration.

Operating Parameters: Table 17 compares the effect of flowrate on the performance of the device in washing frozen-thawed cells. These results, on average suggest the following:

a. Increasing the flowrate increases hemolysis as indicated by decreased recovery and relatively elevated post-wash supernatant hgb. This is consistent with the fresh blood results discussed earlier;

b. Pressures are elevated with lower flowrates. However, all samples tested after 3/4/96 were washed

in filters encased in the clam shell. This likely accounts for the increase in the pressure as was seen with the fresh cell results;

c. Wash flowrate had no effect on intracellular potassium levels on average.

Wash Volume: The wash volume is related to the waste volume. Table 18 compares results for washing frozen-thawed cells with the 214 12R 6H filter based on three groupings of waste volume; less than 2000 ml, between 2000-2500 ml, and greater than 2500 ml. Reviewing the results, only intracellular potassium appears to be potentially decreased as the wash volume was increased. All other parameters appear to be independent of wash volume based on this data.

Transmembrane Pressure: Elevation in the pressure has been discussed previously as a potential cause of hemolysis and decreased recovery based on hematocrit with fresh cells. Table 19 compares results for washing frozen-thawed cells with the 214 12R 6H filter grouped in sets according to the maximum pressure achieved during the wash process. The only parameter which may be dependent on the pressure is the recovery. Somewhat unexpectedly, the recovery appears to increase with filter transmembrane pressure elevation, with the highest average recovery observed for Sets C and D with maximum pressures in excess of 400mmHg. This observation will need to be investigated further.

Rotor Design: Two additional rotor configurations have been evaluated compared to the 214 12R 6H rotor using pooled frozen-thawed blood. Table 20 lists three sets of results for pooled tests. The filters evaluated with frozen blood were the 214 12R and the 214 6R 6H rotor configurations. Comparison of the results in each set suggests that reducing the number of ridges may improve the recovery and decrease hemolysis as evidenced by post wash supernatant hgb (sets B and C). This rotor configuration will be evaluated further.

Increased Filter Volume

The results with the 214 12R 6H filter lead us to begin preliminary evaluation of washing large unit volumes of blood with a 320 ml filter configured with this rotor. The goal was to determine if increasing the filter size would affect performance with a 12R6H filter. The filter's internal volume was increased by adding to the height of the center ring. Increasing the ring height also required an increase in the height of the rotor in order to keep it in the center of the casings.

We evaluated the effect of rotor speed vs. the transmembrane pressure for the 320 12R 6H rotor filter at both 100 and 150 ml/min flowrates. Results for these experiments are shown in Figure 7. These results are encouraging, since the pressure vs rotor speed with the 320 ml filter is comparable to the 214 ml filter. There appears to be a small increase in the operating pressure at a 150 ml/min flowrate with the increased filter size. These results suggest that increasing the flowrate above 100 ml/min for routine washing of a full unit may be possible.

The 320 12R 6H rotor has also been evaluated for routine washing of calcium-doped RBCs. Results of tests are listed in Table 21. In 4 of 5 of these experiments the target hematocrit of 60% was met. This hematocrit is consistent with those achieved for 214 filters. The pressure was low in each filter. Post-wash supernatant hgb results were mixed. These results strongly suggest that with modified rotors, such as the 12R6H, increasing the final production filter volume to accommodate the "average and range" of frozen blood units should be possible.

Two Unit Washing

One of the issues we have addressed is how to wash a second unit with the same filter. Two approaches have been examined.

a. Washing two units simultaneously in one filter, yielding a super unit.
b. Back to back washing in series in a filter which holds one unit.

Simultaneous Washing The original intent was for the 518 ml plain filter (WUP) to be used for washing a single full unit. With the change in final hematocrit specification from 40% to 60-70% from the Phase I to Phase II of this program, the 518 plain filter became obsolete for single unit washing. However, it did allow for evaluation of washing two unit simultaneously in one filter.

Results from four tests with the 518 plain rotor suggest that the filter as designed did not give adequate performance with a maximum actual hematocrit achieved of less than 55% (Table 22). This approach has not been pursued further due to the results and a preference for washing units in series compared together.

Washing in Series in the same filter. To wash two units back to back in the same filter. We have evaluated washing two units with the 214 plain disposable and the 214 12R 6H disposable (Table 23). Results with the 214 plain filter showed decreased recovery and increased transmembrane pressure for the second units washed. We investigated whether the improved mixing in the 214 12R 6H filter would allow for such washing. Experiments 114/115 indicated that the improved mixing did not significantly improve the performance of washing a second unit due to increased pressure and decreased post-wash hematocrit. The likely cause for this reduction in performance is debris occluding the membrane, which was not removed in flushing the filter between units.

To address this a backflush procedure was developed for washing the filter following washing the first unit. This was performed by gravity feeding 1l of saline through the waste line to backflush the membrane with the rotor spinning at 300rpm. The flushed debris was collected in a satellite bag for disposal. Experiment 128/129 was performed in this manner using the 214 12R 6H rotor and show that for the first time comparable results are obtained for the first and second units washed. Given this result we repeated the experiment with the 320 12R 6H rotor filter. These results for experiment 130/131 were similar, with little increase in pressure and only a slight decrease in the post hematocrit, relative to the first unit. We believe that this backflush procedure can be incorporated into the final device to allow for automation of this step and maintenance of sterility. These results will be confirmed during year two.

TBPS System Development

The TBPS hardware consists of three major components: the disposable filter interface, the fluid flow path, and the console. Development of the three major components and the software for the console has taken place during this business year. Each major component, including the software, will be presented and discussed.

Disposable Filter Interface

The interface with the disposable filter includes the magnet coupled drive for the rotor and the tubing connections between the filter and any external bags. The magnet drive consists of a motor, motor controller, and magnetic drive coupler. The tubing connections made are between the filter and waste bag,

filter and processed blood bag, filter and thawed/diluted blood bag, filter and process saline solution bag, and filter and sterile vent port.

Filter Rotor Drive

The motor is coupled to the magdrive which drives the rotor inside the filter, which in turn washes the blood being processed. The motor and motor controller used in the magnetic drive consist of a Reliance Electric (Robbins Meyers) 12 Volt DC servo motor with feedback coming from a Hewlett Packard tachometer. The motor maintains 75 ounce inches (oz-in) of torque at all times and may be controlled at speeds up to 2500 revolutions per minute (rpm). The motor is manufactured in compliance with US FDA GMP requirements, and in testing, has proven to have a long life and high reliability. The motor is relatively light and quiet, and it is easily mounted in a bracket welded into the console with a bearing to seal the motor from the outside. The tachometer provides for simple feedback control from the microprocessor and is easily mounted on a tachometer shaft at the bottom of the motor.

The magnetic drive coupler (magdrive) is used to indirectly couple the motor to the rotor of the filter. The ingenious aspect of the coupler is that it does not require the magnetic drive of the motor to directly attach to the magnetic drive of the rotor inside the filter. This is one of the primary aspects that ensure sterility for the TBPS. The filter remains closed and the driving and driven magnets couple by magnetic force through the Acrylonitrile/Butadiene/Styrene (ABS) casing of the filter. The magdrive attaches directly to the shaft of the motor with a brass key and is locked down with a spindle that aligns the filter with the magdrive. The magdrive consists of a cylinder that has six magnetic poles inserted, three North and three South alternating. The filter contains a coated magnet that contains corresponding poles, three North and three South. The magnet in the filter is attached to the rotor. When the filter is placed in the magnetic drive coupler, the spindle aligns the filter to the center, and the magnet of the filter will align to opposite poles of the magnetic drive coupler. The magnetic force between the coupler and rotor is strong enough to allow the motor to turn the rotor without breaking the coupling, allowing for indirect control of the rotor.

Fluid Circuit

Figure 8 is a diagram of the tubing attachments to the filter. Cyclohexanone is used as a solvent to attach the tubing to the filter and connectors to the tubing (all of which are EtO sterilized). Sterile 0.22 micron hydrophobic filters are used to create sterile barriers for the pressure tap and vent port, while sterile 0.22 micron hydrophilic filters are used to create sterile barriers for the saline solution spikes. The processed blood bag, waste bag, and feed line are connected to the filter with Luer connectors. The feed tubing line (thawed/diluted blood bag, saline solution bags) is run through the pump, and a pressure tap is taken off after the pump but before the filter in order to determine the pressure inside the filter. A vent port is located at the top of the filter to allow for complete filling.

The fluid flow path is determined by the fluid schematic and the sequence of events. The fluid schematic provides for automated delivery of all solutions involved in the processing. The sequence of events lays out the fluid cycle to allow for the processing of the thawed blood.

The fluid schematic, presented in Figure 9, demonstrates automatic delivery of the diluents to the blood bags prior to filtration. The blood is automatically delivered to the filter along with processing solution and the processed blood is automatically delivered to the processed blood bag after processing. The schematic also allows for the automatic delivery of waste to the waste bag during processing.

The TBPS will utilize valves that are simple two-way units, allowing the valves to be solenoid pinch valves for minimized disposable cost. The pinch valves are also isolated from the fluid by acting on the exterior surface of the tubing in order to occlude the flow. Also, the placement of the valves in the schematic were carefully considered in order to reduce the number of valves used (also reducing cost). The pump is located so that it can pump both into and out of the thawed blood bags, allowing for the precise metering of diluents to the blood. The strategic location of the pump allows the system to be automated and operational with minimal incorporated equipment.

The TBPS will utilize sterile docking devices between the blood bags and the feed tubing as requested. A pressure sensor, located at the filter inlet, is provided to record pressure data on the filter. A hemolysis sensor and glycerol sensor are to be placed on the waste line with a hematocrit sensor on the processed blood line.

The Process

The sequence of events, shown in Table 24, includes the dilution of the thawed blood, the processing of the diluted/thawed blood and the draining of the processed blood. The sequence of events is critical in determining the effectiveness of the processing of the thawed blood. The firing sequence of the valves, pump, shaker, and motor determine the sequence of events.

The processing starts with the dilution of the thawed blood. The thawed blood bag is placed on the shaker on the console. The thawed blood is diluted in the blood bag with 50 milliliters (ml) of 12% saline solution delivered at 100 milliliters per minute (ml/min) with the shaker on. The shaker is turned off and the thawed blood is allowed to equilibrate for two minutes. With the shaker on, 100 ml of 0.9% saline / 0.2% glucose solution is pumped into the thawed blood bag at 100 ml/min. The shaker is turned off and the thawed blood is allowed to equilibrate for two minutes. With the shaker on, 150 ml of 0.9% saline / 0.2% glucose solution is pumped into the thawed blood bag at 100 ml/min. The shaker is turned off and the thawed blood is allowed to equilibrate for two minutes.

The filter is primed with 300 to 400 ml of 0.9% saline / 0.2% glucose solution delivered at 100 ml/min with the waste line open. The motor is started, and the rotor is brought up to speed. The diluted/thawed blood is pumped into the filter at a rate of 100 ml/min. A solution of 0.9% saline / 0.2% glucose is pumped into the filter to wash the blood until the hemolysis (hemoglobin) sensor detects an acceptable transfusion level of free hemoglobin, when this occurs, the pump and rotor are stopped. The filter is drained into the processed, washed blood bag and the operator will be alerted. The operator will seal the waste bag and discard the waste bag and disposables according to universal precautions and biohazard waste procedures. The processed blood bag will be taken and used or stored for later use.

Console Hardware

The console hardware includes the hardware components, man/machine components, monitoring devices and software design. The hardware components shall include the pump, pinch valves, flow meter and shaker (also the motor and magnetic drive which has already been discussed in the disposable filter interface section). The man/machine components include the display, printer, keyboard, bar code reader and speakers. The monitoring devices include the hematocrit, hemolysis (hemoglobin), glycerol and pressure sensors. The software design includes the microprocessor, programming code, control boards and power board.

The general concept instrument hardware layout is shown in Figures 10 and 11. The instrument is arranged to provide maximum user access with minimum space usage. During prototyping phases the unit's internal workings will be easily accessible through the side panel. All access to user functions is done from the front, top and right sides of the unit. The user interface consists of a keypad for data entry, a VGA display monitor for information display a bar code scanner for easy tracking of blood bags and an external printer output port. The keyboard display, and bar code scanner are located on the front panel of the instrument for easy access in typical use. The keyboard is located on a folding shelf to protect the keyboard from damage and to save space. The printer will be a small stand alone unit sitting next to the unit connected by cable or infra-red.

The pump is used to meter and deliver all fluids to the bags and disposable filter. The pump includes the pump head, pump motor and encoder. The encoder allows for precise metering, eliminating the need for a flow meter. The pump used is a Minntech Renal Systems peristaltic pump system. The pump can occlude 0.06 wall tubing and has a variable flow rate between 50 to 500 ml/min. Loading tubing is simple and easy to do, while the occlusion is adjustable. The pump is quiet and, in testing, has proven to have a long life and high reliability. The pump is manufactured in compliance with US FDA GMP requirements and is able to handle multiple tubing assemblies if the need should arise. The pump has the added feature of having an interlock mechanism on the pump door for user safety.

The pinch valves will be used to control the direction of flow throughout the system. The pinch valves include a solenoid, valve head, and occluder. The solenoid receives a 12 Volt DC stroke signal to actuate the valve and occlude the tubing. The valve is easily microprocessor controlled and acts only on the external surface of the tubing. The shaker to be used on the console is a laboratory type shaker, similar to an Eberbach shaker, and it is used to dilute the thawed blood prior to processing. The shaker will oscillate at approximately 180 oscillations per minute only during the dilution steps and it will be turned off at all other times (especially during equilibration).

A cooling fan is required to cool the electronics inside the casing of the unit. The cooling fan is a single unit that only requires power when the main unit power switch is turned on. The fan is able to cool 5 cubic feet of space 10 degrees Fahrenheit. The fan is easily mounted with four screws into the bottom of the console and it meets US FDA GMP requirements.

The display on the console is a vacuum fluorescent 4 line by 20 character display. Currently the pressure, hematocrit, pump flow rate, rotor speed and status of the unit are displayed. More will be displayed as systems and sensors in development come on-line. The unit beeps when any type of failure occurs and a message is displayed detailing the failure. For example, if the pump door is opened during processing, the unit beeps and "Pump Door Open" is displayed. The user runs the machine by pressing keys on a sealed membrane keypad. A thermal or label printer and bar code reader are systems under development that will come on-line by May 31, 1996.

The monitoring devices have been sub-contracted to a group at the Institute for Innovation and Design in Engineering at Texas A&M University. The system being developed for measuring hemolysis will determine the amount of free hemoglobin in the waste line. After many technologies were investigated and evaluated, an optical system to measure the light absorption at a wavelength of 805 nanometers (nm) in the waste stream was chosen to be developed to measure the free hemoglobin. The waste stream tubing will be sandwiched between two optical glass plates (1/4 inch thick) that will aid in the determination of the absorption. An 805 nm laser-diode will be directed at the plates. The light will be reflected from the first glass surface, and its intensity will be measured for reference. The light that passes will be refracted, and its intensity will be measured on the second glass surface. The hemoglobin concentration may be

calculated using the intensities of the reflected and refracted light along with the length of the optical path and the absorptivity factor of hemoglobin. In order to reduce costs and conserve space, the glycerol sensor is being combined with the hemoglobin sensor. The glycerol causes a change in the index of refraction of the light beam, and this can be utilized to determine the glycerol concentration. The same 805 nm laser-diode is utilized to give a displacement after the light has passed through the stream. This displacement is used to determine the percent concentration glycerol.

The hematocrit sensor being utilized and developed for the processed blood line is a resistive / capacitance measurement system. The resistivity of the processed blood is a function of the hematocrit. The resistance over a determined length of tubing with flowing processed blood or the capacitance of the processed flowing blood is easily related to resistivity and is therefore measured. Measuring the resistance or capacitance is currently done with a multimeter or capacitance meter; however, more stable measurement systems are being investigated. A Fabry-Perot Interferometer and Honeywell pressure transducer switch are being investigated for use as the pressure sensor.

Software for the unit is being developed in general accordance with ANSI / IEEE standard 730-1989 as detailed in the IEEE Standards Collection, Software Engineering, 1993 edition. Included in Appendix 1 is a copy of the Software Quality Assurance Plan (SQAP) for the TBPS. The SQAP covers, among other things, the documentation, reviews, and audits to be performed throughout the development process. Some or all of the software code may be sub-contracted; as such, the SQAP includes contractor control methods and required performance reviews.

The Software Requirements Specification (SRS) has been outlined and is in Appendix 2. The SRS is in general accordance with IEEE Standard 830-1984 as detailed in the IEEE Standards Collection, Software Engineering, 1993 edition.

Conclusions

Preliminary work with the 214 plain filter demonstrated that there was inadequate mixing within the filter. This was concluded based on an apparent limit in the maximum hematocrit which could be achieved post-washing. There also appeared to be excessive shear from the relatively fast rotor speed potentially leadng to decreased intracellular potassium levels.

To address these limitations, the filter rotor was modified in several forms. Results from the rotor modification studies lead to the 214 12R 6H design. Testing with this filter for washing fresh and frozen thawed blood indicated; an improvement in the post wash hematocrit relative to the 214 plain filter; that the rotor speed could be reduced without sacrificing mixing, thereby reducing shear stress and resulting hemolysis; and that the ability to remove free hemoglobin is dependent on the level of hemolysis and the build-up of an apparant gel layer which selectively retains hemoglobin within the filter during washing. Further studies with additional modified rotors suggest that reducing the number of ridges may improve recovery and decrease hemolysis.

Future Directions:

Year two will focus on finalizing the filter design and operation. Addition development will address the optimum rotor configuration, post wash hematocrit and cellular hemolysis. The console development will continue according to plan. Additionally, experimental series 3,4,5, and 6 will be completed in year two.

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Tables

Table 3: Effect of Target Hct on 214 Plain Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Target Hct. 55 or less									
28	15.3	100	1200	73	42.1	48.0	114.1	82	263
29	12.5	120	1200	76	42.1	48.0	114.1	334	
30	10.8	140	1200	76	42.1	48.0	114.1	334	160
25	14.0	100	1200	69	46.5	54.0	116.1	60	124
27	12.5	120	1200	90	50.5	55.0	109.0		186
26	10.7	120	1200	83	52.7	58.0	110.0		391
21	15.8	100	1200	86	54.7	58.4	106.8	88	459
22	15.0	100	800	333	54.7	48.9	89.4	88	201
24	15.8	100	1200	75	55.0	62.0	112.8	60	
AVG	13.6		1155.6	106.8	48.9	53.4	109.6	149.4	254.9
SD	2.0		133.3	85.1	5.8	5.4	8.1	126.6	125.2
(B) Target Hct. 55 - 65									
19	12.8	100	1200	113	58.6	61.0	104.1		
20	10.4	150	1200	195	58.6	58.8	100.3		256
56	16.1	100	1200	108	58.6	54.8	93.5	806	168
48	16.0	100	1200	79	58.9	54.6	92.6	444	1154
49	16.8	100	1200	78	58.9	57.1	96.9	444	699
53	16.0	100	1500	433	58.9	56.8	96.4	67	419
50	16.0	100	1200	252	59.6	54.0	90.6	67	522
51	16.0	100	1200	173	59.6	57.5	96.4	67	578
52	16.0	100	1200	132	59.6	58.1	97.4	67	541
44	16.5	100	1200	92	59.9	61.7	103.1	5	652
45	16.5	100	1200	295	59.9	51.8	86.5	5	221
46	16.3	100	1200	77	62.0	56.3	90.8	5	132
47	16.0	100	1200	108	62.0	55.4	89.4	5	443
23	16.0	100	1200	134	63.4	67.0	105.6	60	451
83	12.0	100	1200	114	64.0	59.7	93.2		
AVG	15.3		1220.0	158.9	60.2	57.6	95.8	170.2	479.7
SD	1.9		77.5	99.5	1.8	3.7	5.6	255.5	272.1
(C) Target Hct. 66 - 80									
82	12.0	100	1200	157	65.6	56.4	85.9		
81	13.0	100	1200	167	66.5	56.7	85.2		
79	14.0	100	1200	94	67.0	54	80.5		
80	15.0	100	1200	156	67.1	55.3	82.4		
36	25.0	100	1200	267	67.4	60.0	89.0	49	337
18	16.8	100	1200	199	67.6	65.0	96.1	61	662
39	16.8	100	1200	311	68.3	58.6	85.7	92	453
57	16.3	100	1500	99	68.7	55.5	80.8	800	376
58	17.3	100	1500	219	68.7	55.9	81.4	800	376
176	17	100	1100	514	68.7	58.9	85.8	37	144
178	17	100	1100	401	68.7	59.4	86.5	37	83
59	17.5	100	1200	224	70.4	54.7	77.7	800	668
42	16.3	100	1200	288	72.9	57.9	79.4	220	753
37	24.0	120	1200	380	74.6	61.3	82.2		399
60	17.5	100	1200	356	76.7	64.5	84.1	205	740
61	26.0	50	1200	521	77.1	60.5	78.5	205	249
43	16.8	100	1200	397	77.8	59.3	76.3	220	375
AVG	17.5		1223.5	279.4	70.2	58.5	83.4	293.8	431.9
SD	3.9		109.1	132.8	4.0	3.2	4.8	313.6	217.0
(D) Target Hct. > 80									
38	21.5	140	1200	405	81.8	62.6	76.5		373
97	16.5	100	1200	271	84.8	55.4	65.3		
77	22.0	100	1200	433	86.1	54.5	63.3		
78	18.5	100	1200	382	87.5	52.3	59.7		
91	18.0	100	1200	307	87.0	52.0	59.8		
94	16.0	100	1200	321	87.1	49.0	56.2		
AVG	18.8		1200.0	353.2	85.7	54.3	63.5		373.0
SD	2.5		0.0	62.9	2.2	4.6	7.1		

Table 4 : Marker Washout Results for Ca - Doped Fresh RBC Washed w/ 214 Plain Filters

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Hct. Recovery	Calcium Washout (%)
18	16.8	100	1200	199	96.1	98.95
19	12.8	100	1200	113	104.1	98.83
20	10.4	150	1200	195	100.3	98.76
21	15.8	100	1200	86	106.8	97.61
22	15.0	100	800	333	89.4	97.06
23	16.0	100	1200	134	105.6	99.45
24	15.8	100	1200	75	112.8	99.36
25	14.0	100	1200	69	116.1	99.23
26	10.7	120	1200	83	110.0	98.93
27	12.5	120	1200	90	109.0	98.87
28	15.3	100	1200	73	114.1	98.31
29	12.5	120	1200	76	114.1	98.31
30	10.8	140	1200	76	114.1	98.31
36	25.0	100	1200	267	89.0	99.08
37	24.0	120	1200	380	82.2	98.96
38	21.5	140	1200	405	76.5	99.28
39	16.8	100	1200	311	85.7	99.51
42	16.3	100	1200	288	79.4	99.33
43	16.8	100	1200	397	76.3	99.35
44	16.5	100	1200	92	103.1	99.31
45	16.5	100	1200	295	86.5	99.28
46	16.3	100	1200	77	90.8	85.19
47	16.0	100	1200	108	89.4	99.33
48	16.0	100	1200	79	92.6	99.27
49	16.8	100	1200	78	96.9	99.31
50	16.0	100	1200	252	90.6	99.30
51	16.0	100	1200	173	96.4	99.35
52	16.0	100	1200	132	97.4	99.36
53	16.0	100	1500	433	96.4	99.08
56	16.1	100	1200	108	93.5	98.17
57	16.3	100	1500	99	80.8	98.19
58	17.3	100	1500	219	81.4	98.21
59	17.5	100	1200	224	77.7	98.16
60	17.5	100	1200	356	84.1	98.57
61	26.0	50	1200	521	78.5	99.28
77	22.0	100	1200	433	63.3	96.44
78	18.5	100	1200	382	59.7	96.65
AVG	17		1214	208	93	98
SD	4		108	135	14	2

Table 5 : Effect of Transmembrane Pressure on 214 Plain Filter Performance for Washed Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Maximum Transmembrane Pressure < 300									
25	14.0	100	1200	69	46.5	54.0	116.1	60	124
28	15.3	100	1200	73	42.1	48.0	114.1	82	263
24	15.8	100	1200	75	55.0	62.0	112.8	60	
29	12.5	120	1200	76	42.1	48.0	114.1	334	
30	10.8	140	1200	76	42.1	48.0	114.1	334	160
46	16.3	100	1200	77	62.0	56.3	90.8	5	132
49	16.8	100	1200	78	58.9	57.1	96.9	444	699
48	16.0	100	1200	79	58.9	54.6	92.6	444	1154
26	10.7	120	1200	83	52.7	58.0	110.0		391
21	15.8	100	1200	86	54.7	58.4	106.8	88	459
27	12.5	120	1200	90	50.5	55.0	109.0		186
44	16.5	100	1200	92	59.9	61.7	103.1	5	652
79	14.0	100	1200	94	67.0	54	80.5		
57	16.3	100	1500	99	68.7	55.5	80.8	800	376
47	16.0	100	1200	108	62.0	55.4	89.4	5	443
56	16.1	100	1200	108	58.6	54.8	93.5	806	168
19	12.8	100	1200	113	58.6	61.0	104.1		
83	12.0	100	1200	114	64.0	59.7	93.2		
52	16.0	100	1200	132	59.6	58.1	97.4	67	541
23	16.0	100	1200	134	63.4	67.0	105.6	60	451
80	15.0	100	1200	156	67.1	55.3	82.4		
82	12.0	100	1200	157	65.6	56.4	85.9		
81	13.0	100	1200	167	66.5	56.7	85.2		
51	16.0	100	1200	173	59.6	57.5	96.4	67	578
20	10.4	150	1200	195	58.6	58.8	100.3		256
18	16.8	100	1200	199	67.6	65.0	96.1	61	662
58	17.3	100	1500	219	68.7	55.9	81.4	800	376
59	17.5	100	1200	224	70.4	54.7	77.7	800	668
50	16.0	100	1200	252	59.6	54.0	90.6	67	522
36	25.0	100	1200	267	67.4	60.0	89.0	49	337
97	16.5	100	1200	271	84.8	55.4	65.3		
42	16.3	100	1200	288	72.9	57.9	79.4	220	753
45	16.5	100	1200	295	59.9	51.8	86.5	5	221
AVG	15.2		1218.2	143.0	60.5	56.5	95.2	246.2	440.5
SD	2.7		72.7	71.9	9.2	4.2	12.8	292.8	247.0
(B) Maximum Transmembrane Pressure > 301									
91	18.0	100	1200	307	87.0	52.0	59.8		
39	16.8	100	1200	311	68.3	58.6	85.7	92	453
94	16.0	100	1200	321	87.1	49.0	56.2		
22	15.0	100	800	333	54.7	48.9	89.4	88	201
60	17.5	100	1200	356	76.7	64.5	84.1	205	740
37	24.0	120	1200	380	74.6	61.3	82.2		399
78	18.5	100	1200	382	87.5	52.3	59.7		
43	16.8	100	1200	397	77.8	59.3	76.3	220	375
178	17	100	1100	401	68.7	59.4	86.5	37	83
38	21.5	140	1200	405	81.8	62.6	76.5		373
53	16.0	100	1500	433	58.9	56.8	96.4	67	419
77	22.0	100	1200	433	86.1	54.5	63.3		
61	26.0	50	1200	521	77.1	60.5	78.5	205	249
176	17	100	1100	514	68.7	58.9	85.8	37	144
AVG	18.7		1178.6	392.4	75.4	57.0	77.2	118.9	343.6
SD	3.3		142.4	67.4	10.5	5.0	12.6	78.2	187.6

Table 6: BUP I Evaluation of the Effect of Inputting Blood and Saline Simultaneously

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Calcium Washout (%)	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
47 B-S	16.0	100	1200	108	62.0	55.4	89.4	98.77	5	443
46 Sim	16.3	100	1200	77	62.0	56.3	90.8	72.30	5	132
48 B-S	16.0	100	1200	79	58.9	54.6	92.6	98.68	444	1154
49 Sim	16.8	100	1200	78	58.9	57.1	96.9	98.68	444	699

Table 7: Performance of 214 Plain Filter for Washing Frozen Thawed Cells

Name	Rotor Speed	Nominal Flow Rate	Max. Pressure	Thaw / Dilute Recovery	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Post-Dilute - Weight	Post-Wash - Weight	Post-dilute Sup Hb	Post -wash - Sup Hb	Waste Sup Hb.	Post - wash Hct	Osm.	Waste volume	Intra K+ mEg/ml
Brenda Mayer	1200	100	56	97	81.8	55.7	550	180	348	288	388	49.5	296	1599	
David Mayer	1200	100	57	91.7	69.8	47.4	500	170	1077	280	689	44	298	1548	
Joe Caroll	1200	100	362	95.4	69.4	44.4	655	123	466	901	523	63	314	1670	
Paul Carroll	1200	100	202	93.5	60.2	28.4	675	210	1322	717	850	56	314	1730	
P. Pitt	1200	100	363	96.1	71.9	78.4	660	306	554	760	685	56	336	2930	
T. Pitt	1200	100	251	96	76	73.5	710	287	597	460	685	49	310	2113	
S. Jones	1000	100	47	95.9	73.9	55.1	433	183	484	274		36.5	307	1619	4.07
B. Miller	1200	100	104	96.1	77.6	63.4	615	178	356	395		56	298	1673	3.67
B. Jones	1200	100	107	98.1	84.8	80.3	570	217	202	395		58	301	1767	4.27
Schwinger	1200	100	60	97.5	71.6	49.9	427	187	447	401	616	50	299	1475	4.87
Lee	1200	100	230	97.6	82.5	76.7	590	236	278	727	529	60	354	1388	4.27
Miller	1200	100	72	95.4	61.4	43	529	188	752	544	894	49	313	1802	4.48
AVG	1183.3		159.3	95.9	73.4	58.0	576.2	205.4	573.6	511.8	651.0	52.3	311.7	1776.2	4.3
SD	57.7		118.6	1.8	7.7	16.5	92.5	50.9	331.1	214.3	159.7	7.4	17.4	406.7	0.4

Table 8 : Effect of Rotor Modification on 214 Filter Performance for washing Fresh Blood Cells

Test #	Filter Type	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery
91	214 pln	18.0	100	1200	307	87.0	52.0	59.8
94	214 pln	16.0	100	1200	321	87.1	49.0	56.2
97	214 pln	16.5	100	1200	271	84.8	55.4	65.3
AVG		16.8		1200.0	299.7	86.3	52.1	60.4
93	214 3R	17.0	100	900	398	87.1	48.2	55.3
95	214 3R	17.0	100	800-1100	303	84.8	51.0	60.1
AVG		17.0		900.0	350.5	86.0	49.6	57.7
96	214 3R 2H	15.5	100	1000	220	84.8	59.5	70.2
127	214 12R 6H	20.8	100	900	192	80.8	66.6	82.4
132	214 12R 6H	17.0	100	900	206	75.5	68.2	90.3
133	214 12R 6H	17.0	100	900	283	80.5	65.1	80.9
134	214 12R 6H	17.0	100	700	224	75.1	65.7	87.4
135	214 12R 6H	18.0	100	700	376	80.5	62.1	77.2
AVG		18		820	256	78	66	84

Table 9: Effect of Increasing Flowrate on Performance with a 214 12R6H Filter for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery
102	11.5	100-200	900	145	58.5	50.0	85.4
104	11.5	100-150	900	128	58.5	60.3	103.0
AVG	11.5		900.0	136.5	58.5	55.2	94.2
SD	0.0		0.0	12.0	0.0	7.3	12.4

Table 10 : Effect of Target Hct on 214 12R6H Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Target HCT 55 - 70%									
102	11.5	100-200	900	145	58.5	50.0	85.4	309	801
103	10.5	150	900	143	58.5	58.1	99.3	309	825
104	11.5	100-150	900	128	58.5	60.3	103.0	309	795
105	10.5	150	800	136	58.5	58.3	99.6	309	395
106	14.5	100	800	94	63.1	58.1	92.0	306	694
107	9.5	150	900	118	63.1	57.9	91.7	306	398
125	16.3	100	900	108	64.6	61.1	94.6		
128	15.0	100	900	117	64.7	62.5	96.6		
114	18.0	100	900	153	67.6	63.2	93.4		
101	15.5	100	900	133	68.4	65.8	96.2		
AVG	13.3		880.0	127.5	62.6	59.5	95.2	308.0	651.3
SD	2.9		42.2	18.3	3.9	4.3	5.0	1.5	202.4
(B) Target HCT 71 - 80%									
163	24.0	60	600	206	72.0	61.5	85.5	35	201
164	15.0	100	600	369	72.0	60.2	83.7	35	263
165	16.0	100	800	203	72.0	62.3	86.6	35	344
172	18	100	800	577	72.1	64.7	89.7	34	1170
175	17	100	800	432	73.5	63.8	86.9	37	308
161	15.0	100	600	282	73.8	56.6	76.7	16	480
162	15.0	100	800	242	73.8	62.0	84.1	16	106
144	12.0	150	800	374	75.7	60.3	79.6	461	2445
145	10.0	150	800	330	75.7	58.3	77.0	461	2399
142	11.0	150	800	350	78.3	60.9	77.8	818	2119
AVG	15.3		740.0	336.5	73.9	61.1	82.7	194.8	983.5
SD	4.0		96.6	113.7	2.1	2.4	4.6	283.1	970.9
(C) Target HCT > 80%									
134	17.0	100	700	224	80.4	65.7	81.7	331	73
127	20.8	100	900	192	80.8	66.6	82.4		
132	17.0	100	900	206	80.8	68.2	84.4	331	67
133	17.0	100	900	283	86.1	65.1	75.6	331	725
135	18.0	100	700	376	86.1	62.1	72.1	331	460
AVG	18.0		820.0	256.2	82.8	65.5	79.3	331.0	331.3
SD	1.6		109.5	75.4	3.0	2.3	5.2	0.0	320.5

Table 11: Summary Fresh Blood Washing w/ 214 12R6H Filter

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct Recovery %	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) 100ml/min Flowrate W/O Clam Shell									
106	14.5	100	800	94	60.1	58.1	96.7	306	694
128	15.0	100	900	117	61.5	62.5	101.6		
125	16.3	100	900	108	61.4	61.1	99.5		
114	18.0	100	900	153	64.4	64.0	99.4		
101	15.5	100	900	133	64.8	65.8	101.5		
127	20.8	100	900	192	76.8	66.6	86.8		
114	18.0	100	900	153	64.4	63.2	98.1		
128	15.0	100	900	117	61.5	62.5	101.6		
132	17.0	100	900	206	71.7	68.2	95.1	331	67
133	17.0	100	900	283	76.4	65.1	85.2	331	725
134	17.0	100	700	224	71.3	65.7	92.1	331	73
135	18.0	100	700	376	76.4	62.1	81.3	331	460
136	14.0	100	800	111	87.3	61.4	70.3	320	76
161	15.0	100	600	282	65.4	56.6	86.5	16	480
162	15.0	100	800	242	65.4	62.0	94.8	16	106
163	24.0	60	600	206	63.8	61.5	96.4	35	201
164	15.0	100	600	369	63.8	60.2	94.4	35	263
165	16.0	100	800	203	63.8	62.3	97.7	35	344
Average	16.7	97.8	805.6	198.3	67.8	62.7	93.3	189.7	317.2
(B) 100ml/min Flowrate With Clam Shell									
172	18	100	800	577	63.9	64.7	101.3	34	1170
175	17	100	800	432	65.0	63.8	98.2	37	308
177	17	100	600	475	65.0	60.7	93.4	37	1542
Average	17.3	100.0	733.3	494.7	64.6	63.1	97.7	36.0	1006.7
(C) 150ml/min Flowrate W/O Clam Shell									
103	10.5	150	900	143	55.5	58.1	104.7	309	825
105	10.5	150	800	136	55.5	58.3	105.0	309	395
107	9.5	150	900	118	60.1	57.9	96.3	306	398
137	12.0	150	800	145	87.3	61.2	70.1	320	885
142	11.0	150	800	350	69.5	60.9	87.6	818	2119
144	12.0	150	800	374	67.2	60.3	89.8	461	2445
145	10.0	150	800	330	67.2	58.3	86.8	461	2399
Average	11	150	829	228	66	59	91	426	1352

Table 12: Effect of Transmembrane Pressure on 214 12 R6H Plain Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Transmembrane Pressure < 300 mmHg									
106	14.5	100	800	94	63.1	58.1	92.0	306	694
125	16.3	100	900	108	64.6	61.1	94.6		
128	15.0	100	900	117	64.7	62.5	96.6		
107	9.5	150	900	118	63.1	57.9	91.7	306	398
104	11.5	100-150	900	128	58.5	60.3	103.0	309	795
101	15.5	100	900	133	68.4	65.8	96.2		
105	10.5	150	800	136	58.5	58.3	99.6	309	395
103	10.5	150	900	143	58.5	58.1	99.3	309	825
102	11.5	100-200	900	145	58.5	50.0	85.4	309	801
114	18.0	100	900	153	68.0	64.0	94.1		
127	20.8	100	900	192	80.8	66.6	82.4		
165	16.0	100	800	203	72.0	62.3	86.6	35	344
132	17.0	100	900	206	80.8	68.2	84.4	331	67
163	24.0	60	600	206	72.0	61.5	85.5	35	201
134	17.0	100	700	224	80.4	65.7	81.7	331	73
162	15.0	100	800	242	73.8	62.0	84.1	16	106
161	15.0	100	600	282	73.8	56.6	76.7	16	480
133	17.0	100	900	283	86.1	65.1	75.6	331	725
AVG	15.3		833.3	172.9	69.2	61.3	89.4	226.4	454.2
SD	3.7		102.9	58.7	8.8	4.4	8.0	139.8	289.4
(B) Transmembrane Pressure > 300 mmHg									
145	10.0	150	800	330	75.7	58.3	77.0	461	2399
142	11.0	150	800	350	78.3	60.9	77.8	818	2119
164	15.0	100	600	369	72.0	60.2	83.7	35	263
144	12.0	150	800	374	75.7	60.3	79.6	461	2445
135	18.0	100	700	376	86.1	62.1	72.1	331	460
175	17	100	800	432	73.5	63.8	86.9	37	308
172	18	100	800	577	72.1	64.7	89.7	34	1170
AVG	14.4		757.1	401.1	76.2	61.5	81.0	311.0	1309.1
SD	3.4		78.7	83.6	4.9	2.2	6.1	297.3	997.6

Table 13: Effect of Flowrate on 214 12R6H Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery
(A) Flowrate <= 100							
163	24.0	60	600	206	72.0	61.5	85.5
101	15.5	100	900	133	68.4	65.8	96.2
106	14.5	100	800	94	63.1	58.1	92.0
114	18.0	100	900	153	68.0	64.0	94.1
125	16.3	100	900	108	64.6	61.1	94.6
127	20.8	100	900	192	80.8	66.6	82.4
128	15.0	100	900	117	64.7	62.5	96.6
132	17.0	100	900	206	80.8	68.2	84.4
133	17.0	100	900	283	86.1	65.1	75.6
134	17.0	100	700	224	80.4	65.7	81.7
135	18.0	100	700	376	86.1	62.1	72.1
161	15.0	100	600	282	73.8	56.6	76.7
162	15.0	100	800	242	73.8	62.0	84.1
164	15.0	100	600	369	72.0	60.2	83.7
165	16.0	100	800	203	72.0	62.3	86.6
172	18	100	800	577	72.1	64.7	89.7
175	17	100	800	432	73.5	63.8	86.9
AVG	17.0	97.6	794.1	246.9	73.7	63.0	86.0
SD	2.4	9.7	114.4	129.3	7.1	3.0	7.2
(B) Flowrate = 150 ml/min							
103	10.5	150	900	143	58.5	58.1	99.3
105	10.5	150	800	136	58.5	58.3	99.6
107	9.5	150	900	118	63.1	57.9	91.7
142	11.0	150	800	350	78.3	60.9	77.8
144	12.0	150	800	374	75.7	60.3	79.6
145	10.0	150	800	330	75.7	58.3	77.0
AVG	10.6		833.3	241.8	68.3	59.0	87.5
SD	0.9		51.6	121.0	9.3	1.3	10.7

Table 14 : Marker Washout Results for Ca Doped Fresh RBC washed w/ 214 12R6H Filter

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Hct. Recovery	Calcium Washout (%)
101	15.5	100	900	133	96.2	99.35
102	11.5	100-200	900	145	85.4	99.24
103	10.5	150	900	143	99.3	99.36
104	11.5	100-150	900	128	103.0	99.31
105	10.5	150	800	136	99.6	99.27
106	14.5	100	800	94	92.0	99.29
107	9.5	150	900	118	91.7	99.29
125	16.3	100	900	108	94.6	99.50
127	20.8	100	900	192	82.4	99.23
128	15.0	100	900	117	96.6	99.52
AVG	14		880	131	94	99
SD	3		42	27	6	0

Table 15: Comparison of Filter Performance w/ Fresh Blood as a function of Rotor Design w/214 ml Filter

Test #	Filter Type	Dilute Blood Volume In (mL)	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct Recovery %	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) 214 12R 6H vs 214 Pln											
175	214 12R 6H	794	17	100	800	432	65.0	63.8	98.2	37	308
176	214 pln	794	17	100	1100	514	65.0	58.9	90.7	37	144
178	214 pln	794	17	100	1100	401	65.0	59.4	91.4	37	83
(B) 214 12R 6H vs 214 Pln 6H											
172	214 12R 6H	731	18	100	800	577	63.9	64.7	101.3	34	1170
171	214 Pln 6H	731	16	100	1100	242	63.9	58.9	92.2	34	211
173	214 Pln 6H	731	16	100	1100	300	63.9	57.4	89.9	34	339
(C) 214 12R 6H vs 214 12R											
181	214 12R-6H	638	30	100	800	444	59.7	65.7	110.1	9	1573
180	214-12R	636	30	100	800	409	59.4	67	112.8	9	1024
(D) 214 12R 6H vs 214 6B											
181	214 12R-6H	638	30	100	800	444	59.7	65.7	110.1	9	1573
179	214 6B	696	17	100	700	254	65.1	62	95.3	9	1405
(E) 214 12R 6H vs 214 6B 6H											
172	214 12R 6H	731	18	100	800	577	63.9	64.7	101.3	34	1170
170	214 6B 6H	731	23	70	700	386	63.9	60.9	95.4	34	1311
(F) 214 12R 6H vs 214 Pln 12B 6H											
175	214 12R 6H	794	17	100	800	432	65.0	63.8	98.2	37	308
177	214 12B 6H	794	17	100	600	475	65.0	60.7	93.4	37	1542

Table 16: Compilation of all Frozen Thawed Blood Testing Results for the Fourth Quarter

Unit #	Filter Type	Date of Test	Rotor Speed	Nominal Flow Rate	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post-dilute Sup Hb	Post - wash - Sup Hb	Waste Sup Hb.	Post - wash Hct	Waste volume	Intra K+ mEg/ml
95-02217	214 12R-6H	1/4/96	800	100	13	294	61.9	44.8	862	1454	935	1068	43.5	1638	
95-02220	214 12R-6H	1/4/96	800	150	13	470	76.9	70.5	618	414	1158	753	55	1801	
95-02227	214 12R-6H	1/11/96	800	150	13	352			371	442	864	530	45	1963	
95-02218	214 12R-6H	1/11/96	800	150	12	267			408	661	719	566	45.5	1942	
95-02224	214 12R-6H	1/22/96	800	150	18	537	72.2	63.3	603	545	882	560	51.5	2683	4.47
95-02230	214 12R-6H	1/22/96	800	150	15	372	73.7	66.4	641	438	652	504	51	2453	4.44
	214 12R-6H	2/1/96	800	150	18	352	69.6	65		182	420	455	54	2816	4.24
8060243	214 12R-6H	2/1/96	800	150	25	251	69.8	66.7		294	882	663	51.5	2284	4.67
8091485	214 12R-6H	2/6/96	800	150	18	458	70.3	66.2		245	1158	543	49	2626	4.86
95-01585	214 12R-6H	2/8/96	800	150	15	157	75.5	65.8	697		678	410	57	2417	5.1
95-01634	214 12R-6H	2/8/96	800	150	15	396	66.3	50.8	1111		556	700	56	2373	5.51
95--02205	214 12R-6H	2/12/96	800	150	11	195	68.2	64.3	284		606	630	46.5	1931	4.87
95-01568	214 12R-6H	2/12/96	800	150	20	580	69.2	71.5	729		731	784	56	1845	4.94
1628/1631 b	214 12R-6H	2/22/96	800	100	20.0	366	73.5	73.9	560		220	555	58	2264	4.68
1628/1631a	214 12R-6H	2/22/96	600	100	20.0	252	73.9	69	2633		386	547	51	2279	4.66
1583/1616b	214 12R-6H	3/4/96	800	100	30	444	71.8	70.3	1798		522	814	65	1924	5.07
1583/1616a	214-12R	3/4/96	800	100	30	409	72.8	69.3	1798		642	763	66	1801	4.37
379/89/1411	214 6R-6H	3/7/96	800	100	38	602	73.6	76	1518		422	551	59	2439	4.55
379/89/1411	214 12R-6H	3/6/96	800	75	47	435	75.1	68.7	1518		424	470	56	2490	4.76
373/85/1429	214 12R-6H	3/7/96	800	50	32	689	74.3	71.9	1202		1049	515	64	2566	5.18
373/85/1429	214 12R-6H	3/7/96	800	50	34	678	73.9	70.1	1202		723	536	63.5	2538	4.81
373/85/1429	214 6R-6H	3/7/96	800	60	28	769	75.9	73.3	1202		630	494	62	2490	5.31
Average			790.9	119.8	22.0	423.9	71.9	66.9	1039.7	519.4	693.6	609.6	54.8	2252.9	4.8

Table 17: Effect of Operating Conditions on Performance of the 214 12R6H Filter w/ Frozen thawed cells

Unit #	Date of Test	Rotor Speed	Nominal Flow Rate	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post - wash - Sup Hb	Waste Sup Hb.	Post - wash Hct	Intra K+ mEg/ml
(A) Flow rate 100 ml min or less												
95-02217	1/4/96	**800**	**100**	13	294	61.9	44.8	862	935	1068	43.5	
1628/1631 b	2/22/96	**800**	**100**	20.0	366	73.5	73.9	560	220	555	58	4.68
1583/1616b	3/4/96	**800**	**100**	30	444	71.8	70.3	1798	522	814	65	5.07
1379/89/1411a	3/6/96	**800**	**75**	47	435	75.1	68.7	1518	424	470	56	4.76
1373/85/1429a	3/7/96	**800**	**50**	32	689	74.3	71.9	1202	1049	515	64	5.18
1373/85/1429b	3/7/96	**800**	**50**	34	678	73.9	70.1	1202	723	536	63.5	4.81
Average					484.3	71.8	66.6	1190.3	645.5	659.7	58.3	4.9
(B) Flow rate												
95-02220	1/4/96	**800**	**150**	13	470	76.9	70.5	618	1158	753	55	
95-02227	1/11/96	**800**	**150**	13	352			371	864	530	45	
95-02218	1/11/96	**800**	**150**	12	267			408	719	566	45.5	
95-02224	1/22/96	**800**	**150**	18	537	72.2	63.3	603	882	560	51.5	4.47
95-02230	1/22/96	**800**	**150**	15	372	73.7	66.4	641	652	504	51	4.44
	2/1/96	**800**	**150**	18	352	69.6	65		420	455	54	4.24
8060243	2/1/96	**800**	**150**	25	251	69.8	66.7		882	663	51.5	4.67
8091485	2/6/96	**800**	**150**	18	458	70.3	66.2		1158	543	49	4.86
95-01585	2/8/96	**800**	**150**	15	157	75.5	65.8	697	678	410	57	5.1
95-01634	2/8/96	**800**	**150**	15	396	66.3	50.8	1111	556	700	56	5.51
95--02205	2/12/96	**800**	**150**	11	195	68.2	64.3	284	606	630	46.5	4.87
95-01568	2/12/96	**800**	**150**	20	580	69.2	71.5	729	731	784	56	4.94
Average					365.6	71.2	65.1	606.9	775.5	591.5	51.5	4.8

Table 18: Effect of Wash Volume on performance of 214 12R6H Filter w/ Frozen Thawed Cells

Unit #	Date of Test	Waste volume	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post - wash - Sup. Hb	Waste Sup Hb.	Intra K+ mEg/ml
(A) Waste Volume < 2000 ml										
95-02217	1/4/96	**1638**	13	294	61.9	44.8	862	935	1068	
95-02220	1/4/96	**1801**	13	470	76.9	70.5	618	1158	753	
95-02227	1/11/96	**1963**	13	352			371	864	530	
95-02218	1/11/96	**1942**	12	267			408	719	566	
95--02205	2/12/96	**1931**	11	195	68.2	64.3	284	606	630	4.87
95-01568	2/12/96	**1845**	20	580	69.2	71.5	729	731	784	4.94
1583/1616b	3/4/96	**1924**	30	444	71.8	70.3	1798	522	814	5.07
Average		**1863.4**	16.0	371.7	69.6	64.3	724.3	790.7	735.0	5.0
(B) Waste Volume 2000 - 2500 ml										
95-02230	1/22/96	**2453**	15	372	73.7	66.4	641	652	504	4.44
8060243	2/1/96	**2284**	25	251	69.8	66.7		882	663	4.67
95-01585	2/8/96	**2417**	15	157	75.5	65.8	697	678	410	5.1
95-01634	2/8/96	**2373**	15	396	66.3	50.8	1111	556	700	5.51
1628/1631 b	2/22/96	**2264**	20.0	366	73.5	73.9	560	220	555	4.68
1628/1631a	2/22/96	**2279**	20.0	252	73.9	69	2633	386	547	4.66
379/89/1411	3/6/96	**2490**	47	435	75.1	68.7	1518	424	470	4.76
373/85/1429	3/7/96	**2490**	28	769	75.9	73.3	1202	630	494	5.31
Average		**2381.3**	23.1	374.8	73.0	66.8	1194.6	553.5	542.9	4.9
(C) Waste Volume > 2500 ml										
95-02224	1/22/96	**2683**	18	537	72.2	63.3	603	882	560	4.47
	2/1/96	**2816**	18	352	69.6	65		420	455	4.24
8091485	2/6/96	**2626**	18	458	70.3	66.2		1158	543	4.86
373/85/1429	3/7/96	**2566**	32	689	74.3	71.9	1202	1049	515	5.18
373/85/1429	3/7/96	**2538**	34	678	73.9	70.1	1202	723	536	4.81
Average		**2645.8**	24.0	542.8	72.1	67.3	1002.3	846.4	521.8	4.7

Table 19: Effect of pressure on 214 12R6H Filter Performance w/ Frozen/Thawed Cells

Unit #	Date of Test	Max. Pressure	Rotor Speed	Nominal Flow Rate	Washing Time	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-Dilute Sub Hb	Post - wash - Sup Hb	Waste Sup. Hb.	Waste volume	Intra K+ mEg/ml
(A) Transmembrane pressure <300 mm Hg												
95-02217	1/4/96	**294**	800	100	13	61.9	44.8	862	935	1068	1638	
95-02218	1/11/96	**267**	800	150	12			408	719	566	1942	
8060243	2/1/96	**251**	800	150	25	69.8	66.7		882	663	2284	4.67
95-01585	2/8/96	**157**	800	150	15	75.5	65.8	697	678	410	2417	5.1
95--02205	2/12/96	**195**	800	150	11	68.2	64.3	284	606	630	1931	4.87
1628/1631a	2/22/96	**252**	600	100	20.0	73.9	69	2633	386	547	2279	4.66
Average		**236.0**	766.7	133.3	16.0	69.9	62.1	976.8	701.0	647.3	2081.8	4.8
(B) Transmembrane pressure 300 - 400 mm Hg												
95-02227	1/11/96	**352**	800	150	13			371	864	530	1963	
95-02230	1/22/96	**372**	800	150	15	73.7	66.4	641	652	504	2453	4.44
	2/1/96	**352**	800	150	18	69.6	65		420	455	2816	4.24
95-01634	2/8/96	**396**	800	150	15	66.3	50.8	1111	556	700	2373	5.51
1628/1631 b	2/22/96	**366**	800	100	20.0	73.5	73.9	560	220	555	2264	4.68
Average		**367.6**	800.0	140.0	16.2	70.8	64.0	670.8	542.4	548.8	2373.8	4.7
(C) Transmembrane pressure 401 - 500 mm Hg												
95-02220	1/4/96	**470**	800	150	13	76.9	70.5	618	1158	753	1801	
8091485	2/6/96	**458**	800	150	18	70.3	66.2		1158	543	2626	4.86
1583/1616b	3/4/96	**444**	800	100	30	71.8	70.3	1798	522	814	1924	5.07
379/89/1411	3/6/96	**435**	800	75	47	75.1	68.7	1518	424	470	2490	4.76
Average		**451.8**	800.0	118.8	27.0	73.5	68.9	1311.3	815.5	645.0	2210.3	4.9
(D) Transmembrane pressure > 500 mm Hg												
95-02224	1/22/96	**537**	800	150	18	72.2	63.3	603	882	560	2683	4.47
95-01568	2/12/96	**580**	800	150	20	69.2	71.5	729	731	784	1845	4.94
373/85/1429	3/7/96	**689**	800	50	32	74.3	71.9	1202	1049	515	2566	5.18
373/85/1429	3/7/96	**678**	800	50	34	73.9	70.1	1202	723	536	2538	4.81
Average		**621.0**	800.0	100.0	26.0	72.4	69.2	934.0	846.3	598.8	2408.0	4.9

Table 20: Effect of Filter Rotor Design on Performance of washing Frozen - Thaw Blood

Unit #	Filter Type	Date of Test	Rotor Speed	Nominal Flow Rate	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post -wash - Sup Hb	Waste Sup Hb.	Intra K+ mEg/ml
Set (A)												
1583/1616b	214 12R-6H	3/4/96	800	100	30	444	71.8	70.3	1798	522	814	5.07
1583/1616a	214-12R	3/4/96	800	100	30	409	72.8	69.3	1798	642	763	4.37
Set (B)												
1379/89/1411a	214 12R-6H	3/6/96	800	75	47	435	75.1	68.7	1518	424	470	4.76
1379/89/1411b	214 6R-6H	3/7/96	800	100	38	602	73.6	76	1518	422	551	4.55
Set (C)												
1373/85/1429a	214 12R-6H	3/7/96	800	50	32	689	74.3	71.9	1202	1049	515	5.18
1373/85/1429b	214 12R-6H	3/7/96	800	50	34	678	73.9	70.1	1202	723	536	4.81
1373/85/1429c	214 6R-6H	3/7/96	800	60	28	769	75.9	73.3	1202	630	494	5.31

Table 21: Performance of 320 12R6H Filter for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
116	20.0	100	900	178	63.8	60.8	95.3		
130	18.5	100	900	88	64.7	60.8	93.9		
124	36.0	150			64.9	53.1	81.8		
136	14.0	100	800	111	65.5	61.4	93.7	320	76
137	12.0	150	800	145	65.5	61.2	93.4	320	885

Table 22: Performance of 518 Plain (WUPI) Filter for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash PHb (mg/dL)
32	21.4	80	1200	153	43.1	42.3	98.2	33	216
33	21.0	150	1200	124	44.9	45.9	102.3	42	270
31	23.8	80	1200	280	51.7	52.7	101.9	33	244
35	16.5	50	1200	380	56.6	50.1	88.5	49	471

Table 23: Comparison of Results for Washing Two Units in Series with Each Filter Design

Test #	Filter Type	Back Flushed	Dilute Blood Volume In (mL)	Saline Wash Volume (mL)	Total Wash Time (min)	Average Flowrate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Calcium Washout (%)
44	214 pln	no	700	1000	16.5	103	1200	92	59.9	61.7	103.1	99.31
45	214 pln		700	1000	16.5	103	1200	295	59.9	51.8	86.5	99.28
60	214 pln	no	878	1000	17.5	107	1200	356	76.7	64.5	84.1	98.57
61	214 pln		882	1000	26.0	72	1200	521	77.1	60.5	78.5	99.28
114	214 12R 6H	no	735	1000	18.0	96	900	153	68.0	63.2	92.9	
115	214 12R 6H		733	1000	19.0	91	900	339	67.8	48.7	71.8	
128	214 12R 6H	yes	602	1000	15.0	107	900	117	64.7	62.5	96.6	99.52
129	214 12R 6H		601	1000	15.3	105	900	133	64.6	60.5	93.6	99.50
130	320 12R 6H	yes	903	1000	18.5	103	900	88	64.7	60.8	93.9	99.50
131	320 12R 6H		901	1000	17.8	107	900	113	64.6	57.5	89.0	99.24

Table 24 - Sequence of Events for TBPS Console

Event #	Description	Pumped Amount (ml)	Pump Rate (ml/min)	Rotor	Shaker	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
1	Process Start															
2	Dilution of Thawed Blood Unit #1 with 12% Saline Solution	50	100		X			X		X						
3	Equilibration of Unit #1 for 2 minutes	0	0													
4	Dilution of Thawed Blood Unit #1 with 0.9% Saline/0.2% Glucose Solution	100	100		X				X	X						
5	Equilibration of Unit #1 for 2 minutes	0	0													
6	Dilution of Thawed Blood Unit #1 with 0.9% Saline/0.2% Glucose Solution	150	100		X				X	X						
7	Equilibration of Unit #1 for 2 minutes	0	0													
8	Priming of Filter with 0.9% Saline/0.2% Glucose Solution	300-400	100						X			X		X		
9	Spin-Up of Filter Rotor	0	0	X										X		
10	Pumping of Diluted Unit #1 into Filter	1300	100	X		X						X		X		
11	Pumping of 0.9% Saline/0.2% Glucose Solution into Filter	Note 1	100	X					X			X		X		
12	Spin-Down of Filter Rotor	0	0													
13	Draining of Filter to Washed Unit #1	0	0										X		X	
14	Process End															

NOTES 1) Delivery Continues Until S4 Threshold Obtained
2) Refer to Figure * for V1-V11 and S4
3) X = Actuation of Valves, Shaker, and/or Rotor
4) Rotor Speed when Actuated is 800 RPM

Figures

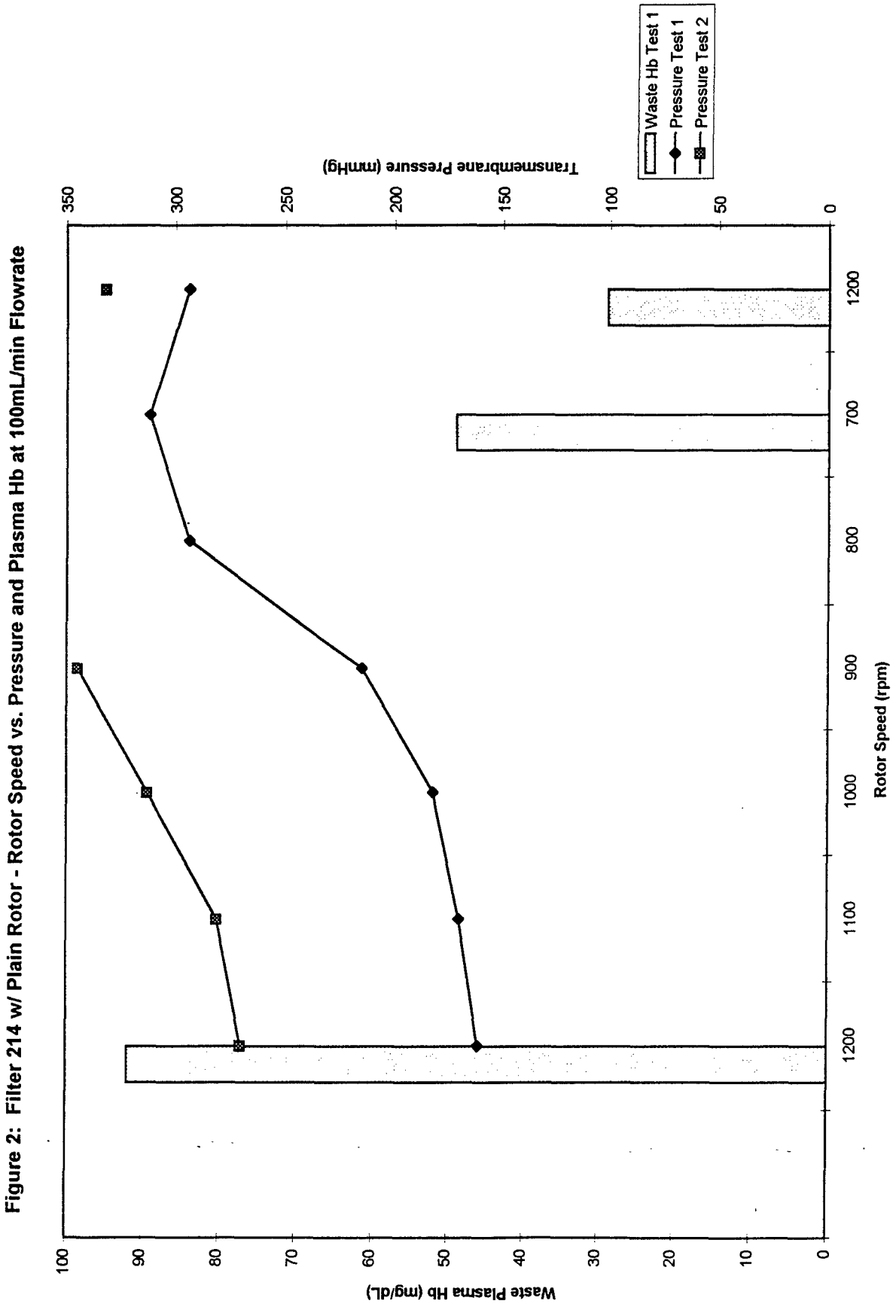
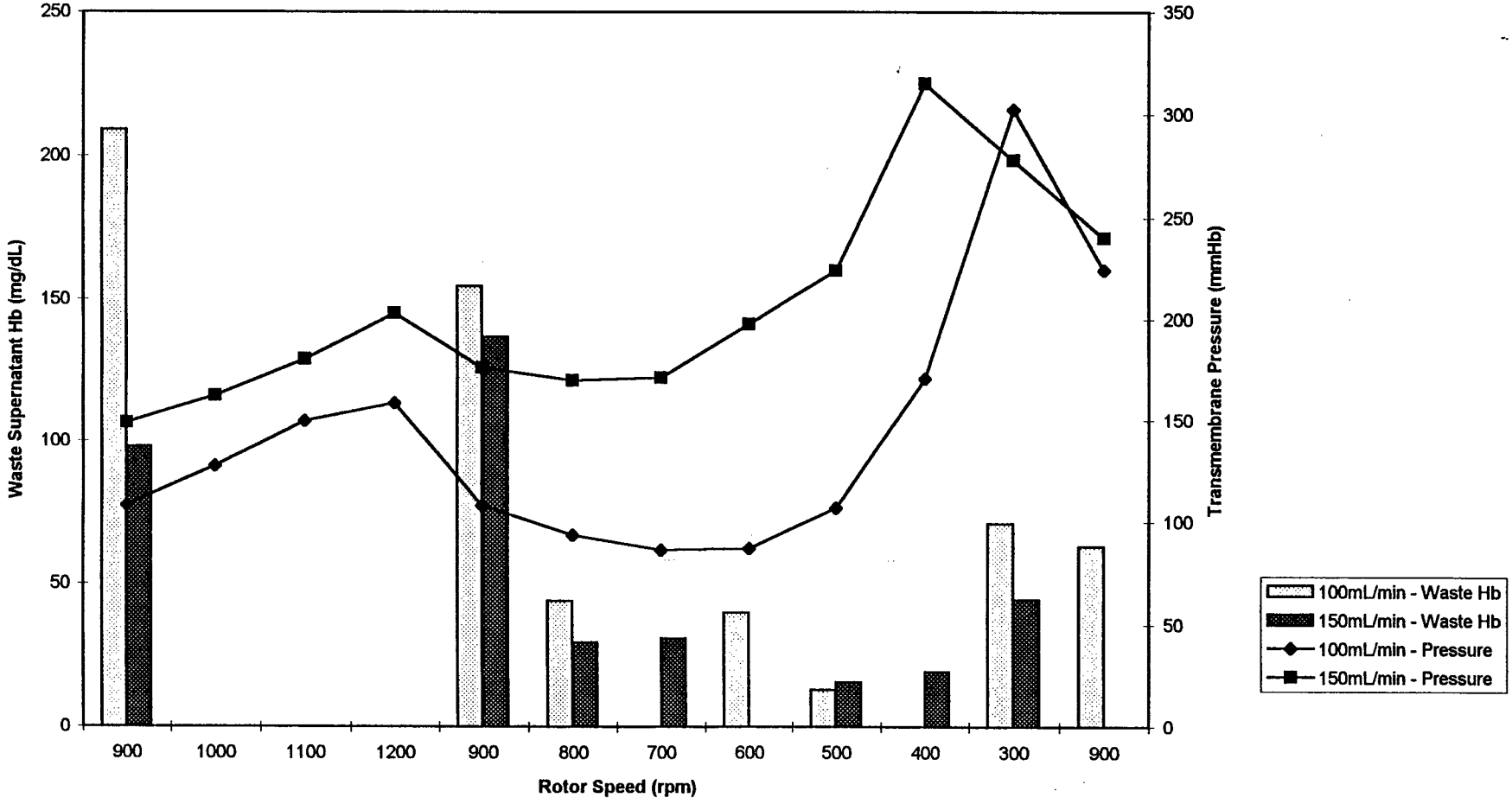


Figure 2: Filter 214 w/ Plain Rotor - Rotor Speed vs. Pressure and Plasma Hb at 100mL/min Flowrate

Figure 3: Filter 214 w/ 12R 6H Rotor - Comparison of Averaged Data for Rotor Speed vs Plasma Hb at 100mL/min vs 150mL/min Flowrate



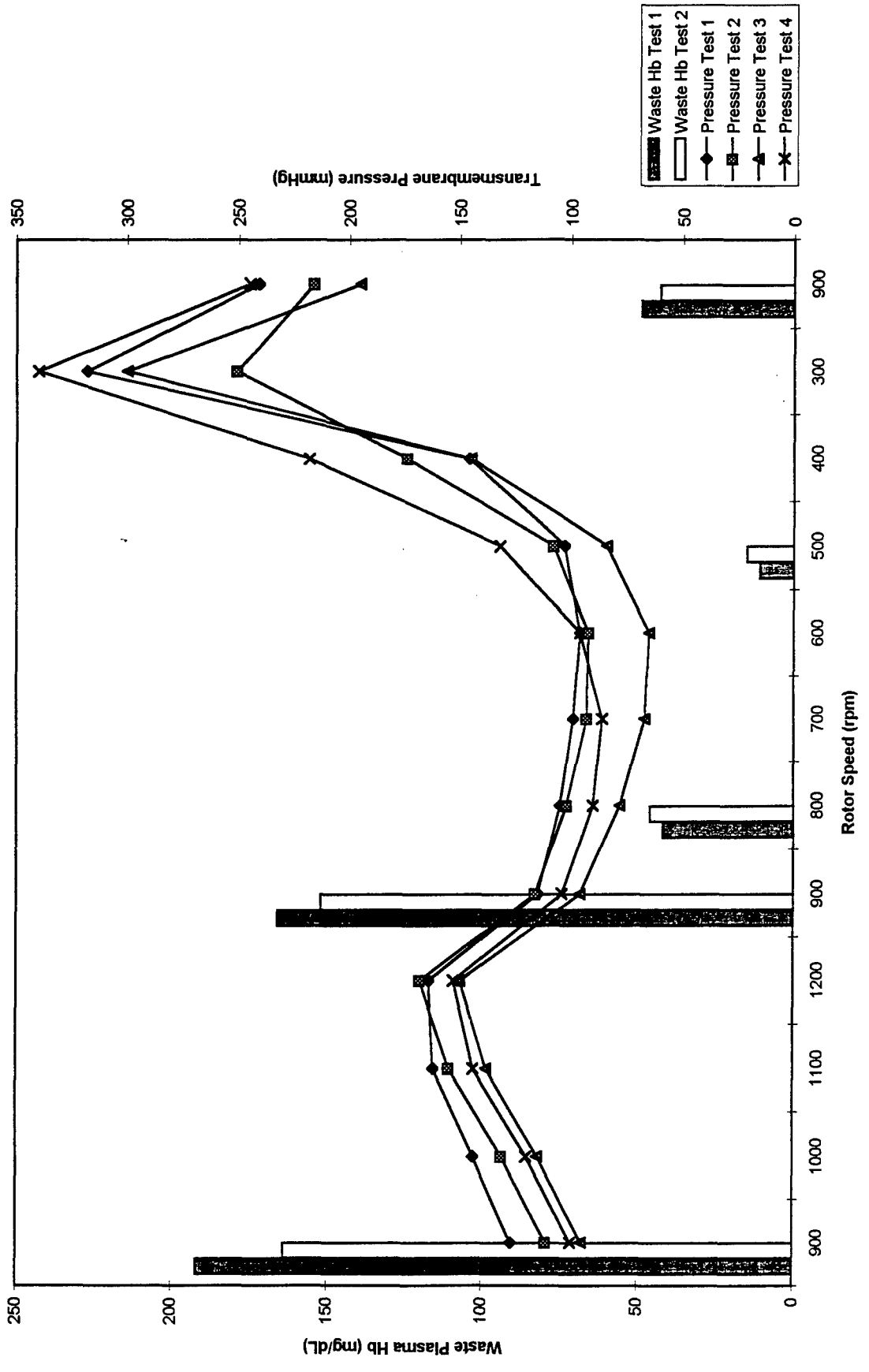


Figure 4: Filter 214 w/ 12R 6H Rotor - Rotor Speed vs. Pressure and Plasma Hb at 100mL/min Flowrate

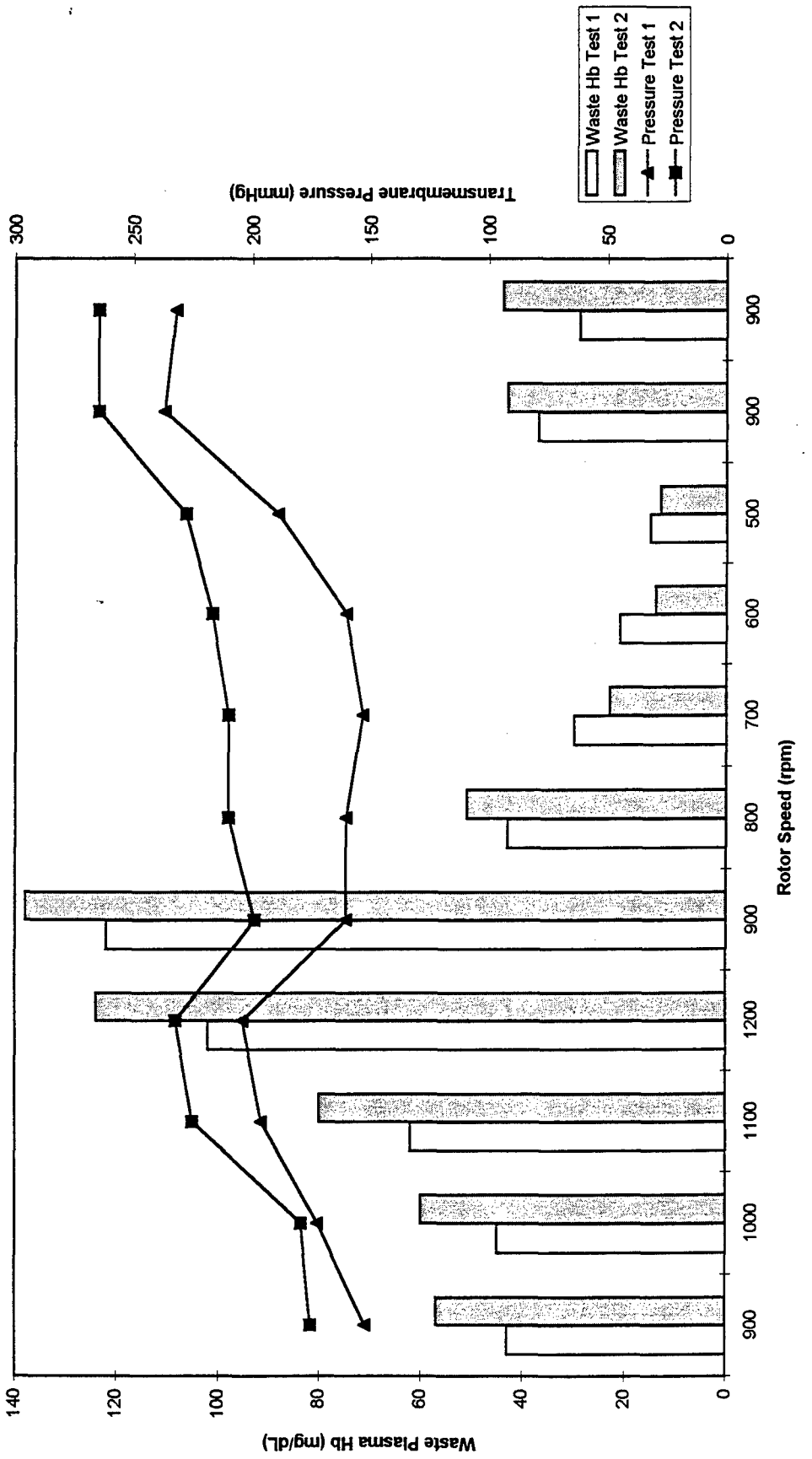


Figure 5: Filter 214 w/ 12R 6H Rotor - Rotor Speed vs Plasma Hb at 150mL/min Following Equilibrium

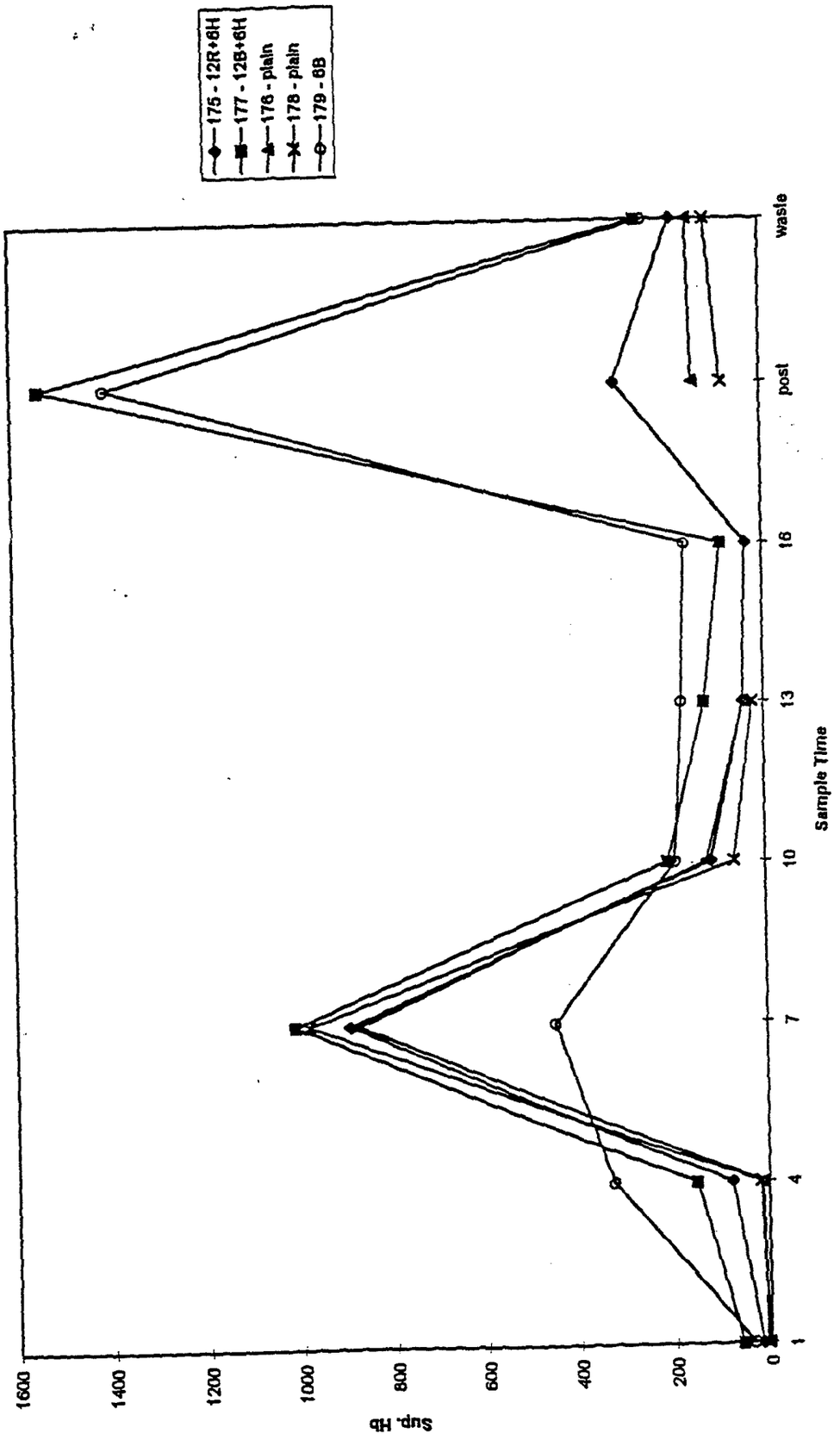


Figure 6: Supernatant Hb vs. Washing Time as Function of Rotor Type

Figure 7: Filter 320 w/ 12R 6H Rotor - Rotor Speed vs Pressure and Plasma Hb at 100 and 150 mL/min Flowrates

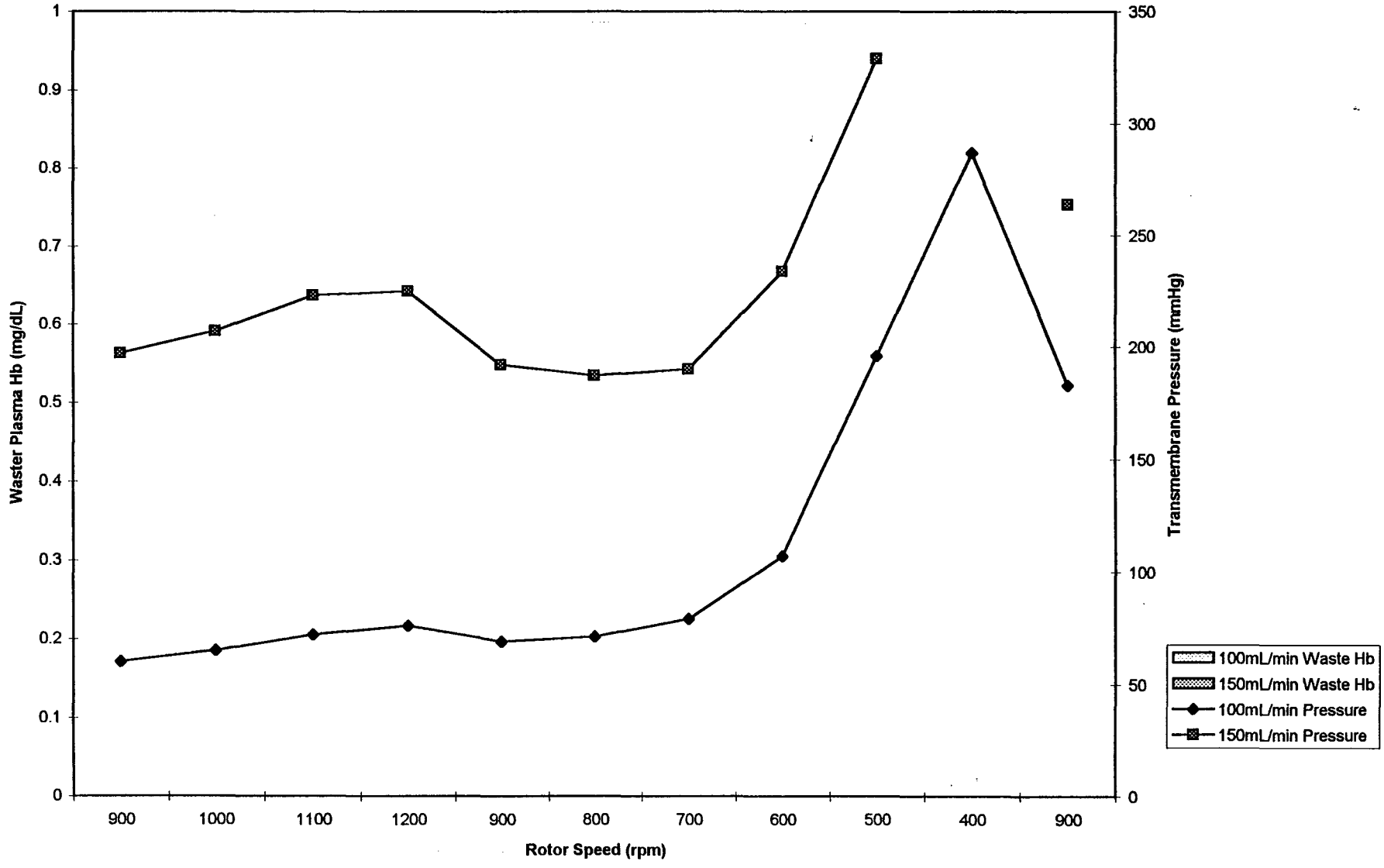
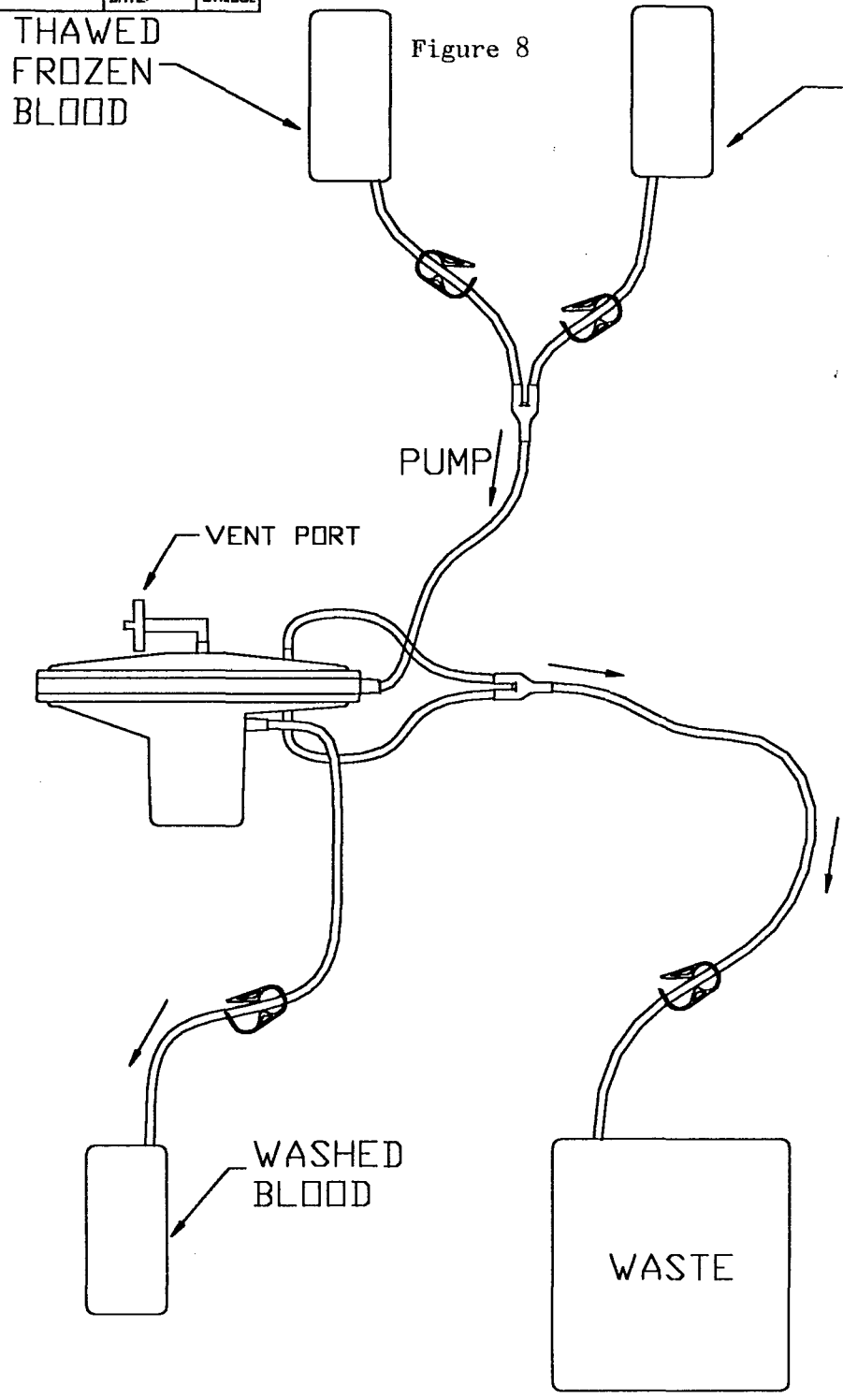


Figure 8



E.C.N.	REV	CHANGE MADE	DATE	SYMBOL

ITEM	QTY	PART NUMBER	DESCRIPTION

MATERIAL: N/A

SURGIMEDICS

TITLE: FILTER DISPOSABLES

PAGE: 1 OF 1

SCALE: NONE

DATE CREATED: 1/25/96

DWN: S. Walker

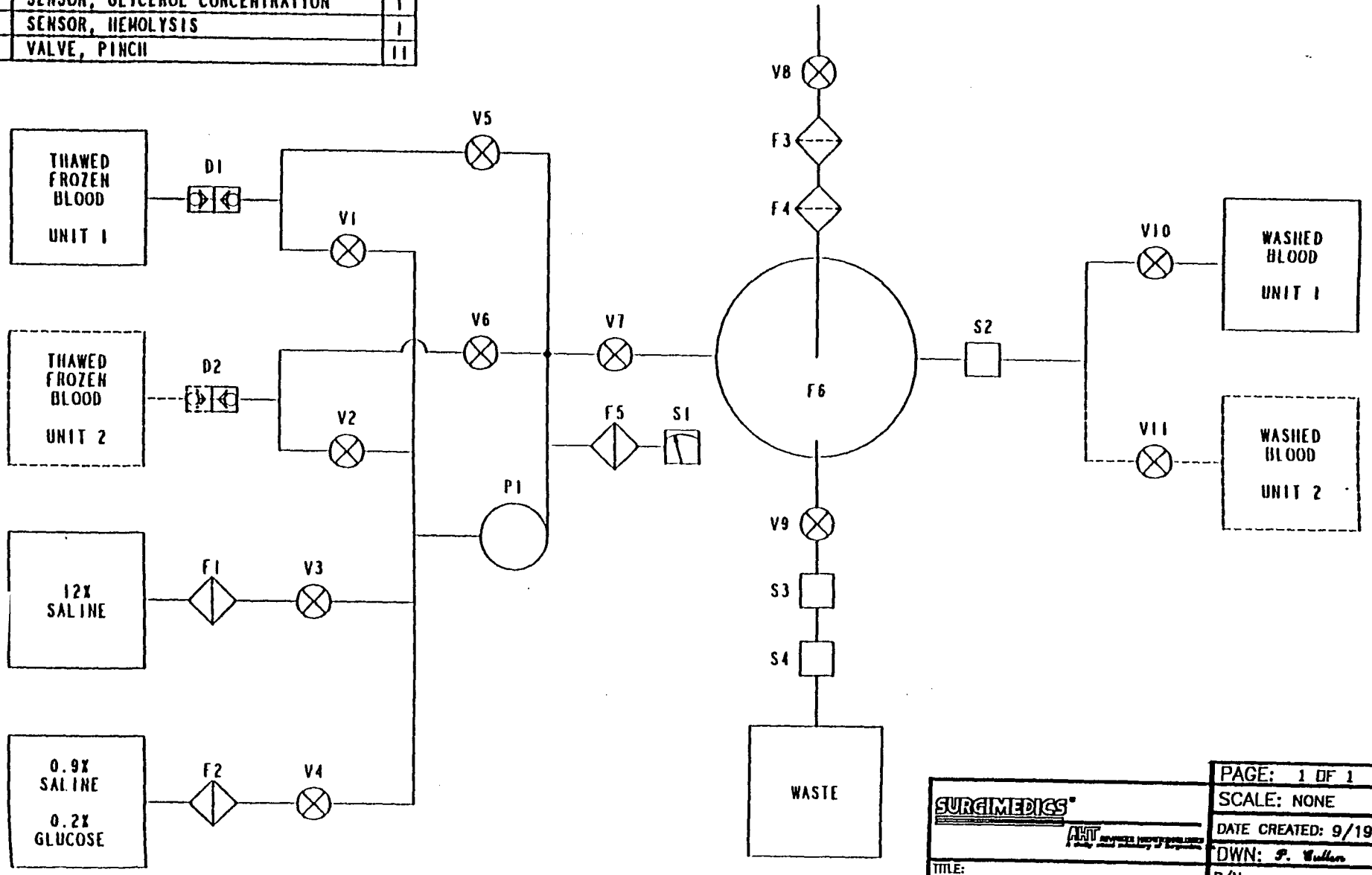
P/N N/A

REV. IR

Figure 9

SYMBOL	DESCRIPTION	QTY
D1-2	DOCKING DEVICE, STERILE	2
F1-2	FILTER, 0.22 MICRON HYDROPHILIC	2
F3-5	FILTER, 0.22 MICRON HYDROPHOBIC	3
F6	FILTER, TBPS	1
P1	PUMP, PERISTALTIC	1
S1	SENSOR, PRESSURE	1
S2	SENSOR, HEMATOCRIT	1
S3	SENSOR, GLYCEROL CONCENTRATION	1
S4	SENSOR, HEMOLYSIS	1
V1-11	VALVE, PINCH	11

BY	REV	DATE	APPR	ECO	DESCRIPTION
SHD	0.1	13 SEP 95		N/A	FIRST ISSUE
SHD	0.2	13 SEP 95		N/A	REVISED SYMBOLOGY
SHD	0.3	19 SEP 95		N/A	PER 14 SEP 95 DISCUSSION



SURGIMEDICS®
AHT

PAGE: 1 OF 1
SCALE: NONE
DATE CREATED: 9/19/95
DWN: P. Wallen

TITLE: TBPS FLUID SCHEMATIC

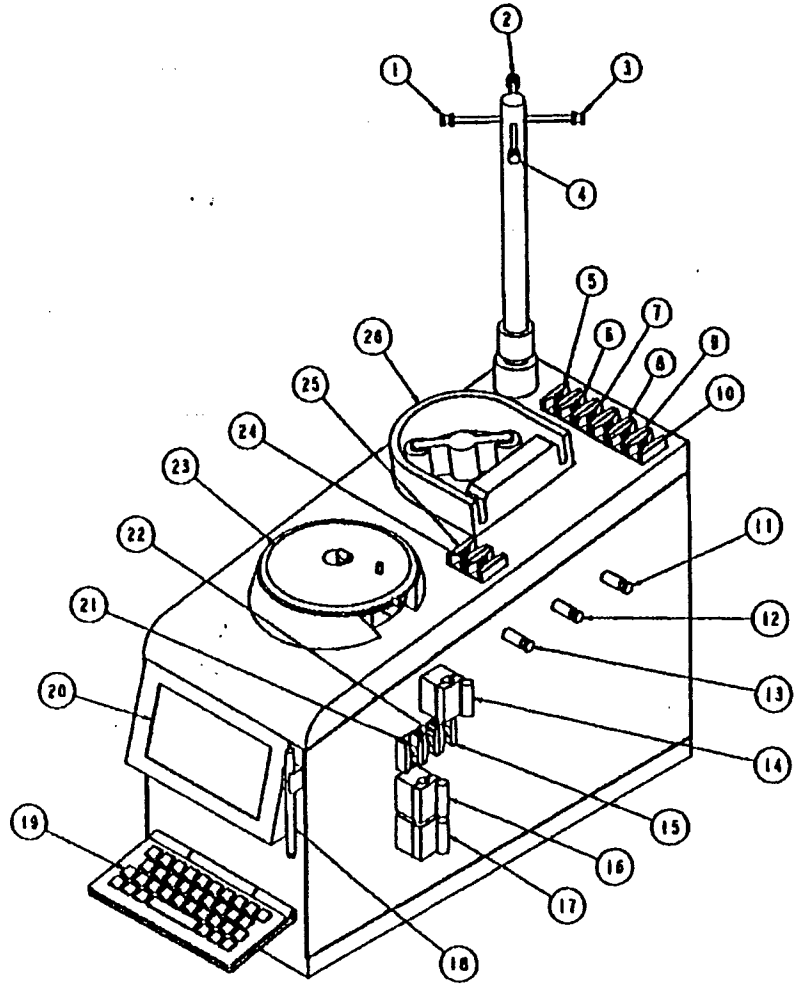
P/N N/A

REV. IR

Figure 10

INDEX	DESCRIPTION	SYMBOL IN D10-1011
1	HANGER, THAWED BLOOD UNIT #1 BAG	N/A
2	HANGER, THAWED BLOOD UNIT #2 BAG	N/A
3	HANGER, 12% SALINE BAG	N/A
4	HANGER, 0.9% SALINE / 0.2% GLUCOSE BAG	N/A
5	VALVE, PINCH	V1
6	VALVE, PINCH	V2
7	VALVE, PINCH	V3
8	VALVE, PINCH	V4
9	VALVE, PINCH	V5
10	VALVE, PINCH	V6
11	HANGER, WASHED BLOOD UNIT #1 BAG	N/A
12	HANGER, WASHED BLOOD UNIT #1 BAG	N/A
13	HANGER, WASTE BAG	N/A
14	SENSOR, HEMATOCRIT	S2
15	VALVE, PINCH	V10
16	SENSOR, GLYCEROL CONCENTRATION	S3
17	SENSOR, HEMOLYSIS	S4
18	SCANNER, BAR CODE	N/A
19	KEYBOARD	N/A
20	DISPLAY	N/A
21	VALVE, PINCH	V9
22	VALVE, PINCH	V11
23	FILTER, TBPS	F6
24	VALVE, PINCH	V8
25	VALVE, PINCH	V7
26	PUMP, PERISTALTIC	P1

BY	REV	DATE	APPR	ECO	DESCRIPTION
SMD	0.1	26 SEP 95		N/A	FIRST ISSUE



MATERIAL: N/A

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES
DIMENSION LIMITS
ONE PLACE OR FRACTION .X ±.060
TWO PLACES .XX ±.030
THREE PLACES .XXX ±.010
ANGULAR DIMENSIONS ±1/2°
ENGINEERING DEPARTMENT APPROVAL REQUIRED ON SUBSTITUTIONS.

SURGIMEDICS®
AHT

TITLE: TBPS CONCEPT LAYOUT

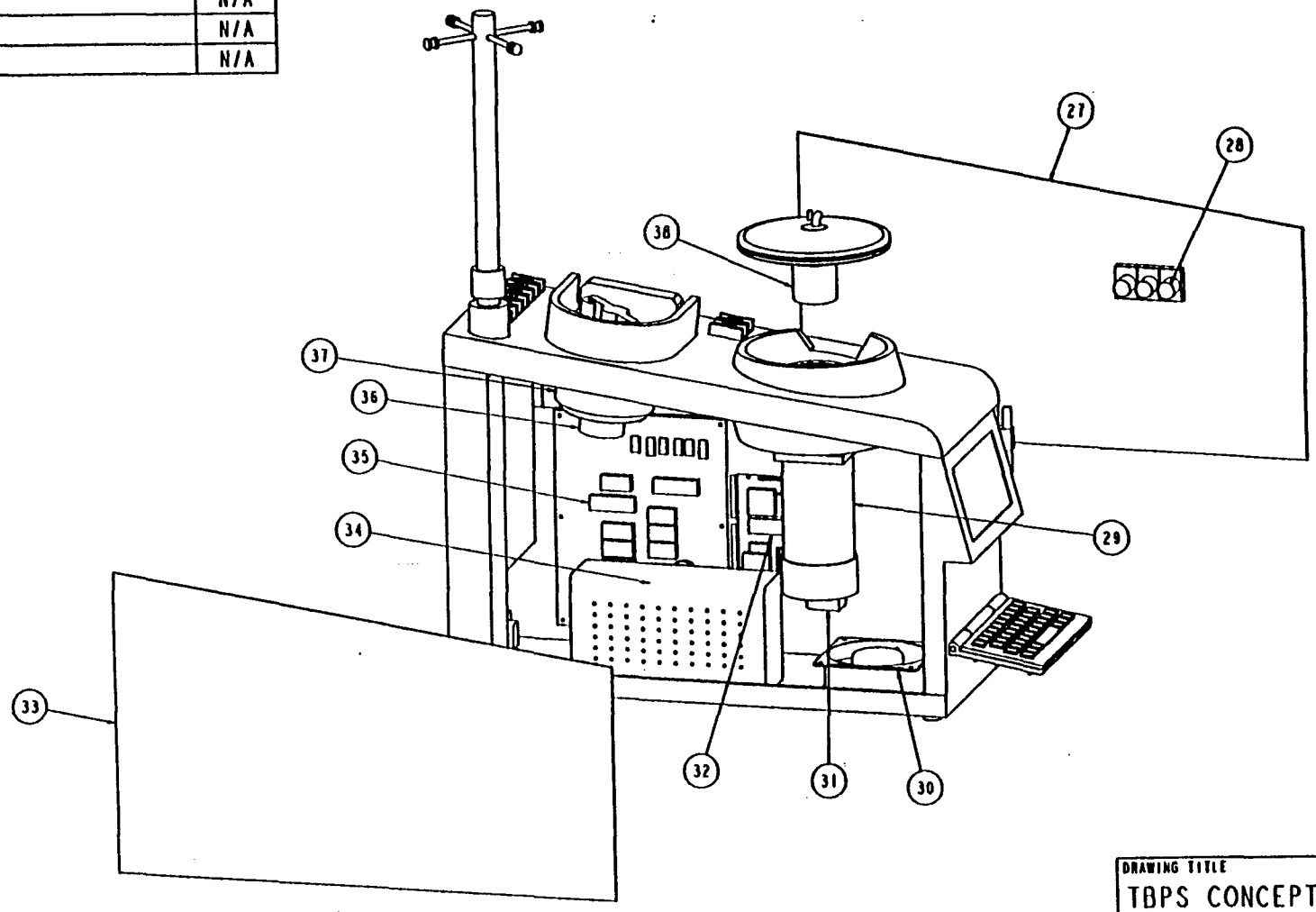
P/N N/A

REV. IR

PAGE: 1 OF 2
SCALE: NONE
DATE CREATED: 9/26/95
DWN: P. Wallace

INDEX	DESCRIPTION	SYMBOL IN D10-1811
27	SIDE PANEL, RIGHT	N/A
28	ACTUATOR, PINCH VALVE (TYPICAL)	N/A
29	MOTOR, BRUSHED DC SERVO	N/A
30	FAN, COOLING	N/A
31	ENCODER MODULE, OPTICAL	N/A
32	MICROCONTROLLER	N/A
33	SIDE PANEL, LEFT	N/A
34	POWER SUPPLY	N/A
35	PCB, INTERFACE	N/A
36	ENCODER, OPTICAL	N/A
37	MOTOR, BRUSHED DC SERVO	N/A
38	COUPLING, MAGNETIC	N/A

Figure 11



REV

DRAWING TITLE
TBPS CONCEPT LAYOUT

SIZE	DRAWING NUMBER
C	D10-1813

TYPE: ILLUSTRATION
SCALE: NONE
SHEET 2 OF 2

Appendix 1

Software Quality Assurance Plan

Software Quality Assurance Plan

for the
ADVANCED HAEMOTECHNOLOGIES
Thawed Blood Processing System

CONTENTS

1.0 Purpose

This document comprises the Software Quality Assurance Plan (SQAP) for development of the software to be used in the Thawed Blood Processing System (TBPS) manufactured by Surgimedics - Advanced Haemotechnologies (AHT). The purpose of this plan is to specify the actions to be taken and the documents to be produced to insure that the following objectives are met:

1. The finished software product implements an agreed upon, unambiguous set of functional requirements.

2. The implementation of these requirements is verifiable by a defined set of test procedures.

3. Changes and maintenance updates to the software can be performed in a controlled manner.

4. AHT management is an active participant in the specification, review, and approval phases of this project.

2.0 Reference Documents

The following documents are referenced in other sections of this manual:

1. <Code Supplier>, Standards for Software Documentation and Coding

2. Advanced Haemotechnologies, SOP-1000, Change Control

3. Advanced Haemotechnologies, SOP-1002, Device Master Record

4. Modern Structured Analysis, Edward Yourdon, Yourdon Press, 1989

3.0 Management

This project is to be implemented on a turn key basis by <Code Supplier> with oversight and project phase approval performed by AHT. <Code Supplier> will assign a technical project manager for the duration of the project and at least one additional staff member at the beginning of the coding phase. In addition, <Code Supplier> will provide one person, not involved with the design and development of the software, to produce the in-house test plan document and perform the in-house test procedures. AHT will assign a project coordinator who will act as a technical liaison for the duration of the project. This person along with any others that AHT may specify will also participate in all formal reviews as specified in Section 6.

The tasks to be accomplished under this plan and their sequence are listed below.

1. Prepare Software Requirements Specification
2. Conduct Software Requirements Review
3. Prepare preliminary Software Design Document
4. Conduct preliminary Design Review
5. Prepare final Software Design Document
6. Conduct Critical Design Review
7. Prepare Software Validation Plan
8. Prepare Software Validation Plan Review
9. Conduct Code Reviews
10. Conduct Functional Audit
11. Perform Software Configuration Control Plan review
12. Perform Post Mortem Review

4.0 Documentation

The following documents will be produced as part of the implementation of this plan:

1. Software Requirements Specification (SRS) - This document will describe the context of the software in terms of its interface with the system hardware and the functionality of the software in terms of its response to inputs from the hardware. The SRS forms the basis for all the remaining documents, thus it is critical that it be complete and correct. The SRS will be produced based on information provided by AHT and will be checked during the Software Requirements Review (see Section 6). No subsequent phases of the project will be started until AHT has approved the SRS.

2. Software Design Description (SDD) - This document describes all the software modules, internal interfaces, and database structures required to implement the functionality described by the SRS. A preliminary version of the SDD will include all database structures and top level module definitions. This will be checked during the Preliminary Design Review. The final version of the SDD will include definitions of all system modules and will be checked during Critical Design Review. The completed SDD is the basis for the development of the system source code.

3. Software Validation Plan (SVP) - This document describes a series of test procedures which will be used to validate that the finished product implements the functionality described by the SRS and conversely that it does not implement behavior not described by the SRS. Each procedure in the test document will include the expected results. The SVP will be produced from the SRS and the SDD by person(s) not involved with the SDD and coding phases of the project. The adequacy and completeness of these procedures will be determined by the Software Validation Plan Review. The SVP will be executed during Functional Audit.

4. Software Validation Report (SVR) - This document is a log of the results of all reviews, audits, and tests required by the SQAP.

5. User Documentation - All user's manuals will be produced by AHT.

6. Software Configuration Management Plan (SCMP) - This document describes the methods and procedures to be used for identifying software items, controlling and implementing changes, and recording and reporting change implementation status (see Appendix A of this document).

5.0 Standards, Practices, and Conventions

Documentation and coding standards to be applied to this project are those described in <Code Supplier>, Standards for Software Documentation and Coding (subject to review and approval by AHT).

6.0 Reviews and Audits

The following reviews and audits will be conducted:

1. Software Requirements Review - This review is held to ensure the correctness and completeness of the SRS (section 4). Participants will include the <Code Supplier> project manager, AHT project coordinator, and if possible at least one other member of AHT management. The SRS should be distributed to all participants at least 3 days prior to the initial review to allow adequate preparation time. It is anticipated that, while some iteration will be required to complete the SRS, the subsequent reviews will be done without a group meeting. The end result of this review process will be an approved SRS with signatures of representatives from both AHT and <Code Supplier>.

2. Preliminary Design Review - This review is held to evaluate the technical adequacy of the preliminary design of the software as described in the preliminary SDD (section 4). Participants will include, at a minimum, <Code Supplier> project manager, and AHT project coordinator. The preliminary SDD should be distributed to all participants at least 2 days prior to the review to allow adequate preparation time.

3. Critical Design Review - This review is held to ensure that the SDD implements the functionality described in the SRS. Participants will include all <Code Supplier> project staff, and the AHT project coordinator. The SDD should be distributed to all participants at least 9 days prior to the initial review to allow adequate preparation time. The end result of this review will be an approved SDD with the signatures of representatives from both AHT and <Code Supplier>.

4. Software Validation Plan Review - This review is held to ensure that the SVP adequately tests the functionality described in the SRS. The objective of these tests is not only to demonstrate that the system does what it is supposed to do, but that it doesn't do what it's not supposed to do. Participants will include the <Code Supplier> project manager, SVP author, and the AHT project coordinator. The SVP should be distributed to all participants at least 2 days prior to the initial review to allow adequate preparation time. The end result of this review process will be an approved SVP with the signatures of representatives from both AHT and <Code Supplier>.

5. Code Reviews - During the coding phase of the project, code walk through will be held by <Code Supplier> personnel. These reviews are part of the unit test procedure and will precede computer based testing of the modules.

6. Functional Audit - This audit will comprise the software acceptance test procedure and will be performed by executing the SVP and recording the results. These tests will be run by AHT personnel with a representative from <Code Supplier> present. When all tests have been performed and been found to give the correct results, AHT will indicate acceptance of the software by signing the acceptance page of the SVP.

7. Managerial Reviews - AHT will be responsible for periodically assessing, or having a third party assess, the execution of this plan during the project.

8. Software Configuration Management Plan Review - This review is held to evaluate the adequacy and completeness of the configuration management methods defined in the SCMP. Participants will include the <Code Supplier> project manager, and the AHT project coordinator.

9. Post Mortem Review - This review will be held at the end of the project to assess the development activities implemented on the project and to provide recommendations for appropriate actions.

7.0 Testing

Testing shall be done in accordance with the SVP.

8.0 Problem Reporting and Corrective Action

In the event that a software problem is encountered that cannot be resolved by the normal testing procedures, the individual(s) who discover the problem shall immediately report it to the project manager. The project manager may elect to resolve such problem, if it falls within his/her authority, or forward it to the software committee for resolution. In no case may the project manager alter the SRS, or any other certified documents and/or specifications without the designated reviews and approvals.

9.0 Tools, Techniques, and Methodologies

The SRS and SDD documents will be done using structured analysis and design techniques as discussed in the book Modern Structured Analysis; or equivalent techniques. This effort will be supported by PC based tools such as Microsoft Word for Windows, Micrografix Designer, and Dbase; or equivalent tools.

The design will be implemented in the C language using an industry recognized PC based text editor, compiler, and object file linker.

Testing will be done on the target hardware.

10.0 Code Control

Revision control of source code and documentation will be done using the Polytron Version Control System (PVCS), or equivalent process. Refer to the SCMP in appendix A of this document.

11.0 Media Control

Two floppy disk backups of all system files will be made whenever a new version is created. One of these backup sets will be maintained on site for protection against a hard disk failure on the source code control computer. The other set will be maintained in another physical location to protect against loss due to fire, flood, etc.

Backup of checked out modules which are in the process of being modified will be the responsibility of the person doing the modification.

The MASTER software copy will be released by Engineering only after completion of the SVP and certification to that effect. The MASTER software copy becomes a part of the Device Master Record and is controlled by Regulatory Affairs/Document Control (ref. SOP-1002, Device Master Record). Revision follows standard change request/authorization procedures (ref. SOP-1000, Change Control).

12.0 Supplier Control

<Code Supplier> will be reviewed and audited for compliance with this SQAP. See section 3 and 6 for a description of the reviews and audits to be performed.

13.0 Record Collection, Maintenance, and Retention

All documents produced under this plan shall be assembled into a binder (or binders) and kept by AHT for the lifetime of the TBPS product, along with any changes or additions to these documents, and copies of ECN's applicable to this product.

14.0 Training

This section is not applicable to this plan as all personnel involved in the software development process will be familiar with the tools and techniques necessary to implement this plan.

15.0 Risk Management

Any individual(s) discovering a previously unknown risk, or new factors relating to a known risk, shall immediately notify the project manager. The project manager shall perform a preliminary risk analysis and submit it, along with the risk statement, to the software committee.

The software committee shall have the responsibility for performing a detailed risk analysis and making recommendations to management when appropriate.

Appendix A

Software Configuration Management Plan (SCMP)

When the software developed under this plan is produced, each software (source file, header file, etc.) any "make" or system build control files, and the SRS, SDD, and SVP document files will be installed in a source control system (see section 10). AHT will assign one person to be responsible for managing the controlled modules. The responsibilities of this person will include managing / monitoring the defined backup and physical security procedures, reviewing the procedures involving the check out and check in of controlled modules, and verifying the creation and retrieval of system versions.

The controlled modules will be initially installed with the revision number 1,0 and the version label "VER 1.0". Any changes to the software from that point on will be initiated by an Engineering Change Request (ECR). All ECR's are reviewed by the Software Committee and, if approved, become an Engineering Change Notification (ECN).

The implementation of an ECN will typically require changes to existing software modules, possible addition of new software modules, and changes to the SRS, SDD, and SVP. The implementor will determine which modules are affected and check them out using the source code control system. When the changes are made and tested, and the documentation files updated, all affected modules are checked back in. The check-in process for each module requests a description of the changes made, which becomes part of the header of that module, and automatically creates a new revision number for the module. Note that the revision number applies only to a single module and reflects the revision history of that module.

One or more ECN's may be incorporated into a new version of the software. A version is composed of a single revision level of each module required to build the system. To facilitate the recreation of a version, a label. e.g. "VER 1.1", is applied to each module revision comprising that version. The build process then becomes one of extracting all module revisions with the desired version label; which performs the appropriate compile to produce the run-time program.

Appenidix 2

Software Requirements Specification

Software Requirements Specification

1.0 Introduction
- 1.1 Purpose
- 1.2 Scope
- 1.3 Definitions, Acronyms, and Abbreviations
- 1.4 References
- 1.5 Overview

2.0 General Description
- 2.1 Product Perspective
- 2.2 Product Functions
- 2.3 User Characteristics
- 2.4 General Assumptions
- 2.5 Assumptions and Dependencies

3.0 Specific Requirements
- 3.1 System Requirements
  - 3.1.1 Functional Requirement 1
    - 3.1.1.1 Introduction
    - 3.1.1.2 Inputs
    - 3.1.1.3 Processing
    - 3.1.1.4 Outputs
    - . . . .
  - 3.1.2 Functional Requirement 2
    - . . . .
  - . . . .
  - 3.1.n Functional Requirement n
    - . . . .
- 3.2 External Interfaces
  - 3.2.1 User Interfaces
  - 3.2.2 Hardware Interfaces
  - 3.2.3 Software Interfaces
  - 3.2.4 Communications Interfaces
  - . . . .
- 3.3 Performance Requirements
  - . . . .
- 3.4 Design Constraints
  - 3.4.1 Standards Compliance
  - 3.4.2 Hardware Limitations
  - . . . .
- 3.5 Attributes
  - 3.5.1 Security
  - 3.5.2 Maintainability
  - . . . .

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 19 Apr 00

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for Accession Document Numbers ADB210895, ADB223532, ADB209674, ADB231094, and ADB249633, be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Virginia Miller at DSN 343-7327 or by email at Virginia.Miller@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Received 4-28-00