AD NUMBER

ADB190895

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to DoD only; Specific Authority; 13 Sep 94. Other requests shall be referred to Commander, US Army Medical Research and Material Command, Attn: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

AUTHORITY

U.S. Army Medical Research and Materiel Command ltr., dtd January 25, 2000.

AD-B190 895

AD_____

CONTRACT NO: DAMD17-94-C-4049

TITLE: AN AUTOMATED HOLLOW FIBER SYSTEM FOR THE
          DEGLYCEROLIZATION OF THAWED FROZEN HUMAN BLOOD

PRINCIPAL INVESTIGATOR: Thomas C. Robinson, Ph.D.

CONTRACTING ORGANIZATION: Razor Associates, Inc.
                           253 Humboldt Court
                           Sunnyvale, California 94089

REPORT DATE: September 13, 1994

TYPE OF REPORT: Phase I Final Report

DTIC
ELECTE
OCT 17 1994
S B D

PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick
               Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to DOD
Components only, Specific Authority, September 13, 1994. Other
requests shall be referred to the Commander, U.S. Army Medical
Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick,
Frederick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

DTIC QUALITY INSPECTED

94-32337

REPORT DOCUMENTATION PAGE

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 13 SEPT 94	3. REPORT TYPE AND DATES COVERED Phase I FINAL REPORT

4. TITLE AND SUBTITLE

AN AUTOMATED HOLLOW FIBER SYSTEM FOR THE DEGLYCEROLIZATION OF THAWED FROZEN HUMAN BLOOD

5. FUNDING NUMBERS

DAMD17-94-C-4049

6. AUTHOR(S)

THOMAS C. ROBINSON, Ph.D.
THOMAS P. SAHINES
ROBERT FERNANDEZ   /   ADOLF SANCHEZ

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

RASOR ASSOCIATES, INC.
253 Humboldt Court
Sunnyvale, CA 94089

8. PERFORMING ORGANIZATION REPORT NUMBER

94-0905

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

Covers Phase I research for period March 15, 1994 - September 14, 1994.

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution authorized to DOD Components only, Specific Authority, September 13, 1994. Other requests shall be referred to the Commander, U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, MD 21702-5012

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

This Phase I program demonstrated the feasibility and potential advantages of a new technical approach for the deglycerolization of thawed frozen human blood. This approach uses a hollow fiber separator, modified roller pumps, and a new blood-saline mixing device incorporated into a fully automated system. The hollow fiber separator removes glycerol and saline wash solution in an efficient recirculation process using serial dilutions followed by fluid and glycerol removals. System requirements and specifications were established; system performance was analytically modeled; the system and its key components were designed; the new hollow fiber separators, recirculation bag, and blood-saline mixer were fabricated and tested; a complete prototype manually-operated system was constructed; and this system was tested with bovine and thawed frozen human blood. The requirements for glycerol removal with acceptable levels of hemolysis and intracellular potassium were achieved. All of the Phase I tasks were successfully completed and the objectives were met. All of the requirements established by the military for the future complete deglycerolization system appear to be achievable based on the results of this Phase I program

14. SUBJECT TERMS

DEGLYCEROLIZATION,   FROZEN BLOOD,   HOLLOW FIBER, SEPARATOR,   RECIRCULATION,   AUTOMATED

15. NUMBER OF PAGES

69

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

TCR Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

TCR Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

_____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

TCR For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

_____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

_____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Thomas C. Robinson   9/13/94
————————————————   ————
PI - Signature   Date

ii

4.0   TABLE OF CONTENTS

Final Phase I Program Report:
AN AUTOMATED HOLLOW FIBER SYSTEM FOR THE
DEGLYCEROLIZATION OF THAWED FROZEN HUMAN BLOOD

5.0 INTRODUCTION

5.1 The Problem and the Background of Clinical and Military Experience

Current manually controlled centrifugal methods for the deglycerolization of frozen thawed human blood have limitations which restrict the use of this blood, require the intensive efforts of trained technicians, result in greater than desired variations in blood quality, and costs more and takes longer than desired. The military has determined that a fully automated, faster system with a less expensive disposable and longer processed blood storage time is necessary to meet the needs of future military or civilian occurances where many casualties require blood transfusions. Blood banks can benefit from such a system in reducing deglycerolization time and costs for their frozen blood programs. The military has defined specific objectives, requirements, and design goals for this new deglycerolization system.

5.2 The Purpose of the Phase I Effort

The purpose of the Phase I effort was to demonstrate the feasibility and potential advantages of a new technical approach for the deglycerolization of thawed frozen human blood. This approach uses a hollow fiber separator to remove glycerol and saline wash solution from red cells in an efficient recirculation process that uses serial dilutions followed by fluid removal. The basic objectives, requirements, and design goals for a fully-automatic system that performs the deglycerolization process were established by the military. The Phase I effort was performed to establish more complete specifications, analyze system performance, design a complete system using hollow fiber separation, construct and test a prototype of this system, and demonstrate the feasibility of this approach and of meeting the requirements established for this system.

5.3 The Basic Approach

The basic requirement for a deglycerolization system is to reduce the glycerine-cryopreservative level in the blood to less than 1% without damaging red blood cells. This is done by washing the red cells with saline and carrying away the glycerine. The RAI approach uses a technique of serial dilution and hollow fiber membrane separation to accomplish this goal.

The membrane separator consists of several parallel microporous hollow tubes having an inner diameter of 200-350 μm and a specific length for the fiber bundle. The optimum pore size for high waste flux and low hemolysis is likely to be about 0.2 μm. The surface area is computed based on the inside diameter and will probably be 0.5 to 0.8 m².

The red cell, glycerol, and saline solution enters the hollow fiber separator and flows along the inner diameter. The red cells are carried along the fiber bore as the effluent is removed across the walls of the fibers. The effluent removal is partially regulated by the differential pressure between the inside and outside of the fibers. This is referred to as the transmembrane pressure or TMP. Red cells are prevented from depositing on the inner wall and plugging the fibers by the shear rate and radial forces created by the fluid/red cell solution flow. The greater the flow the higher the shear rate and radial forces. Higher shear rates require less TMP to establish a specific plasma flow. However, as shear rates increase, so does the pressure drop along the fibers. The optimum geometry balances shear rate, pressure drop, and TMP to optimize plasma removal and minimize red cell damage which can occur if any of the above parameters are excessive. This optimized geometry includes hollow fiber inner diameter, number of fibers, and fiber length.

The blood is first prepared for washing, as prescribed in the Standard Operating Procedure [Ref. 41]. The blood is then pumped by the roller pump into the hollow fiber separator. The saline roller pump provides 0.9% sodium chloride, 0.2% glucose solution, referred to herein as saline, at a specific rate to provide a constant inlet hematocrit to the separator. Effluent flow is controlled by a roller pump

referred to as the waste pump. The blood pump, waste pump, and saline pump flow rates are controlled to adjust the outlet hematocrit of the separator and achieve efficient glycerol removal.

Saline is introduced into the blood flow stream via a mixing tee. Blood and saline continue to mix and flow into the recirculation bag. Glycerol diffuses out of the red cell and into the saline. The recirculation and serial dilution process is then repeated until the remaining glycerine is less than 1% of the effluent volume.

5.4　The Specific Technical Approach

The key element in the technical approach is the use of a hollow fiber separator to efficiently remove glycerol from red cells. It performs this function by removing the fluid surrounding the red cells through the pores in the wall of the hollow fiber. This fluid contains glycerol that diffused out of the red cells and was originally in equilibrium concentration with glycerol in the red cells. A saline solution is added to the red cells, and the saline containing glycerol is removed in the separator using a recirculation process that can involve several such passes of red cells.

The RAI technical approach uses three roller pumps to precisely control fluid flow rates: a blood pump, saline pump, and waste pump. The automatic control of these three flow rates fully defines the operation of the hollow fiber separator and fixes the glycerol concentration and hematocrits at any stage in the glycerol removal process. The separator transmembrane pressure is a dependent variable which remains consistently low with proper separator design.

The technical approach includes automatically adjusting the three fluid flows at the end of the deglycerolization process to concentrate red cells and then pump them into the output blood bag.

A single saline pump is used to deliver the 12% NaCl solution, the 0.9% NaCl solution, and the long-term storage solution to either the frozen blood bag or the output blood bag, as required. The thawed frozen blood bag is supported vertically in a blood-saline mixer, eliminating any manual movement of this bag after set-up and permitting all of the initial dilution steps to be performed automatically.

Roller pump function is controlled using fluid pressure sensing to detect empty or full bags. For example, a sufficiently negative pressure detected at the inflow to the blood roller pump indicates that the thawed blood bag is empty and stops the pump. Microprocessor-based electronic controls perform all pump speed, control, monitoring, and detailed diagnostic functions via software that implements the necessary logic algorithms.

Much of this technical approach was based on the familiarity with the similar technical approaches used with current autotransfusion, apheresis, and hemoconcentration systems. The development of the RAI autotransfusion system used a very similar technical approach to perform very similar functions meeting similar requirements.

6.0 GOALS, EXPERIMENTAL METHODS, AND RESULTS OBTAINED

6.1 Phase I Program Goals and Program Plan

A primary goal of this Phase I program was to demonstrate the feasibility and potential advantages of a new technical approach for the deglycerolization of thawed frozen human blood. This approach uses a hollow fiber separator to remove glycerol and saline wash solution from red cells in an efficient recirculation process that uses serial saline dilutions and removals.

Another major objective was to show that it is feasible to remove the glycerol cryopreservative from human red cells using a fully automated, user-friendly system and a sterile, rapid (under 30 minutes) process yielding red cells capable of refrigerated storage for up to 21 days.

The more detailed objectives, requirements, design goals, and specifications are given in Table 1. These were established in the SBIR initial solicitation and the Phase I contract. Requirements established more recently are given in Table 2.

The Phase I program plan was designed to evaluate technical feasibility by a progressive series of tasks that culminated in the testing and demonstration of feasibility with a prototype manually-operated TBPS (Thawed Blood Processing System). These tasks were: the definition of detailed system objectives, requirements, and specifications; analytical modeling of system performance to guide the design and testing effort; design of a prototype test system and its key components, and a preliminary TBPS product design; the design fabrication and testing of hollow fiber separators specifically for the needs of the TBPS; assembly of a prototype system; and testing of this prototype with optimization of system performance using bovine and thawed human blood.

The methods used and results obtained for each task are described below.

6.2 The Definition of System Specifications

The purpose of this task was to clearly define in detail the objectives, requirements, and specifications in addition to those of Tables 1 and 2 needed for a fully automatic deglycerolization system (or TBPS, Thawed Blood Processing System) having major improvements in performance and decreases in cost compared to current systems. These specifications include "external" requirements seen by the user and "internal" requirements defining the disposable components, the control module hardware, and control software.

A detailed literature search has been performed covering the entire field of "frozen blood", including freezing, thawing, and deglycerolization methods; platelet freezing, thawing, washing, and storage methods; long-term blood (RBC) storage solutions and methods; and related procedures and descriptions of equipment. This has included the clinical, technical, patent, commercial, and military report literature. The references (Section 8.0) describe all literature obtained and reviewed. The key reference [Ref. 41] describing the process to be automated is the "Standard Operating Procedure" (SOP) from the Naval Blood Research Laboratory at the Boston University School of Medicine. The video of this SOP was obtained through the Army program office and was viewed by RAI staff.

Discussions were held with Dr. Valeri of the Naval Blood Research Laboratory. These discussions included: a review of the SOP to be implemented; a discussion of the RAI concept for automated deglycerolization using this SOP; a description by Dr. Valeri of methods to achieve blood sterility and store blood for about three weeks after deglycerolization; and a description by Dr. Valeri of methods for freezing, storing, thawing, and washing platelets.

Discussions were also held with Dr. Meryman of the Red Cross. These discussions included: a review by Dr. Meryman of the FDA concerns and actions regarding blood sterility in the collection, freezing, and deglycerolization of blood; a description by Dr. Meryman of requirements and methods to achieve blood sterility and long-term storage after deglycerolization; a review of the RAI procedure for

Table 1

OBJECTIVES, REQUIREMENTS AND DESIGN GOALS

A. **OBJECTIVES AND REQUIREMENTS**
(Taken directly from the SBIR Topic A93-235 description)

1. **Objective:**
To remove the glycerin cryopreservative from human red blood cells in a sterile, rapid (<30 minutes), labor non-intensive manner.

2. **Requirements:**
   a. The glycerine must be removed from the red cells by a sterile procedure which does not harm the cells, is quick, and low in labor requirements.
   b. Final equipment should be less than 8 cubic feet in size and have expendable components competitively priced with the currently-approved centrifugal techniques.
   c. Device should require minimal operator interaction and minimize maintenance.
   d. Use closed automatable techniques to safely separate red cells from glycerin solution.
   e. Saline wash solutions should be used.
   f. Performance/costs must be superior to centrifugal technology.

B. **DESIGN GOALS**
(Taken directly from the Phase I Contract)

1. **General:**
   a. Fully automated, user-friendly design.
   b. A sterile closed system licensed by FDA for 21-day storage of output blood products.
   c. Capable of washing 1 unit of glycerolized blood in 30 minutes or less.
   d. Capable of washing 1 unit of platelets in 15 minutes or less.

2. **Physical Characteristics:**
   a. Weight of 50 kg (110 pounds) or less.
   b. Tabletop style having footprint of 25 square decimeters (2.7 sq. ft.).
   c. Volume of 250 cubic decimeters (8.8 cu. ft.) maximum, not to exceed 1 meter (3.3 ft.) in height.
   d. Ruggedized for MIL STD 810 Environmental Tests.
   e. Multiple power inputs 100, 220, volts A/C, 50-60 Hz. Commercial or tactical electrical power.
   f. Disposables which are 450 grams (1 lb.), 4 cubic liters (244 cu. in.) and cost $30 or less.

Table 2

THAWED BLOOD PROCESSING SYSTEM (TBPS)
PERFORMANCE REQUIREMENTS (Summarized)
(From Naval Command, Control & Ocean Surveillance Center RDT&D Div.,
Marine Environment Branch, San Diego, California).

1. Fully automated system requiring less than 5 minutes of operator involvement per unit of thawed red blood cells. This time includes mounting disposables onto the system, initiating the wash process, entering identification data, sealing the bags of washed cells and removing the disposables from the system.
2. Total processing time for each unit of blood will be as short as possible; not to exceed 30 minutes including operator involvement time.
3. A single operator will be able to operate up to 6 systems at one time.
4. The TBPS will be a closed sterile system. Sterile docking techniques will be used to connect the thawed blood bag and wash solution bags to the disposable set. A 0.22 micron bacterial filter may be used to ensure sterility of wash solutions and long-term storage solutions instead of using sterile docking for these connections, if acceptable to the FDA.
5. The TBPS includes an off-the-shelf sterile docking device as an integral component.
6. The initial FDA license request should be for a minimum of 5-day life for the washed red cells. A 21-day shelf-life is the ultimate goal of this development; FDA licensing and approval is essential.
7. Capability of washing one and/or two units of thawed cells with one disposable filter cartridge. When two units are washed with one filter, the units must be identified as sister units and are physically attached to each other to ensure that both units are used for one patient.
8. The goal is to use no more than one liter of wash solution per unit of red blood cells.
9. Provide the capability to process thawed frozen platelets.
10. A nutritive additive or storage solution is added to the cells to achieve prolonged shelf-life. The additive could be metered by the system, already contained in the sterile product bag or contained in a small bag attached to the product bag and forced into the bag once the cells are washed.
11. A bag sealer capability is not required as part of the TBPS but is assumed available.
12. Recovery and survivability of washed cells will, at a minimum, meet the requirements as stated in FM8-70, the Standards for Blood Banks and Transfusion Services of the American Association of Blood Banks.
13. Acceptable range of final hematocrit before adding nutritive additions is 70-80%. Acceptable range of final hematocrit after adding additives is 60-70%.
14. 85% or more of the RBCs should be recovered in the deglycerolization process.
15. Tubing should be color-coded the same as the Haemonetics system for reduced potential for operator error.
16. TBPS must monitor and control the quality of the cell washing process. It is desired that the system measure and provide to the user on a printout the hemolysis, hematocrit, and residual glycerol for each unit washed.
17. If the processing is interrupted, the system must provide the operator with both visual and auditory alerts.
18. For any unit of cells not successfully washed, the system will provide a printout detailing, to the degree possible, the reason for the failure of the wash process.
19. For every unit of cells processed, the TBPS must provide a printout containing the following information:

- unit number of the thawed frozen red cell unit
- unit number of the washed RBC unit
- unit number of the sister unit
- lot number of the 12% saline
- lot number of the 0.9% saline
- name and lot number of additive used for shelf-life extension
- filter number (bowl lot number equivalent)
- serial number of TBPS unit
- expiration date of deglycerized blood
- start and stop time of wash process
- quality control values (hemolysis, hematocrit, and glycerol)

Note: Required fields may change depending upon product processing (e.g., RBCs, platelets).

If some of the numbers do not change from deglycing one unit to another, then it is preferred to have the choice to keep the same number without having to re-enter it again (e.g., the saline lot numbers).

To allow for the entry, display and printing of this information, TBPS must have either a bar code reader or a keyboard, display and printer. The bar code reader must be capable of reading CODABAR and Code 128 formats.

All number strings entered by the operator will contain a two-character checksum to help prevent invalid numbers from being entered. TBPS must be able to parse the operator entries to ensure validity.

20. TBPS footprint is to be as small as possible, not to exceed 2 square feet.

21. TBPS must operate from 110 or 220 volts AC, +/- 15%, single phase, 50/60 Hz, commercial or tactical power sources. System may shut down or go into a standby condition but not be damaged when operating outside these limits. Frequent power problems aboard ship are transient overvoltages of about 150%-250% of the AC supply voltage. Line spike voltages could be up to 100 microseconds duration. Momentary loss of power for about 50 microseconds could be encountered.

22. Volatile memory should be retained during power interruptions.

23. TBPS is to be a tabletop style system with a height of 1 meter (3.3 ft.) or less, a volume of 250 cu. decimeters (8.8 cu. ft.) or less, and weigh no more than 50 kg (110 lb.).

24. TBPS must operate without degradation in temperatures from 10 to 37 degrees C. The system will survive storage without degradation in temperatures from -40 to 70 degrees C.

25. TBPS must be capable of operation in environmental humidity conforming to the full range of requirements for data processing spaces of the American Society of Heating and Air Conditioning Engineers (ASHRAE) Handbook.

26. TBPS will be designed to FCC Class A regulations, at a minimum, to reduce potential electromagnetic interference (EMI) problems. The addition of EMI filters and shielding may be necessary to meet the requirements of shipboard application.

27. TBPS must be in accordance with the Type I requirements of MIL-STD-167-1 for shipboard vibration.

28. TBPS must have a Mean Time to Repair (MTTR) at the unit level of 1 hour or less. MTTR at Direct/General Support will not exceed 3 hours.

29. TBPS must have a Mean Time between Operational Mission Failures of at least 320 hours.

automated deglycerolization; and a discussion of the potential for such an automated system to wash red cells after long-term storage, up to six months without freezing, and up to three months after freezing and deglycerolization.

These discussions helped to define the system specifications and confirmed the technical approach being taken by RAI.

6.3   Analytical Modeling of System Performance

6.3.1   Objectives

Analytical modeling is useful in developing, selecting, and optimizing alternative glycerol removal processes. It is especially important in the RAI concepts because all fluid flows (blood, waste, and saline) in the hollow fiber separator are fixed by pumps. This means that the analytical models can accurately predict the performance of the system for any deglycerolization process, except for the effect of glycerol diffusion out of the red cells.

The analytical modeling of the system operation has emphasized the more efficient "two-bag" and the mixed and unmixed blood "one-bag" system concepts. The objectives of this modeling are to: achieve 1% or less glycerol concentration in washed outlet blood; achieve 40% hematocrit outlet blood following the SOP, or 75% hematocrit before storage solution addition for long-term storage; minimize 0.9% saline consumption to that used in the SOP (1500 ml) or less; and perform the deglycerolization (after saline dilutions) in under 20 minutes.

6.3.2   Blood States

The composition of the blood (red cells mixed with various solutions) varies during the deglycerolization process. The various compositions at each step of the process are called "states" and are defined below.

Thawed Blood Composition: State #1

A total of 450 ml of blood is collected from the donor into the blood bag. Assuming a donor hematocrit of 40%, then the volume of RBCs collected (V_{RBC}) is: $V_{RBC} = 0.4 (450) = 180$ ml. The blood (mostly red blood cells) before glycerolization has a hematocrit of 75% ±5%, with a total volume of 240 ml and a fluid volume of 60 ml.

A total of 450 ml of glycerol is added to this blood, increasing total volume to 690 ml and decreasing the hematocrit to 26%. This mixture is spun down in a centrifuge, ending up with a hematocrit of 60% ± 5% and a total volume of 300 ml. This is the mixture that is frozen and then thawed.

Assume conservatively that the glycerol concentration is the same within the RBCs as it is in the solution. Then the mixture after centrifugation has a glycerol content of: 450 ml glycerol added (300 ml final total volume/690 ml initial total volume) = 196 ml of final glycerol in the thawed blood. Then this is the quantity of glycerol that must be removed, to below a final concentration of less than 1% by volume.

Summarizing, the thawed blood composition (State #1) is:

V_{RBCs} =   180 ml
V_1 =   300 ml total volume
H_1 =   60% hematocrit
V_{G1} =   196 ml glycerol volume
V_{G1}/V_1 =   0.65, the glycerol concentration
V_{F1} =   120 ml fluid volume

Saline-Diluted Blood Composition: State #2a

Three saline dilutions are made to the thawed blood.

First dilution: 50 ml of 12% sodium chloride solution

Second dilution: 100 ml of 0.9% sodium chloride, 0.2% glucose solution

Third dilution: 150 ml of the same 0.9% saline solution

The total fluid addition is 300 ml. Then the total volume in the blood bag becomes 600 ml with a hematocrit of 30%.

Summarizing, the diluted blood composition (State #2a) is:

V_{RBCs} = 180 ml

V_2 = 600 ml total volume

H_2 = 30% hematocrit

V_{G2} = V_{G1} = 196 ml glycerol volume

V_{F2} = 420 ml fluid volume

V_{G2}/V_{F2} = 0.467, the glycerol concentration, assuming conservatively that all glycerol is now in the fluid outside the cells and none is in the cells.

V_{F2} = 120 ml fluid volume

Saline-Diluted Blood Composition: State #2b

The third saline dilution may be increased to decrease the final State 2 hematocrit as an aid in glycerol removal.

Pre-Separation Blood Composition: State #3

The blood may be concentrated to a hematocrit above 30%. This concentration process removes waste and may add saline to the blood after the waste removal. Waste removal will decrease glycerol concentration. This hematocrit, H_3, is selected to optimize the plasma separation process. This optimization results in the shortest processing time consistent with the smallest and least expensive hollow fiber separator.

Post-Separation Blood Composition: State #4

The separation process uses a single hollow fiber separator. The blood is passed (recirculated) through this separator several times. Blood is concentrated by fluid removal in the separator to a higher hematocrit H_4 at State #4. After each pass the blood is diluted with the 0.9% saline solution to the same hematocrit H_3 of State #3.

Concentrated Blood Composition: State #5

The blood after glycerol removal in the plasma separator is concentrated to a hematocrit H_5 higher than H_4 in a final pass through the hollow fiber separator. This step is in preparation for blood storage in a blood bag. The hematocrit H_5 is 70% to 80% in preparation for dilution with a solution intended for long-term (about three week) refrigerated storage.

Composition of Blood for Long-Term Storage: State #6

The concentrated blood of State #5 is mixed with a solution intended to achieve long-term refrigerated (1 to 6°C) storage of blood. This results in a hematocrit H_6 of about 60% - 70% for sterile blood in a blood storage bag.

6.3.3   The Dual Bag Glycerol Removal Process

Process Description

The deglycerolization process described in this section uses two bags: the thawed frozen blood bag, and a recirculation bag that is part of the disposable (preattached) or is the output blood bag. Blood is first pumped out of the thawed frozen blood bag, flows through the separator where waste flow is removed, a saline flow is added and mixes with the blood flow, and then this mixture enters the recirculation bag. This is the first pass of blood through the separator. The second pass pumps blood out of the recirculation bag, through the separator, saline is added, and the mixture flows into the thawed frozen blood bag. The third (or fifth, seventh, etc.) pass is a repeat of the first pass. The fourth (or sixth, eighth, etc.) pass is a repeat of the second pass. The final pass goes into the output blood bag.

Process Analysis

The hollow fiber separator is utilized in 3 to 9 passes of blood through it. The hematocrits entering (H_3) and leaving (H_4) the separator are the same for each pass. Blood entering the separator is mixed with saline to H_3. Saline flow is controlled by a saline pump, and blood flow into the separator is controlled by a blood pump. Waste flow is controlled by a waste pump. Saline flow equals waste flow for each pass. The waste flow rate, blood flow rate, and H_3 determine H_4. The saline flow rate, blood flow rate, and H_4 determine H_3. The end states of each separation pass are defined, and the volumes of fluids for each pass can be calculated.

The characteristics of blood entering the separator (State #3) are:

V_3 = Total blood volume, ml
H_3 = Hematocrit
V_{RBC} = 180 ml
V_{3F} = Volume of fluid, ml
V_{3G} = Volume of glycerol (depends on number of passes blood has already made through the separator), ml

The characteristics of blood exiting the separator (State #4) are:

V_4 = Total blood volume, ml
H_4 = Hematocrit
V_{4F} = Volume of fluid, ml
V_{4G} = Volume of glycerol (depends on number of passes blood has already made through separator), ml

The ratio by volume of glycerol at State #4 to glycerol at State #3 is the same as the ratio of fluids at States #4 and #3 since the glycerol concentration is the same at each state (for the same pass through the separator). Then,

$$\frac{V_{4G}}{V_{3G}} = \frac{V_{4F}}{V_{3F}} = \frac{\dfrac{V_4}{V_{RBC}} - 1}{\dfrac{V_3}{V_{RBC}} - 1} = \frac{\dfrac{1}{H_4} - 1}{\dfrac{1}{H_3} - 1}$$

The ending glycerol volume is:

$$V_{nG} = V_{2G}\left(\frac{V_{4G}}{V_{3G}}\right)^n$$

where:

n = Total number of identical passes. (A final pass to a higher concentrated hematocrit is considered separately).

V_{nG} = Glycerol volume after n passes, ml

The ending concentration of glycerol after n passes is:

$$C_n = \frac{V_{nG}}{V_{4F}}$$

where:

C_n = Glycerol concentration after n passes.

$$V_{4F} = V_2\frac{H_2}{H_4}(1 - H_4) = V_{RBC}\left(\frac{1}{H_4} - 1\right)$$

Then the complete process (not including any final pass producing a final hematocrit different from H_4) is:

$$C_n = \frac{V_{2G}\left[\dfrac{\dfrac{1}{H_4} - 1}{\dfrac{1}{H_3} - 1}\right]^n}{V_{RBC}\left(\dfrac{1}{H_4} - 1\right)}$$

If a final pass resulting in some hematocrit H_5 is used, then:

$$\frac{V_{5G}}{V_{4G}} = \frac{\dfrac{1}{H_5} - 1}{\dfrac{1}{H_4} - 1}$$

and

$$C_{n+1} = \frac{V_{nG}\left(\dfrac{V_{5G}}{V_{4G}}\right)}{V_{RBC}\left(\dfrac{1}{H_5} - 1\right)}$$

If a dilution with a storage solution is then added (State #6), then:

$$C_{n+1} = \frac{V_{nG}\left(\dfrac{V_{5G}}{V_{4G}}\right)}{V_{RBC}\left(\dfrac{1}{H_6} - 1\right)}$$

The final glycerol concentration C_n or C_{n+1} must be less than 1%.

A Specific Deglycerolization Process: $H_3 > H_2$

* **Process Description**
  This process consists of three steps:

  Step #1   The hematocrit of thawed frozen blood is increased from H_2 to H_3.
  No saline is added after waste removal.

  Step #2   The two-bag deglycerolization process is used in a number of
  recirculation passes between States 3 and 4.

  Step #3   Blood is diluted from State 4 to a lower hematocrit at State 5.
  No waste is removed.

* **Process Analysis**

  Step #1   The glycerol volume can be calculated at State 3:

  $$V_{3G} = V_{2G}\left(\frac{\dfrac{1}{H_3} - 1}{\dfrac{1}{H_2} - 1}\right)$$

  No saline is added from State 3 to State 3.

Step #2   The glycerol volume at the final State 4 after n passes is:

$$V_{4Gn} = V_{3G} \left(\frac{\frac{1}{H_4} - 1}{\frac{1}{H_3} - 1} \right)^n$$

The amount of saline added is:

$$V_{S34} = (n - 1) \, V_{RBC} \left(\frac{1}{H_3} - \frac{1}{H_4} \right)$$

Step #3   No glycerol is removed between States 4 and 5. The final fluid volume at State 5 is:

$$V_{5F} = V_{RBC} \left(\frac{1}{H_5} - 1 \right)$$

The saline added between States 4 and 5 is:

$$V_{S45} = V_{RBC} \left(\frac{1}{H_5} - \frac{1}{H_4} \right)$$

*   The Final Glycerol Concentration
    The final glycerol concentration at State 5 is:

$$C_{5G} = \frac{V_{4Gn}}{V_{5F}} = \frac{V_{2G} \left[\frac{\frac{1}{H_3} - 1}{\frac{1}{H_2} - 1} \right] \left[\frac{\frac{1}{H_4} - 1}{\frac{1}{H_3}} \right]^n}{V_{RBC} \left(\frac{1}{H_5} - 1 \right)}$$

It is useful to solve for the value of H_4 when the desired final glycerol concentration is known:

$$H_4 = \cfrac{1}{\left[\cfrac{1}{H_3} - 1\right]\left[\cfrac{C_{5G} V_{RBC}\left(\cfrac{1}{H_5} - 1\right)\left(\cfrac{1}{H_2} - 1\right)}{V_{2G}\left(\cfrac{1}{H_3} - 1\right)}\right]^{1/n} + 1}$$

* **Total Saline Consumed**

All of the 0.9% NaCl solution consumed by the deglycerolization process can be calculated. This does not include the 300 ml added during the initial dilutions, assuming the current SOP is followed.

$$V_{S25} = (n - 1)\, V_{RBC}\left(\frac{1}{H_3} - \frac{1}{H_4}\right) + V_{RBC}\left(\frac{1}{H_5} - \frac{1}{H_4}\right)$$

Consumption of Solutions

* **12% Saline Solution Consumption**

A solution of 12% sodium chloride is used as an initial dilution of the thawed blood. A total of 50 ml is used, as per the current Naval Blood Research Laboratory deglycerinization SOP.

* **0.9% Saline Solution Consumption**

A solution of 0.9% sodium chloride and 0.2% glucose is used for dilution before and during glycerol removal. A total of 250 ml is used (in two steps) for dilution prior to glycerol removal, as per the current SOP. The blood may be further diluted to a hematocrit H_3.

$$V_{s23} = V_3 - V_2 - V_2\left(\frac{H_2}{H_3} - 1\right)$$

where: V_{s23} = The 0.9% saline solution added between States #2 and #3.

The 0.9% saline solution consumed in one pass through the hollow fiber separator is:

$$V_{s34} = V_3 - V_4$$

$$V_3 H_3 = V_4 H_4$$

$$V_{s34} = V_3\left(1 - \frac{H_3}{H_4}\right) = V_{RBC}\left(\frac{1}{H_3} - \frac{1}{H_4}\right)$$

where: V_{s34} is the 0.9% saline solution added between States #3 and #4, ml. Then, for n passes the saline added is (n-1) times this value:

$$V_{sn} = V_{RBC} \left(\frac{1}{H_3} - \frac{1}{H_4} \right) (n-1)$$

where: V_{sn} = The saline solution added for n passes of blood between States #3 and #4, ml.

* ### The Quantity of Waste Removed
The amount of fluid waste removed by the waste pump to the waste bag at each pass through the separator is identical to the amount of saline added each pass:

$$V_{w34} = V_3 \left(1 - \frac{H_3}{H_4} \right)$$

$$V_{wn} = nV_3 \left(1 - \frac{H_3}{H_4} \right)$$

where: V_{w34} = Volume of waste removed in each separator pass from State #3 to State #4, ml.
V_{wn} = Total volume of waste removed for n identical passes, ml.

Additional waste is removed if the blood is concentrated from State #4 to State #5:

$$V_{w45} = V_4 \left(1 - \frac{H_4}{H_5} \right)$$

where: V_{w45} = Volume of waste removed between States #4 and #5 in one pass, ml.

6.3.4 The Single Bag Glycerol Removal Process: Well-Mixed Blood Bag

Process Description
Deglycerolization can be performed with blood pumped out of a single bag (the initial blood bag or a separate recirculation blood bag) through the hollow fiber separator and back into this bag. Saline is added to the blood stream exiting the separator. Good mixing of blood and saline is assumed. The waste flow rate removed from the blood at the separator is assumed to be equal to the saline flow rate added. Then the hematocrit of blood in the blood bag does not change. The blood entering the blood bag has a lower glycerol concentration than the blood that has just left the blood bag. It is assumed for this analysis that blood in the blood bag is well mixed and has a uniform glycerol concentration that decreases with time.

Process Analysis for Blood Well-mixed in Blood Bag

* **The Initial State of Blood in the Blood Bag**
  - V_3 = Initial Blood Volume, ml
  - H_3 = Initial Blood Hematocrit
  - V_{3G} = Initial Glycerol Volume, ml
  - V_{3F} = Initial Fluid Volume, ml
  - V_{RBC} = Red Blood Cell Volume, ml

* **The Final State of Blood in the Blood Bag**
  - V_4 = Final Blood Volume, ml
  - H_4 = Final Blood Hematocrit, ml
  - V_{4G} = Final Glycerol Volume, ml
  - V_{4F} = Final Fluid Volume, ml

* **Glycerol Concentration vs. Time in the Blood Bag**
  The change in blood parameters in the blood bag for a small increment in time is:

$$\Delta V_W = -Q_W \Delta t$$

$$\Delta V_G = \frac{V_G}{V_F} \Delta V_W$$

where:

- ΔV_W = Incremental change in waste volume, ml
- Q_W = Waste flow rate, ml/min
- Δt = A small time increment, min.
- ΔV_G = An increment of glycerol removed with the waste, ml
- V_G = Glycerol volume dependent on time, ml
- V_F = Fluid volume, ml

Fluid volume does not change with time since hematocrit is assumed constant with time in the blood bag. Then,

$$\Delta V_G = -\frac{V_G}{V_F} Q_W \Delta t$$

$$\frac{\frac{\Delta V_G}{\Delta t}}{V_G} = -\frac{Q_W}{V_F}$$

Integrating this as a differential equation yields:

$$V_G = V_{3G} \, \epsilon \, \exp\left(-\frac{Q_W}{V_F} t \right)$$

$$V_F = V_3 - V_{RBC}$$

$$V_F = V_{RBC}\left(\frac{1}{H_3} - 1 \right)$$

Then,

$$V_G = V_{3G} \, \epsilon \, \exp\left(\frac{-Q_W t}{V_{RBC}\left(\frac{1}{H_3} - 1 \right)} \right)$$

V_{3G} is fixed, $= 196$ ml
V_{RBC} is fixed, $= 180$ ml

$$C_G = \frac{V_G}{V_{5F}} = \frac{V_G}{V_{3G}} \times \frac{V_{3G}}{V_{RBC}\left(\frac{1}{H_5} \right)}$$

$$C_G = \frac{V_{3G} H_5}{V_{RBC}} \, \epsilon \, \exp\left(\frac{-Q_W t}{V_{RBC}\left(\frac{1}{H_3} - 1 \right)} \right)$$

$V_G = V_{4G}$ when $t = t_4$ with $t_3 = 0$.

6.3.5   The Single Bag Glycerol Removal Process: Unmixed Blood

Process Description
This deglycerolization process is conceptually identical to that of the two-bag process described above. The important difference is that the blood flows into and out of the recirculation blood bag are separated, and this bag is designed to prevent blood mixing in the bag. Then blood entering the bag retains its lower glycerol concentration compared to blood already in the bag. This results in the processing of blood batches of successively lower hematocrits. The analytical model for such processing is then identical to the two-bag processing scheme.

Blood in the recirculation bag is always at the same hematocrit since the waste flow rate equals the saline flow rate. Saline is introduced into and mixes with the blood exiting the separator before entering the recirculation bag. The volume of blood in the recirculation bag does not change. Then each batch of blood having a different glycerol concentration is equal to this blood volume. One batch is processed as one pass in the analysis of Section 6.3.3

* **Process Analysis**

The volume of blood in the recirculation bag and processed in one pass is V_{RBC}/H_3. The volume of waste fluid removed and saline added in one pass is:

$$V_w = V_s = V_3 - V_4 = \frac{V_{RBC}}{H_3} - \frac{V_{RBC}}{H_4} = V_{RBC} \left(\frac{1}{H_3} - \frac{1}{H_4} \right)$$

The analysis for glycerol concentration and total waste flow then follows that of Section 6.3.3.

6.3.6 Results of Analyses

The results of these analyses are summarized in the accompanying tables. Table 3 shows the outlet hematocrit versus inlet hematocrit for a range of passes (recirculation) through the hollow fiber separator for the single unmixed bag approach. The analysis is performed for final glycerol concentrations equal to 1% (with a final hematocrit of 40%). Then the fewest number of passes can be identified for separator outlet hematocrits just under 55%, where separator transmembrane pressures and hemolysis tend to increase.

Table 4 shows total waste fluid volume versus inlet hematocrit and the number of passes for the same operating conditions. Waste fluid and saline consumption are acceptable (saline below 1250 ml consumption) for separator inlet hematocrits (H_3) above 30%. Then the optimal conditions range from: $H_1 = 30\%$, $H_4 = 50\%$, $n = 5$, $nV_w = 1209$ ml; to $H_1 = 40\%$, $H_2 = 55\%$, $n = 7$, $nV_w = 865$ ml. For blood flow rates of 300 to 400 ml/min (feasible with 0.4 to 0.8 m^2 separators) the total deglycerolization times, not including initial dilutions, are calculated to be 8 to 13 minutes. This also does not include set-up time or delays due to glycerol diffusion out of the RBCs. Total saline consumption, not including initial dilutions, is calculated to be 864 to 1050 ml.

Table 5 calculates the glycerol removal efficiency and processing time as functions of the blood flow rate and number of passes.

The single mixed bag process analysis is summarized in Table 6. Total blood volume recirculated is given versus separator inlet and outlet hematocrits for a final glycerol concentration of 1%. Here a practical operating condition is $H_3 = 40\%$ and $H_4 = 56\%$. The total recirculated blood volume pumped is calculated to be 4049 ml. For a blood flow rate of 300 ml/min, the deglycerolization time (not counting initial dilutions) is 14 min. This compares to 8 min for the unmixed bag concept for the same conditions, or 75% longer.

6.4 System Design and Development

6.4.1 System Design Overview

The TBPS system design was performed in order to define system characteristics and determine how to meet all system requirements. The technical approach described above was implemented in conceptual and engineering designs for the two basic modules of this system, the Disposable Module and the Control Module.

Table 3 H_4 vs. H_3 and n

H_3 = Inlet Hematocrit H_4 = Outlet Hematocrit

H3	N=NUMBER OF PASSES											
	1	2	3	4	5	6	7	8	9	10	11	12
0.20	0.948	0.681	0.511	0.422	0.371	0.338	0.316	0.299	0.287	0.277	0.270	0.263
0.22	0.953	0.706	0.541	0.452	0.399	0.366	0.342	0.325	0.312	0.302	0.294	0.287
0.24	0.958	0.729	0.569	0.480	0.427	0.392	0.368	0.350	0.337	0.326	0.318	0.311
0.26	0.962	0.750	0.594	0.506	0.453	0.418	0.393	0.375	0.361	0.350	0.342	0.334
0.28	0.966	0.768	0.619	0.532	0.478	0.443	0.418	0.399	0.385	0.374	0.365	0.357
0.30	0.969	0.785	0.641	0.556	0.502	0.467	0.441	0.423	0.408	0.397	0.388	0.380
0.32	0.972	0.800	0.663	0.579	0.526	0.490	0.465	0.446	0.431	0.419	0.410	0.402
0.34	0.974	0.814	0.682	0.601	0.548	0.513	0.487	0.468	0.453	0.442	0.432	0.424
0.36	0.976	0.827	0.701	0.622	0.570	0.535	0.509	0.490	0.475	0.463	0.454	0.446
0.38	0.978	0.839	0.719	0.641	0.591	0.556	0.531	0.512	0.497	0.485	0.475	0.467
0.40	0.980	0.850	0.736	0.661	0.611	0.577	0.551	0.533	0.518	0.506	0.496	0.488

Table 4 nV_w vs. H_3 and n

H_3 = Inlet Hematocrit nV_w = Total Waste Fluid Volume

H3	N=NUMBER OF PASSES											
	1	2	3	4	5	6	7	8	9	10	11	12
0.20	710	1271	1642	1893	2072	2205	2307	2389	2455	2509	2555	2595
0.22	629	1127	1456	1678	1837	1954	2045	2117	2176	2224	2265	2300
0.24	562	1006	1300	1499	1640	1746	1827	1891	1943	1987	2023	2054
0.26	505	904	1169	1347	1474	1569	1642	1700	1747	1786	1818	1846
0.28	456	817	1056	1217	1332	1418	1483	1536	1578	1613	1643	1668
0.30	414	741	958	1105	1209	1286	1346	1393	1432	1464	1491	1514
0.32	377	675	872	1006	1101	1171	1226	1269	1304	1333	1358	1378
0.34	345	617	797	919	1006	1070	1120	1159	1191	1218	1240	1259
0.36	316	565	730	842	921	980	1026	1062	1091	1115	1136	1153
0.38	290	518	670	772	845	899	941	974	1001	1024	1042	1058
0.40	266	477	616	710	777	827	865	896	921	941	958	973

Table 5A
GLYCEROL REMOVAL BY THE SINGLE UNMIXED BAG METHOD

PASS #	BLOOD PUMP RATE (ml/min)	Hct INLET %	INLET VOLUME (ml)	WASTE PUMP RATE (ml/min)	Hct BEFORE SALINE ADD %	OUTLET VOLUME BEFORE SALINE ADD (ml)	SALINE PUMP RATE (ml/min)	SALINE USED (ml)	Hct OUTLET %	OUTLET VOLUME (ml)	% REMOVAL EFFICIENCY	PROCESS TIME (min)
1	250	30%	600	68	41%	437	0	0	41%	437	61.1429%	2.40
2	250	41%	437	68	57%	318	68	119	41%	437	67.1451%	4.15
3	250	41%	437	68	57%	318	68	238	41%	437	82.3456%	5.89
4	250	41%	437	68	57%	318	68	356	41%	437	90.5135%	7.64
5	250	41%	437	68	57%	318	68	475	41%	437	94.9025%	9.39
6	250	41%	437	68	57%	318	68	594	41%	437	97.2609%	11.14
7	250	41%	437	68	57%	318	68	713	41%	437	98.5281%	12.88
8	250	41%	437	68	57%	318	68	832	41%	437	99.2091%	14.63
9	250	41%	437	68	57%	318	68	950	41%	437	99.5750%	16.38
10	250	41%	437	68	57%	318	68	1069	41%	437	99.7716%	18.12
11	250	41%	437	68	57%	318	68	1188	41%	437	99.8773%	19.87
12	250	41%	437	68	57%	318	68	1307	41%	437	99.9341%	21.62
13	250	41%	437	68	57%	318	68	1426	41%	437	99.9646%	23.37
14	250	41%	437	68	57%	318	68	1545	41%	437	99.9810%	25.11
15	250	41%	437	68	57%	318	68	1663	41%	437	99.9898%	26.86
16	250	41%	437	68	57%	318	68	1782	41%	437	99.9964%	28.61
17	250	41%	437	68	57%	318	68	1901	41%	437	99.9987%	30.36
18	250	41%	437	63	57%	318	68	2020	41%	437	99.9996%	32.10
19	250	41%	437	68	57%	318	68	2139	41%	437	99.9998%	33.85
20	250	41%	437	68	57%	318	68	2257	41%	437	99.9999%	35.60

Table 5B
GLYCEROL REMOVAL BY THE SINGLE UNMIXED BAG METHOD

PASS #	BLOOD PUMP RATE (ml/min)	Hct INLET %	INLET VOLUME (ml)	WASTE PUMP RATE (ml/min)	Hct BEFORE SALINE ADD %	OUTLET VOLUME BEFORE SALINE ADD (ml)	SALINE PUMP RATE (ml/min)	SALINE USED (ml)	Hct OUTLET %	OUTLET VOLUME (ml)	% REMOVAL EFFICIENCY	PROCESS TIME (min)
1	300	30%	600	68	39%	464	0	0	39%	464	67.6190%	2.00
2	300	39%	464	68	50%	359	68	105	39%	464	57.4222%	3.55
3	300	39%	464	68	50%	359	68	210	39%	464	73.1900%	5.09
4	300	39%	464	68	50%	359	68	316	39%	464	83.1185%	6.64
5	300	39%	464	68	50%	359	68	421	39%	464	89.3702%	8.19
6	300	39%	464	68	50%	359	68	526	39%	464	93.3067%	9.73
7	300	39%	464	68	50%	359	68	631	39%	464	95.7854%	11.28
8	300	39%	464	68	50%	359	68	736	39%	464	97.3462%	12.83
9	300	39%	464	68	50%	359	68	841	39%	464	98.3290%	14.37
10	300	39%	464	68	50%	359	68	947	39%	464	98.9478%	15.92
11	300	39%	464	68	50%	359	68	1052	39%	464	99.3375%	17.47
12	300	39%	464	68	50%	359	68	1157	39%	464	99.5828%	19.01
13	300	39%	464	68	50%	359	68	1262	39%	464	99.7373%	20.56
14	300	39%	464	68	50%	359	68	1367	39%	464	99.8346%	22.11
15	300	39%	464	68	50%	359	68	1472	39%	464	99.8958%	23.65
16	300	39%	464	68	50%	359	68	1578	39%	464	99.9589%	25.20
17	300	39%	464	68	50%	359	68	1683	39%	464	99.9838%	26.75
18	300	39%	464	68	50%	359	68	1788	39%	464	99.9936%	28.29
19	300	39%	464	68	50%	359	68	1893	39%	464	99.9975%	29.84
20	300	39%	464	68	50%	359	68	1998	39%	464	99.9990%	31.39

Table 5C
GLYCEROL REMOVAL BY THE SINGLE UNMIXED BAG METHOD

PASS #	BLOOD PUMP RATE (ml/min)	Hct INLET %	INLET VOLUME (ml)	WASTE PUMP RATE (ml/min)	Hct BEFORE SALINE ADD %	OUTLET VOLUME BEFORE SALINE ADD (ml)	SALINE PUMP RATE (ml/min)	SALINE USED (ml)	Hct OUTLET %	OUTLET VOLUME (ml)	REMOVAL EFFICIENCY %	PROCESS TIME (min)
1	350	30%	600	95	41%	437	0	0	41%	437	61.2245%	1.71
2	350	41%	437	95	57%	318	95	119	41%	437	67.0262%	2.96
3	350	41%	437	95	57%	318	95	237	41%	437	82.2413%	4.21
4	350	41%	437	95	57%	318	95	356	41%	437	90.4357%	5.46
5	350	41%	437	95	57%	318	95	475	41%	437	94.8489%	6.71
6	350	41%	437	95	57%	318	95	593	41%	437	97.2258%	7.96
7	350	41%	437	95	57%	318	95	712	41%	437	98.5059%	9.21
8	350	41%	437	95	57%	318	95	831	41%	437	99.1953%	10.46
9	350	41%	437	95	57%	318	95	949	41%	437	99.5666%	11.71
10	350	41%	437	95	57%	318	95	1068	41%	437	99.7666%	12.96
11	350	41%	437	95	57%	318	95	1187	41%	437	99.8743%	14.20
12	350	41%	437	95	57%	318	95	1305	41%	437	99.9323%	15.45
13	350	41%	437	95	57%	318	95	1424	41%	437	99.9635%	16.70
14	350	41%	437	95	57%	318	95	1542	41%	437	99.9804%	17.95
15	350	41%	437	95	57%	318	95	1661	41%	437	99.9894%	19.20
16	350	41%	437	95	57%	318	95	1780	41%	437	99.9963%	20.45
17	350	41%	437	95	57%	318	95	1898	41%	437	99.9987%	21.70
18	350	41%	437	95	57%	318	95	2017	41%	437	99.9995%	22.95
19	350	41%	437	95	57%	318	95	2136	41%	437	99.9998%	24.20
20	350	41%	437	95	57%	318	95	2254	41%	437	99.9999%	25.44

Table 5D

GLYCEROL REMOVAL BY THE SINGLE UNMIXED BAG METHOD

PASS #	BLOOD PUMP RATE (ml/min)	Hct INLET %	INLET VOLUME (ml)	WASTE PUMP RATE (ml/min)	Hct BEFORE SALINE ADD %	OUTLET VOLUME BEFORE SALINE ADD (ml)	SALINE PUMP RATE (ml/min)	SALINE USED (ml)	Hct OUTLET %	OUTLET VOLUME (ml)	% REMOVAL EFFICIENCY	PROCESS TIME (min)
1	400	30%	600	110	41%	435	0	0	41%	435	60.7143%	1.50
2	400	41%	435	110	57%	315	110	120	41%	435	67.7679%	2.59
3	400	41%	435	110	57%	315	110	239	41%	435	82.8885%	3.68
4	400	41%	435	110	57%	315	110	359	41%	435	90.9158%	4.76
5	400	41%	435	110	57%	315	110	479	41%	435	95.1774%	5.85
6	400	41%	435	110	57%	315	110	598	41%	435	97.4398%	6.94
7	400	41%	435	110	57%	315	110	718	41%	435	98.6408%	8.03
8	400	41%	435	110	57%	315	110	837	41%	435	99.2784%	9.11
9	400	41%	435	110	57%	315	110	957	41%	435	99.6169%	10.20
10	400	41%	435	110	57%	315	110	1077	41%	435	99.7966%	11.29
11	400	41%	435	110	57%	315	110	1196	41%	435	99.8920%	12.38
12	400	41%	435	110	57%	315	110	1316	41%	435	99.9427%	13.46
13	400	41%	435	110	57%	315	110	1436	41%	435	99.9696%	14.55
14	400	41%	435	110	57%	315	110	1555	41%	435	99.9838%	15.64
15	400	41%	435	110	57%	315	110	1675	41%	435	99.9914%	16.73
16	400	41%	435	110	57%	315	110	1794	41%	435	99.9970%	17.81
17	400	41%	435	110	57%	315	110	1914	41%	435	99.9990%	18.90
18	400	41%	435	110	57%	315	110	2034	41%	435	99.9996%	19.99
19	400	41%	435	110	57%	315	110	2153	41%	435	99.9999%	21.08
20	400	41%	435	110	57%	315	110	2273	41%	435	100.0000%	22.16

Table 6

**TOTAL RECIRCULATED BLOOD VOLUME FOR A RESIDUAL
GLYCEROL LEVEL OF 1%. SINGLE MIXED BAG METHOD.**

H3 \ H4	0.4	0.42	0.44	0.46	0.48	0.5	0.52	0.54	0.56	0.58	0.6
0.2	6170	5890	5656	5458	5289	5142	5013	4900	4799	4709	4628
0.22	6077	5743	5469	5241	5048	4883	4740	4615	4504	4406	4318
0.24	6106	5699	5373	5107	4885	4697	4536	4396	4274	4167	4071
0.26	6272	5762	5366	5049	4790	4573	4390	4234	4098	3979	3874
0.28	6611	5950	5454	5069	4760	4508	4297	4119	3967	3834	3719
0.3	7199	6299	5656	5174	4799	4499	4254	4049	3876	3728	3599
0.32	8195	6884	6010	5385	4917	4553	4261	4023	3824	3656	3512
0.34	9981	7860	6588	5739	5133	4679	4325	4042	3811	3618	3455
0.36	13712	9598	7542	6307	5485	4897	4456	4114	3839	36.5	3428
0.38	25169	13214	9228	7236	6040	5243	4674	4247	3915	3649	3432
0.4		24296	12726	8870	6942	5785	5013	4462	4049	3728	3471
0.42			23433	12249	8521	6657	5539	4793	4260	3861	3550
0.44				22578	11780	8180	6381	5301	4581	4067	3681
0.46					21730	11318	7847	6 2	5070	4376	3880
0.48						20889	10862	7520	5849	4846	4178
0.5							20054	10412	7199	5592	4628

The disposable module mechanical design was performed using the cartridge approach for achieving a small, disposable size, low-cost, ease-of-use, and function advantages. Key standard components were preliminarily selected and non-standard components were designed.

The control module conceptual and overall engineering and mechanical designs were completed. The electromechanical control system functions were defined, key electronic, electromechanical and mechanical hardware components identified, standard components were preliminarily selected, the user interface was preliminarily designed, and the enclosure configuration and construction were conceptually and preliminarily defined and designed. The electronics design approach was defined and is very similar to that used with other microprocessor-based software controlled systems, including current autotransfusion and apheresis equipment and the hollow fiber separator autotransfusion system developed by RAI.

Key software control functions and algorithms were defined. This design effort was sufficient to permit a determination of disposable functions, characteristics, sizes, and costs, and control system functions, size, weight, approximate costs, and other characteristics along with the development needs for the entire TBPS.

6.4.2 Overall System Design and Operating Characteristics

A preliminary design of the complete TBPS was performed in order to address all of the technical, functional, user-related, and design issues and indicate how the TBPS objectives, requirements, and specifications established by the military would be met. Tables 1 and 2 give the initial and recently-established requirements for the TBPS product. The functional design of the system incorporates elements that permit all TBPS objectives, requirements, and specifications to be met.

The disposable module shown in Fig. 1 consists of all fluid-handling components except the frozen blood bag, the saline solutions, and the storage solution used for extended storage of blood after deglycerolization. This system is designed to deglycerolize blood according to the NBRL S.O.P. It is also designed to provide a closed system producing a sterile blood product in a storage or additive solution providing up to 21 days of refrigerated storage before use of the deglycerolized blood. It is also designed, with a modified disposable, similarly to wash thawed frozen platelets in an automatic process using the same control module but a different control software logic, designed for the platelet washing process.

The frozen blood bag connection is attached by sterile docking to the disposable module. The blood output bag and waste bag are pre-attached and the disposable module is packaged and sterilized as a compact assembly.

The saline pump is used to add the saline solutions at the required S.O.P. flow rates and with the required equilibration times to the frozen blood bag. Solenoid operated finger-type pinch valves (on the control module) are used to pinch closed or open the tubing selectively to turn fluid flows on or off. These pinch valves are computer controlled. Identical pinch valves are used successfully and reliably on many autotransfusion, apheresis, and hemodialysis systems. Pinch valves V_1 or V_2 are opened or closed to select the appropriate saline solution for the initial dilutions, and the saline pump pumps the saline into the blood bag.

In the subsequent deglycerolization process the saline pump adds saline to blood as it exits the hollow fiber separator during blood recirculation. The saline pump also is used to prime the system with saline (fill all blood lines with saline and eliminate air) during the pre-dilution stabilization period. The saline pump is used at the end of deglycerolization to purge blood out of the separator and lines by pumping saline through these devices and pushing blood ahead of the saline into the output blood bag.
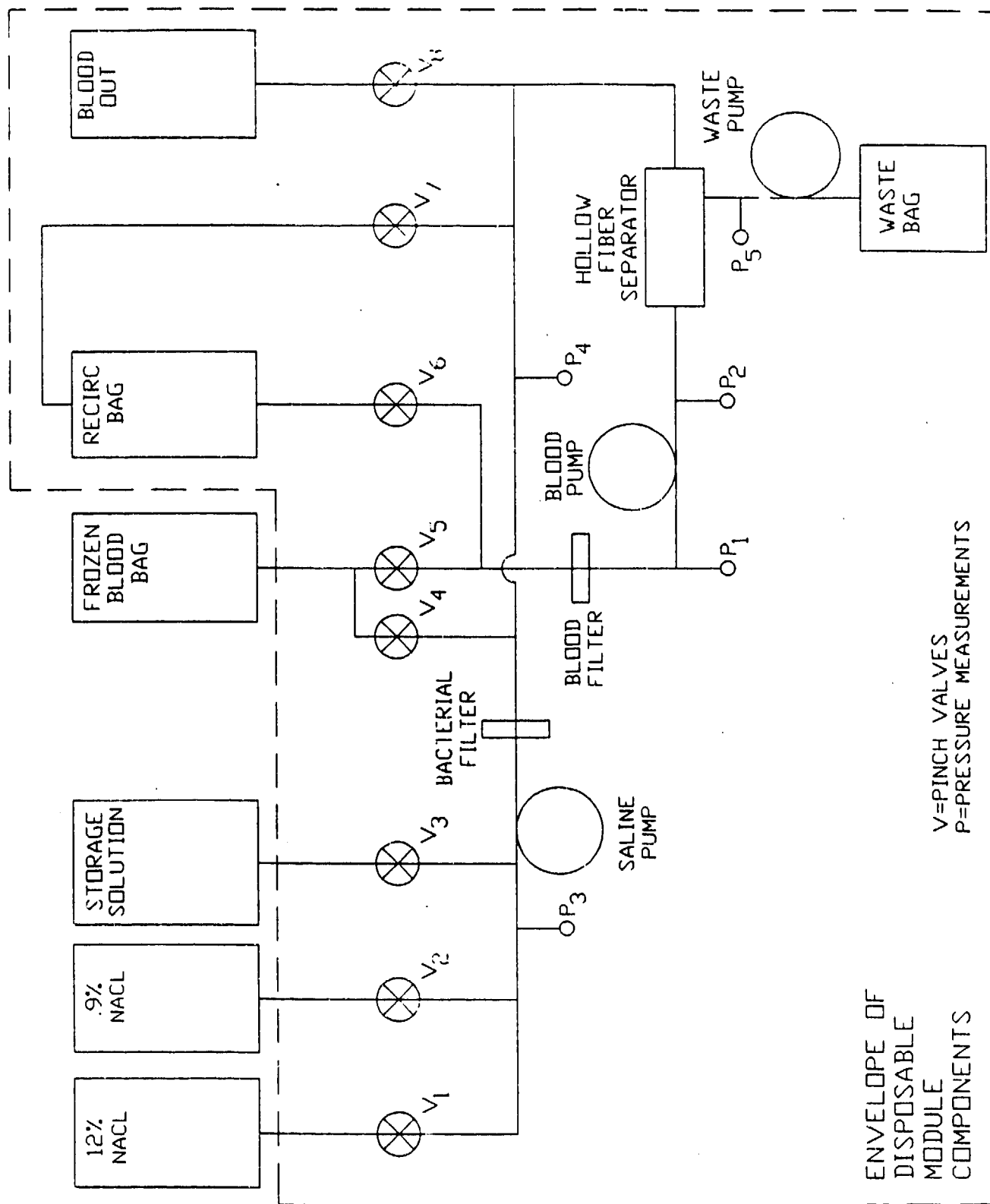
Fig. 1 SCHEMATIC DIAGRAM OF THE DISPOSABLE MODULE

The blood pump is used to pump blood out of the frozen blood bag or out of the recirculation bag, and into either the recirculation bag or the output blood bag. Blood pump timing and flow rates are computer controlled. A blood filter is used to eliminate particulates, clots, and white cell agglomerates that form during the deglycerolization process.

The waste pump controls the waste flow into the waste bag, according to programmed control logic for the deglycerolization process.

The blood pump, saline pump, and waste pump flow rates are controlled to achieve rapid, optimal deglycerolization by recirculation of blood through the separator with waste removal followed by saline addition. At the end of the deglycerolization process the blood is concentrated to higher hematocrits (70% to 80%) by appropriate decreases in saline, blood, and waste flow rates during blood recirculation through the separator. This concentrated blood is then pumped into the output blood bag by the blood pump. The saline pump is used to pump a predetermined amount of storage or additive solution into the concentrated blood as it flows into the output blood bag, to obtain a blood-additive mixture with a hematocrit of 60% to 70%.

Pressure measurements are made by small tubing lines that connect to pressure transducers in the control module. Microporous hydrophobic bacterial filters are used to isolate the transducer from blood and to maintain blood sterility. This is a standard method for measuring pressures in hemodialysis, apheresis, and other blood-handling equipment. If this approach is not acceptable to the FDA, then standard diaphragm pressure isolators with pneumatic pressure measurement will be used to obtain an impervious barrier and ensure sterility. The pressure measurements are made for the following reasons: To determine and terminate pump flow when the frozen blood bag, saline bags, or recirculation bags are empty (by the rate of decrease in pressure level); to control the limits of hollow fiber separator operation (measuring inlet pressure P_2 and transmembrane pressure $P_2 - P_5$); measuring pressure levels to assure proper, safe system operation within anticipated pressure ranges; and informing the user when all bags are full or empty (by pressure increases or decreases).

The pressure measurements, the mode selection by the user, and the control logic for that mode provide inputs to the computer (microprocessor) controller. The outputs controlled by the computer are the timing (ON/OFF) and speeds (fluid flow rates) of the roller pumps and the timing (ON/OFF) of the tubing pinch valves.

6.4.3 Disposable Module and Cartridge Design

The disposable module consists of: interconnected tubing providing the desired pathways for the various solutions, blood, and waste fluid; a blood filter; a bacterial (0.22 micron) solutions filter; a hollow fiber separator; pre-attached waste bag, output blood bag(s), recirculation bag; and pressure lines or isolators.

A major objective of this system is to provide for a simple, rapid set-up and automatically perform the predilutions and deglycerolization, freeing the user to perform additional set-ups or other tasks (see Tables 1 and 2). The technical approach to satisfy this objective, as well as the cost and performance requirements, is to utilize a cartridge approach to combine all of these components in a convenient and very small assembly. This preliminary cartridge design is shown quasi-schematically in Fig 2. The cartridge housing is a partially open plastic structure that supports or attaches to all of the disposable components. The interconnected tubing is precisely located in and by this housing in order to position the appropriate tubing segments within the fingers of the pinch valves and opposite the rollers of the roller pump. This permits the cartridge to quickly and simply plug into the control console with built-in guides for locating the cartridge accurately in the correct position. The attached waste bag, output blood bag(s), and recirculation bag are hung on hooks. The additive and saline bags are hung and attached via spikes. The bacterial filter ensures sterility of these solutions. The blood bag is attached by
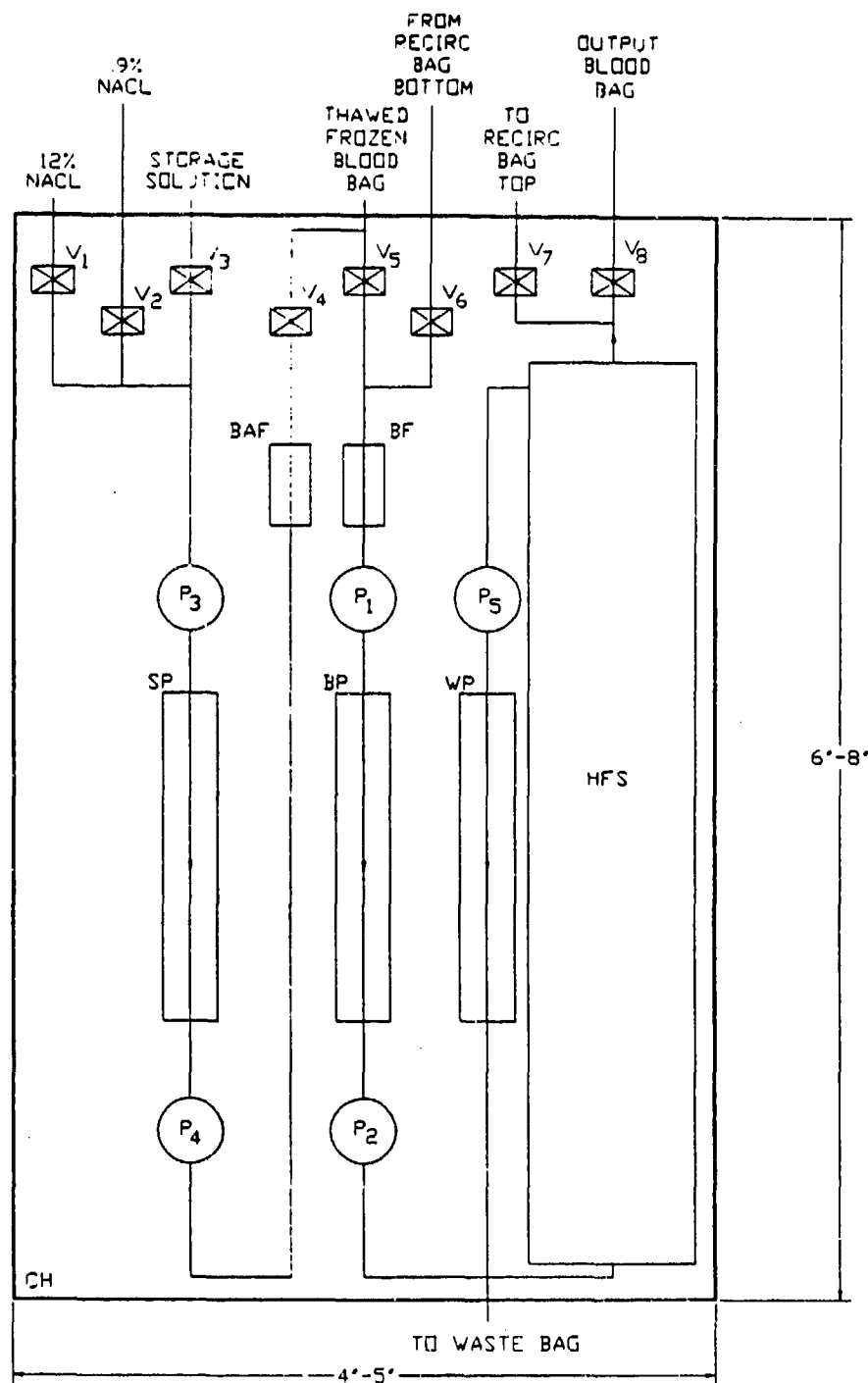
LEGEND:

BF=BLOOD FILTER
BAF=BACTERIAL FILTER
BP=BLOOD PUMP
CH=CARTRIDGE HOUSING
HFS=HOLLOW FIBER SEPARATOR

SP=SALINE PUMP
WP=WASTE PUMP
P1,P2, ETC=PRESSURE SENSORS
V1,V2, ETC=PINCH VALVES

Fig. 2   DISPOSABLE MODULE PLUG-IN CARTRIDGE

sterile docking. The entire operator involvement is described in Table 7. The plug-in of this cartridge to the control module automatically makes the pneumatic connections for each of the pressure lines or isolators.

The roller pump mechanism uses standard, proven designs but is modified to permit straight tubing segments to be used. This permits easier and more precise tubing positioning. The roller pump carriage is spring-loaded to ensure tubing occlusion and sufficient roller engagement along the straight tube segment. The roller pump mechanism is described in more detail below. An advantage of the use of this roller pump concept is the ability to stack separate roller pumps closely together in a parallel array, with about a half-inch separation between pump heads. This, in turn, permits the disposable cartridge, as well as the control module, to be quite small. The disposable cartridge is 4 to 5 inches wide and 6 to 8 inches long, depending on final cartridge component sizes and shapes. It is less than 1.5 inches thick.

A manufacturing cost analysis has been performed on this disposable and is given in Table 8. It appears that the target cost to the Army of $30 per disposable can be met, based on a realistic overall profit margin of 30% to 40%.

6.4.4 Control Module Design

The preliminary control module design is an initial attempt to realistically integrate all specific control module components into a compact, easy-to-use system meeting all objectives and requirements. This overall configuration and enclosure design is expected to change as evaluations are made by users and additional information on components and processes becomes available. Fig. 3 illustrates what such a system might look like.

All bags (except the thawed frozen blood bag) are hung at the top of the system. This includes the 12% NaCl, 0.9% NaCl, long-term storage or additive solution, the blood recirculation bag, and one or two output blood bags. The thawed frozen blood bag is mounted on the blood bag shaker or blood-saline mixer and remains there through the predilution steps and the deglycerolization process. This permits the entire process, including predilutions with mixing, to be performed automatically. The disposable cartridge is mounted below the blood bag shaker. A waste bag is hung below the disposable cartridge and below the front edge of the "table" supporting this tabletop system. Fluid lines are attached between the disposable cartridge and the solution bags by spiking these bags. The output blood bag(s) and waste bag are pre-attached by fluid lines to the cartridge. The thawed frozen blood bag is attached by sterile docking to the cartridge line. A sterile docking device is assumed provided in a pull-out drawer at the bottom of the control module. A user interface panel is located next to the disposable cartridge. The components and their preliminary layout is shown in Fig. 4. Additional printer and bar code reader controls and controls to retrieve detailed diagnostic information may be necessary. The minimum number of user controls appears desirable and will be provided. The roller pumps, tubing pinch valves, and pressure line connections are located under the disposable cartridge, with the mechanisms for these components inside the control module enclosure. The microprocessor-based control electronics, transducer electronics, signal conditioning electronics, control, monitoring, and printer electronics, electric motor drive electronics, and an electrical power supply are located within the control module. The basic control system inputs and outputs are shown in Fig. 5.

The control module will include instrumentation for the measurement of hemolysis (free plasma hemoglobin), hematocrit, and residual glycerol in each unit of blood after processing by the TBPS. The use of optical methods for measuring hemolysis and hematocrit appear feasible, but it is yet to be determined whether there are devices available for measuring residual glycerol.

The control module provides audible and visual warnings or alerts to the operator for failures or other conditions. Power source failure, power disconnect, system failures, clamped lines,

Table 7

OPERATOR INVOLVEMENT WITH THE OPERATION OF THE TBPS

1. **ASSUMPTIONS**
   A. Six system are set up fo ruse; probably side-by-side.
   B. Solution bags, disposable cartridges, thawed blood bags, and the refrigerator for processed blood bags are located nearby, perhaps no more than 20 feet away.
   C. Sterile docking is only needed for the thawed blood bag. Solutions are spiked, and one bacterial (0.22 micron) filter is used for all solutions. Output blood bags (one or two) are pre-attached as part of the disposable, as is the waste bag.
   D. Control Module is "ON" and ready to use.

		Estimated Time (min)
2.	**SET-UP TO DEGLYCEROLIZE ONE UNIT OF THAWED BLOOD**	
A.	Obtain disposable cartridge. Remove and discard packaging from sterile cartridge. Insert cartridge onto control module; lock in place. Hang blood output bag(s) and waste bag.	.5
B.	Remove and discard packaging from sterile solutions; hang bags. 12% NaCl solution, 0.9% NaCl solution, long-term storage solution or additive.	.25
C.	Spike solution bags to attach them to disposable cartridge.	.25
D.	Obtain thawed blood bag and hang. Use sterile docking to attach bag to disposable cartridge.	.5
E.	Use portable bar code reader attached by umbilical to Control Module to enter bar-coded information from: each of the three solution bags; the thawed blood bag; the washed blood bag; the disposable cartridge; and the operator.	.5
F.	Press PROCESS button to begin automatic deglycerolization process.	.2
3.	**POST-PROCESSING REMOVAL**	
A.	Expel any air from processed blood bag. May use manual control of pump (a push-to-activate switch) to pump out air.	.25
B.	Seal off processed blood bag and remove (cut off) from disposable cartridge. May be connected to a sister processed blood bag. Attach or fill out any required labels or printouts.	.25
C.	Remove processed blood bag and put in refrigerator (alone or with sister bag, or do not remove until sister bag has received processed blood).	.50
D.	Disconnect and remove all solution bags except those that have sufficient solution remaining to process another unit of blood. Dispose of removed bags.	.25
E.	Remove disposable cartridge along with waste bag and dispose of as required for biological wastes (blood products).	.25
F.	Perform any clean-up and checks of control module in preparation for the set-up for the next unit of blood.	1.0
	TOTAL TIME..	4.2

Table 8

ENGINEERING ESTIMATE OF TPBS DISPOSABLE MODULE COSTS

Assumption: High volume manufacture with production runs of at least 100k units.

Components per Module	Number	Low	High
Membrane separator	1	$4.45	$6.00
Waste bag	1	$0.62	$1.28
Tubing - feet	20	$0.40	$0.94
Spikes	4	$0.28	$0.36
Transducer Protectors	5	$0.75	$1.00
Blood filter	1	$2.10	$3.60
Bacterial Filter	1	$1.65	$2.60
Recirculating bag	1	$0.85	$1.10
Molded plug in holder	1	$1.30	$1.95
Molded sensor connectors	2	$1.00	$1.60
Four Admin Bags Assembly	1	$3.50	$4.00
Materials cost total		$16.90	$24.43
Packaging & Sterilization		$1.00	$2.40
Labor - Including Benefits		$2.45	$4.20
Manufacturing Overhead		$1.00	$1.85
TOTAL MANUFACTURING COST		**$21.35**	**$32.88**

HAEMONETICS CENTRIFUGAL SYSTEM PRICES - DOMESTIC LIST PRICES

Haemonetics 7499 Bowl & Bag	$44.10
Haemonetics # 842 Dry Quad Pack	$23.10
Total Cost	$67.20
Estimated Manufacturing Cost at 40% margin	**$40.32**

RECIRCULATION BAG AND
OUTPUT BLOOD BAG(S)

FROZEN THAWED
BLOOD BAG

USER INTERFACE
PANEL

PULL-OUT
STERILE DOCKING
DEVICE

SIDE VIEW

SALINE BAGS AND
STORAGE SOLUTION BAG

BLOOD BAG SHAKER

DISPOSABLE MODULE

WASTE BAG

FRONT VIEW
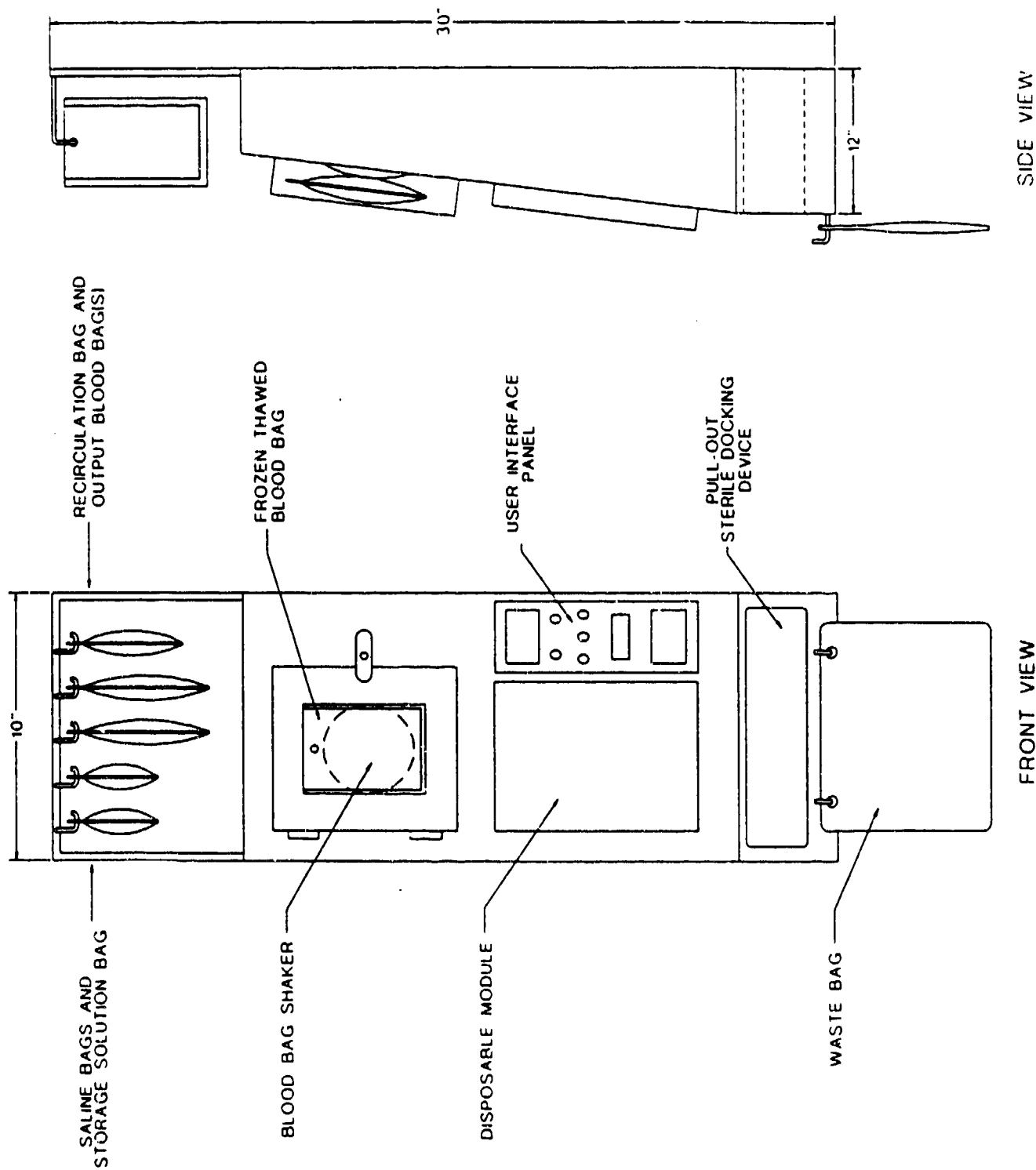
Fig. 3 CONTROL MODULE OVERALL PRELIMINARY DESIGN
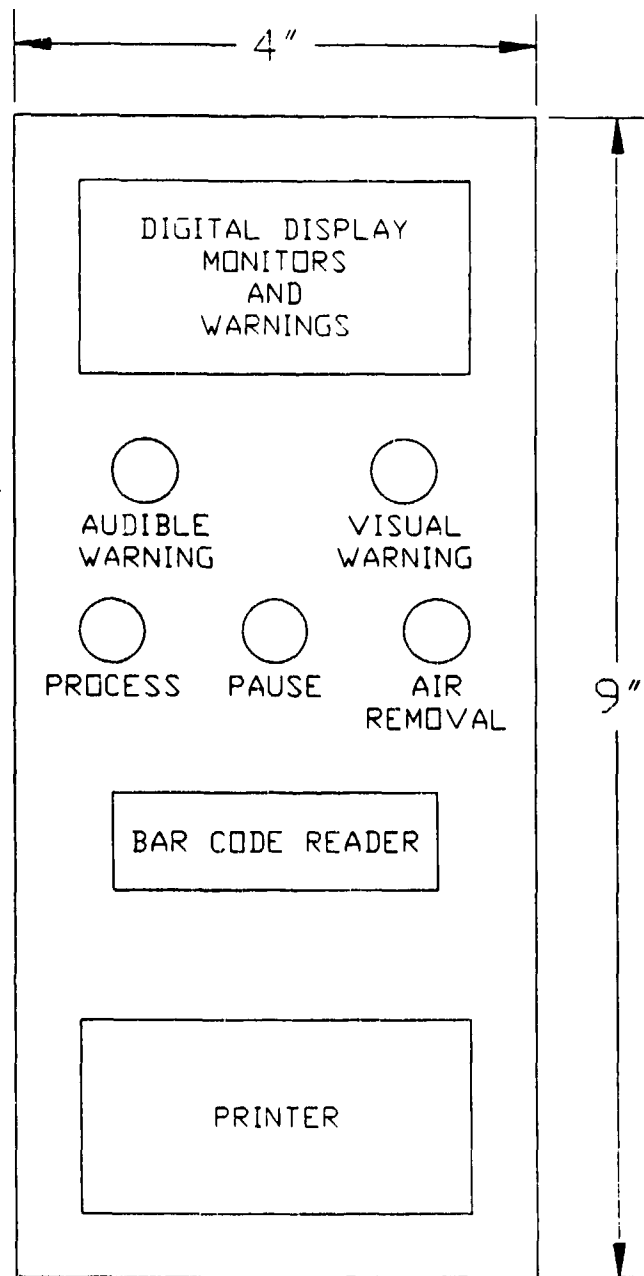
Fig. 4   USER INTERFACE CONTROL AND MONITORING PANEL

INPUTS   OUTPUTS

```
┌──────────────────────┐                    ┌──────────────────────┐
│                      │                    │   ROLLER PUMPS       │
│     PRESSURE         │                    │   SPEED AND          │
│  TRANSDUCERS (6)     │                    │   TIMING (3)         │
└──────────────────────┘   ┌────────────┐   └──────────────────────┘
                           │            │
┌──────────────────────┐   │            │   ┌──────────────────────┐
│     SENSORS          │   │MICROPROCESSOR│  │   PINCH VALVES       │
│     HEMATOCRIT       │   │    AND       │  │   TIMING (8)         │
│FREE PLASMA HEMOGLOBIN│   │   CONTROL    │  └──────────────────────┘
│     % GLYCEROL       │   │  SOFTWARE    │
└──────────────────────┘   │            │   ┌──────────────────────┐
                           │            │   │   MONITOR            │
┌──────────────────────┐   │            │   │   AND                │
│      USER            │   │            │   │   WARNINGS           │
│     CONTROL          │   │            │   └──────────────────────┘
│    SELECTION         │   │            │
└──────────────────────┘   │            │   ┌──────────────────────┐
                           │            │   │   DIAGNOSTIC         │
┌──────────────────────┐   │            │   │   DATA               │
│     BAR CODE         │   │            │   └──────────────────────┘
│     READER           │   │            │
└──────────────────────┘   │            │   ┌──────────────────────┐
                           │            │   │   PRINTER            │
                           └────────────┘   └──────────────────────┘
```
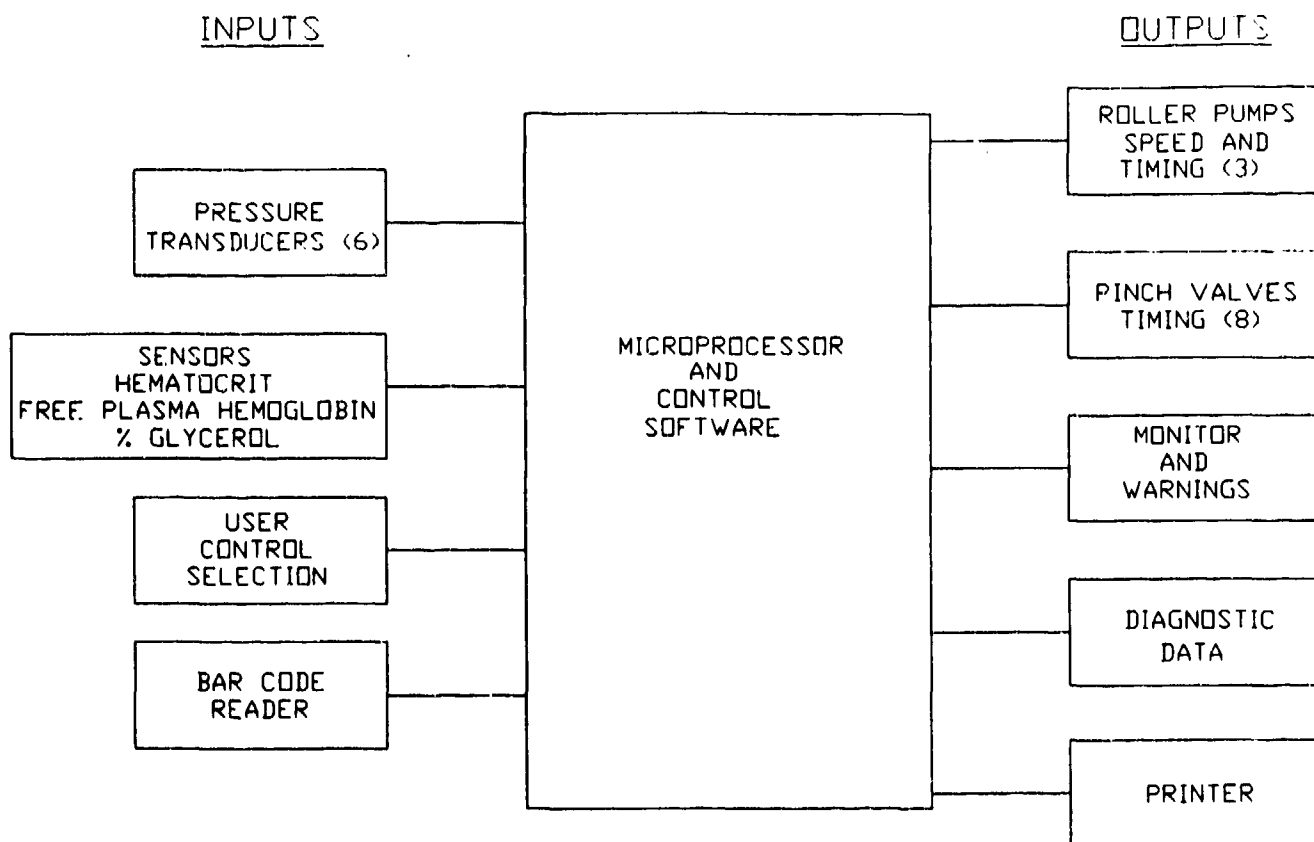
Fig. 5   BASIC CONTROL SYSTEM FUNCTIONS

empty or full bags, and any process interruption are examples of failures or problems that will result in visual and audible warnings. The display will be used to identify the source of the failure or problem, where possible.

The control electronics will store all useful information and parameters about the processing of a specific unit of blood. This data may be retrieved by the user and printed out as an aid to determining the reason for the failure of the wash process.

The TBPS will provide a printout of information for each unit of red cells processed. This information will be available within the TBPS or will be scanned into the TBPS by a bar code reader from bar codes on specific components or containing specific information. The bar code reader may be a wand-type which can be used to scan the bar codes on hung bags and other components. It would be attached by a cable to the control module. Alternatively, the bar code reader could be fixed on the control module, necessitating moving bags and components past the stationary reader. The latter would seem less convenient but all approaches will be investigated with potential users. The information on the printout will include:

* Unit number of the frozen RBC unit (bar coded).
* Unit number for the washed RBC unit (bar code on processed blood bag).
* Unit number on the sister washed RBC unit (bar code on processed blood bag).
* Lot number of the 12% saline (bar code on bag).
* Lot number of the 0.9% saline (bar code on bag).
* Identification and lot number for the long-term storage additive (bar code on bag).
* Filter or disposable cartridge serial number and/or lot number (bar code).
* Serial number of TBPS control module (internal).
* Expiration date of deglycerolized blood (internal clock).
* Start and stop time of wash process (internal clock).
* Quality control values (hemolysis, hematocrit, and residual glycerol) (internal values).
* Other parameters to be determined.

The bar code reader will be capable of reading CODABAR and Code 128 formats and, possible, other formats if necessary. The use of a keyboard or specific data entry controls may also be necessary but will be avoided if possible. Internal battery power will be provided to retain volatile memory during power interruptions. The system is designed to prevent any fluid flow during power interruptions such that processing can be safely resumed when power is restored.

Each control module will have its own internal power supply meeting medical device specifications (FDA, ISO 9000, AAMI, and UL). This supply may be standard or specially constructed by medical device power supply manufacturers. It operates from 110 or 220 volts a.c. ±15% voltage fluctuations, single phase, 50 or 60 Hz, commercial or tactical power sources. The power supply outputs are all d.c. and will not exceed specified limits regardless of large variations or spikes in voltage. Power supply and system will not be damaged by excessive voltages and may shut down or go into standby condition under extreme voltage limits.

The TBPS footprint is realistically estimated to be about 120 square inches compared to the maximum allowed of 288 square inches, or less than half. The TBPS overall height as a tabletop unit, not including the waste bag (see Fig. 3), is about 30 inches, compared to the maximum allowed of 39 inches.

The estimated volume of the TBPS control module, for the enclosure only and including the bag hanging space, is 3300 cubic inches, compared to a maximum allowable of 15,200 cubic inches, or under one-quarter of this maximum. The overall weight is calculated at 50 pounds or about one-half the maximum allowable of 110 pounds.

All of the environmental requirements (temperature, humidity, vibration, EMI) described in Table 2 can be met without difficulty. The repair times (MTTR) and failure requirements from this table can also be met.

6.4.5 TBPS Operation

The TBPS is designed to automatically perform all processes necessary to produce red cells in an additive solution capable of providing 21-day refrigerated storage within an output blood bag, starting with a bag of thawed frozen red cells. The processes performed automatically are: saline dilutions and mixing with the thawed frozen red cells; hollow fiber separator priming; red cell recirculation through the separator with waste removal in the separator followed by saline addition; red cell concentration to a hematocrit of 70% to 80% in the output blood bag; purging of blood remaining in the disposable cartridge; and addition of the long term storage solution to red cells in the output blood bag.

The processes and processing times are summarized in Table 8. The overall processing time, including all operator involvement (not just set-up), is 30 minutes or less and is based on Phase I test results, meeting the requirements and permitting one operator with six systems to produce 12 processed units of red cells per hour. The processes are discussed more fully below:

* **Saline Dilutions**

The NBRL S.O.P. is followed with respect to saline solutions used, sequence of events, solution flow rates into the frozen blood bag, mixing interval, and equilibration times. A roller pump is used to control flow rates of the saline solutions into the thawed frozen blood bag. A unique bag shaker is used to obtain rapid and thorough blood-saline mixing within this blood bag.

* **Separator Priming**

The hollow fiber separator is slowly filled with 0.9% saline in order to displace and remove all air. The saline pump and blood pump together provide this flow. The waste pump is operated to remove any and all displaced air. This process takes no additional time because it occurs during one of the equilibration intervals for the initial dilutions.

* **Red Cell Recirculation Process**

This process uses the hollow fiber separator to remove fluid from the red cell suspension and subsequently replace this fluid with an equal volume of 0.9% saline, keeping the hematocrit in the recirculation bag constant. Tthis process was shown to be effective and rapid for removing glycerol.

* **Red Cell Concentration**

This recirculation process uses appropriate values for blood, saline, and waste flows to rapidly increase the hematocrit of the red cell suspension to the 70% to 80% range without hemolysis.

* **Red Cell Purging**

The tubing and separator blood volume retains red cells at the end of the r .u cell concentration process. These red cells are removed to the output blood bag by pumping 0.9% saline solution through the tubing and separator while removing fluid with the waste pump to keep the hematocrit of these purged red cells as high as possible. This hematocrit decreases as red cells are purged. This process is optimized to recover virtually all red cells without significant dilution of the concentrated red cells. Over 95% of all red cells can be recovered in this way. The manual purge processes used in the

Phase I program were not optimized. However, the automatic purging process has been successfully demonstrated and used in our development of the hollow fiber separator autotransfusion system.

* Long-Term Solution Addition

The additive solution used to obtain long-term storage of red cells is added by using the saline pump to pump this solution from its container into the concentrated (and purged) red cells flowing into the output blood bag. The addition of solution flow to concentrated red cells flowing into the bag promotes good mixing and does not add time to the overall process.

6.4.6 Achieving Effective, Rapid and Atraumatic Deglycerolization

A major technical objective for the TBPS is achievement of a highly effective, rapid, and atraumatic process for thawed red cell deglycerolization. The methods shown to be feasible in the Phase I program are briefly described below:

i. The initial 12% NaCl and 0.9% NaCl dilution processes duplicate the procedures of the NBRL S.O.P., except that blood-saline mixing occurs with the thawed blood bag supported vertically in a specially-designed bag shaker or mixer. This automatic process eliminates any bag handling or other manual operations after the initial set-up.

ii. Deglycerolization takes place by pumping blood at a controlled flow rate through a hollow fiber separator. Waste fluid is removed through the walls of the separator fibers at a controlled flow rate, and 0.9% saline solution is added at the same flow rate at the exit of the separator. Blood recirculation is performed by pumping blood into and out of a specially-designed recirculation bag that prevents blood mixing in the bag. This increases the effectiveness of the saline wash process and decreases deglycerolization time.

iii. The initial priming of the hollow fiber separator with saline is performed using the saline and blood pumps in a simple, rapid process to remove air.

iv. The purging of red cells from the separator is performed using saline to flush the cells into the output blood bag.

v. The use of three roller pumps to control blood, saline, and waste flow rates provides a consistent, reproducible blood product and fixed levels of glycerol in this product.

vi. The adjustment or selection of operating parameters, dead space, pressure drops, transmembrane pressures, and separator fiber surface area minimize red cell damage.

vii. The use of pressure sensing in the fluid lines along with appropriate control algorithms permits safe and effective automatic control of the entire process. This control methodology was successfully used in the RAI autotransfusion system.

6.4.7 Achieving Long-Term Storage of Deglycerolized Red Blood Cells

The TBPS disposable is a closed, sterile system capable of producing washed, sterile red cells stored in an additive solution providing long-term refrigerated storage capability.

The TBPS will deglycerolize red cells and then concentrate them to 70% to 80% hematocrit. The long-term storage solution is assumed to be provided in a separate bag which is hung and

spiked like the saline bags. The saline pump is used to deliver this storage solution to the processed blood bag at a fixed flow rate and volume (depending on red cell volume), mixing with the concentrated red cells as they enter the bag. The final hematocrit in the bag is 50% to 70%. A bacterial (0.22 micron pore size) filter is used downstream of the saline pump to ensure sterility of the storage solution and of the saline solutions. If the storage solution is not available in appropriate bags, or if there are other reasons not to use the approach described above, then the processed blood bag may already contain a volume of the storage solution. This requires dating and inventory control of the TBPS disposable due to a limited shelf life of the storage solution in the processed blood bag. Alternatively, a satellite bag may provide the storage solution to the processed blood bag, requiring manual addition and mixing.

Several storage solutions have been developed and evaluated. Three have been approved by the FDA for red cell refrigerated storage, but not for frozen deglycerolized red cell refrigerated storage. These three solutions are Optisol, Adsol, and Nutracil. These solutions do not need to be removed and can be infused into patients. These solutions may be evaluated first with deglycerolized blood since they may be more easily accepted by the FDA. These solutions are intended to preserve red cell function and limit hemolysis for up to 21 days at $4^\circ C$. The system specifications (Table 2) require a minimum of five days storage. The effective removal of free plasma hemoglobin by a more effective, optimal wash process is anticipated. Red cell damage during deglycerolization is minimized by reducing surface areas, pressure drops, and transmembrane pressures. This may limit hemolysis during storage. Experimental studies will be made of storage solutions added to concentrated washed red cells, and storage life will be measured based on standard assessments of red cell function and hemolysis.

The current NBRL S.O.P. calls for the centrifugation of stored washed blood prior to its use. This centrifugation removes free hemoglobin and other substances and concentrates the red cells to a higher hematocrit. It is very desirable to eliminate this process and the need for this centrifuge at or near the location where the washed blood will be used. This may not be possible, since there may be substantial variability in the levels of free plasma hemoglobin and other substances in the stored blood due to variations in initial glycerolization or due to variations in handling of the stored blood. It is hoped that the storage solution will consistently provide blood storage capabilities to at least 5 days if not 21 days without the need for centrifugation and fluid removal.

An alternative may be to provide each processed blood bag with a small, inexpensive hollow fiber filter and waste bag. This approach would remove most of the fluid from the red cell suspension as blood is infused into the patient. Blood flowing from the bag to the patient flows through the centers of microporous hollow fibers. Excess fluid is removed by pressure differentials caused by gravity. This fluid flows into a small waste bag. This disposable is closed, compact, and sterile. The concept has already been demonstrated in our laboratory.

6.4.8   Development of Blood-Saline Mixing System

A key objective of the overall system for deglycerolizing thawed blood is to automatically perform the initial saline dilutions and the necessary saline-blood mixing. The NBRL S.O.P. for these processes have been assumed as the reference methodology.

It is desirable to mount the thawed blood bag vertically or nearly so at the initial set-up and to avoid any operator involvement until the processing of a unit is completed. To accomplish this a vertical bag mixer was developed. This mixer uses a shaped piston which pushes against the bottom portion of the vertical blood bag. The blood bag is rigidly supported on its opposite side. The piston reciprocates at a given frequency, compressing the blood bag, pushing blood to the top of the bag, and then letting it fall back to the bottom of the bag when the piston moves away from the bag. This process is intended to replace and, to some extent, mimic the horizontal bag shaker used on the Haemonetics 115. The design is shown in Fig. 6.
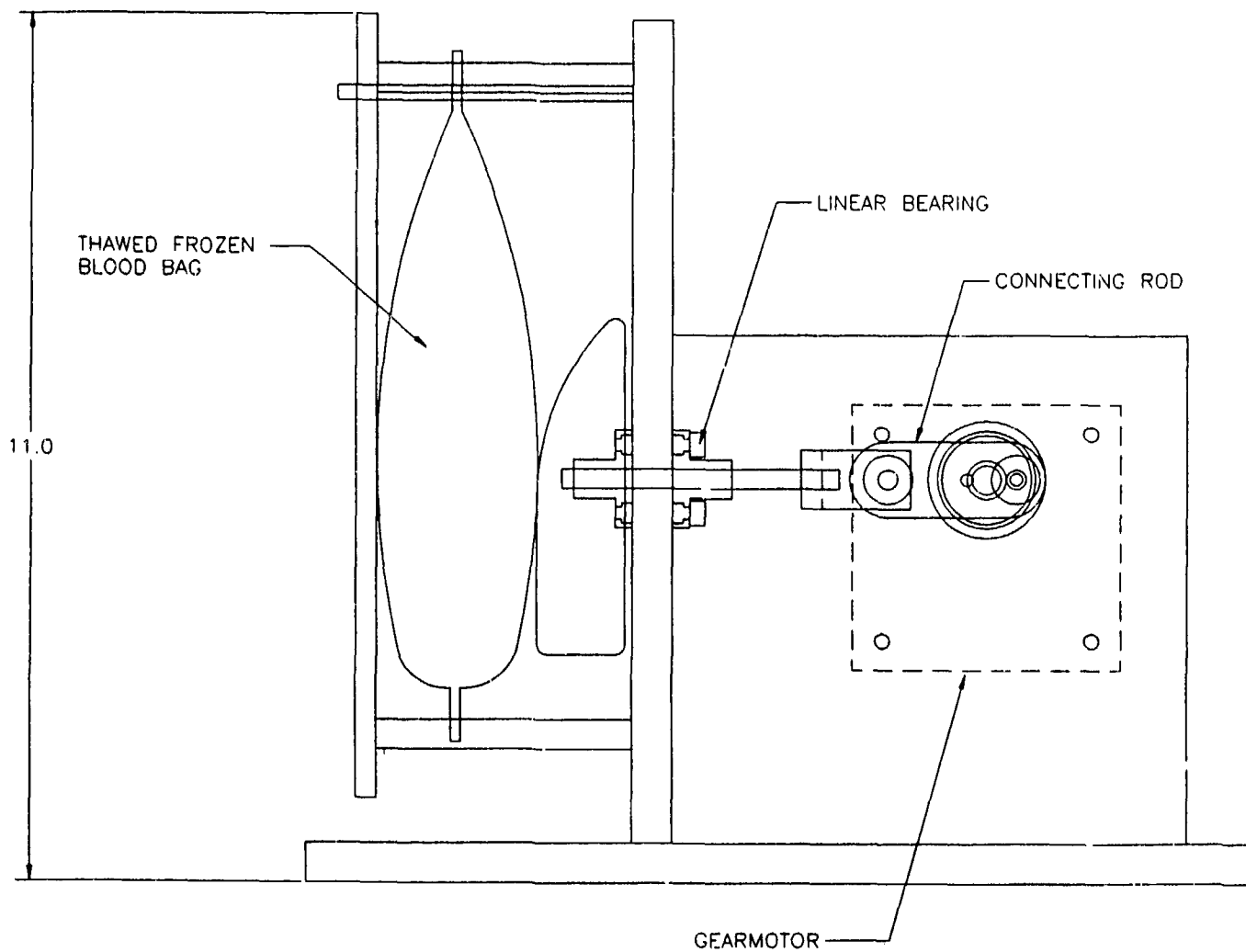
Fig. 6 BLOOD BAG SHAKER FOR BLOOD-SALINE MIXING (Side View)

This bag shaker and blood-saline mixer was designed, constructed, and tested. A variable speed DC motor was used to drive the displacement piston and permit mixing to be observed as a function of cyclic rate. Modifications to the piston size and shape were made, and its location relative to the bottom of the blood bag was varied, as was the piston stroke and amount of compression of the blood bag. These parameters were iteratively tested and evaluated in order to achieve rapid but atraumatic mixing.

The shaker unit was tested using a standard 800 ml frozen blood bag filled with either 300 ml or 600 ml of water at 24^0 C. This simulates the blood bag volumes before and after predilution. Dye was infused into the blood bag while the shaker was operating at various rates. The times required for the dye to fully disperse were noted. Complete mixing occurred in 10 to 20 seconds when the blood bag held 300ml of fluid for cyclic rates of 100 to 380 per minute. When the blood bag is full (600ml) complete mixing occurs in less than 30 seconds if the shaker is operating at 250 cycles per minute or faster; at 100 cycles per minute complete mixing does not occur (see Fig. 7).

In another test 300 ml of bovine blood (36%Hct) was placed into the blood bag and mixed at 380 cycles per minute for 6 minutes. A sample of this blood was centrifuged for 15 minutes and no observable hemolysis was noted. Mixing times are seen to be sufficiently short at moderate-to-high cyclic rates and can probably be decreased with further development.

6.4.9   Modification of a Roller Pump Mechanism

A standard roller pump mechanism has been modified to achieve some specific and important objectives and advantages for this automatic deglycerolization system. These objectives and advantages are:

i.   The use of a disposable cartridge containing all disposable components has the advantages of small size, easy and rapid set-up and removal, easy disposal, reduced inventory volume, convenient packaging, precise alignment of disposable components for mating with the control module pumps and connectors, reduced fluid dead space, reduced control module size, and low cost. Small disposable cartridge size may be achieved by aligning roller pump heads parallel to each other and spacing them close together. Then the fluid tubing engaged by the three roller pumps can be parallel to each other and closely space; the disposable cartridge can consequently be quite small.

ii.   The roller pump heads can be designed to move towards or away from the flexible fluid tubing they engage. They are spring-loaded to provide reliable tubing occlusion without excessive forces.

iii.   The fluid tubing is straight and backed up with a straight rigid support member. It is simpler to hold a straight tube in place than to support a curved tube and ensure that it is centered under the pump rollers.

iv.   The roller pump head is made sufficiently large in diameter and with a sufficient number of rollers that the motion of the head towards and away from the fluid tubing as the head rotates is kept quite small (under 0.2 inches).

v.   The mass of the roller pump head is made sufficiently small that the natural frequency of this spring-mass system is much higher than the maximum operating frequency.
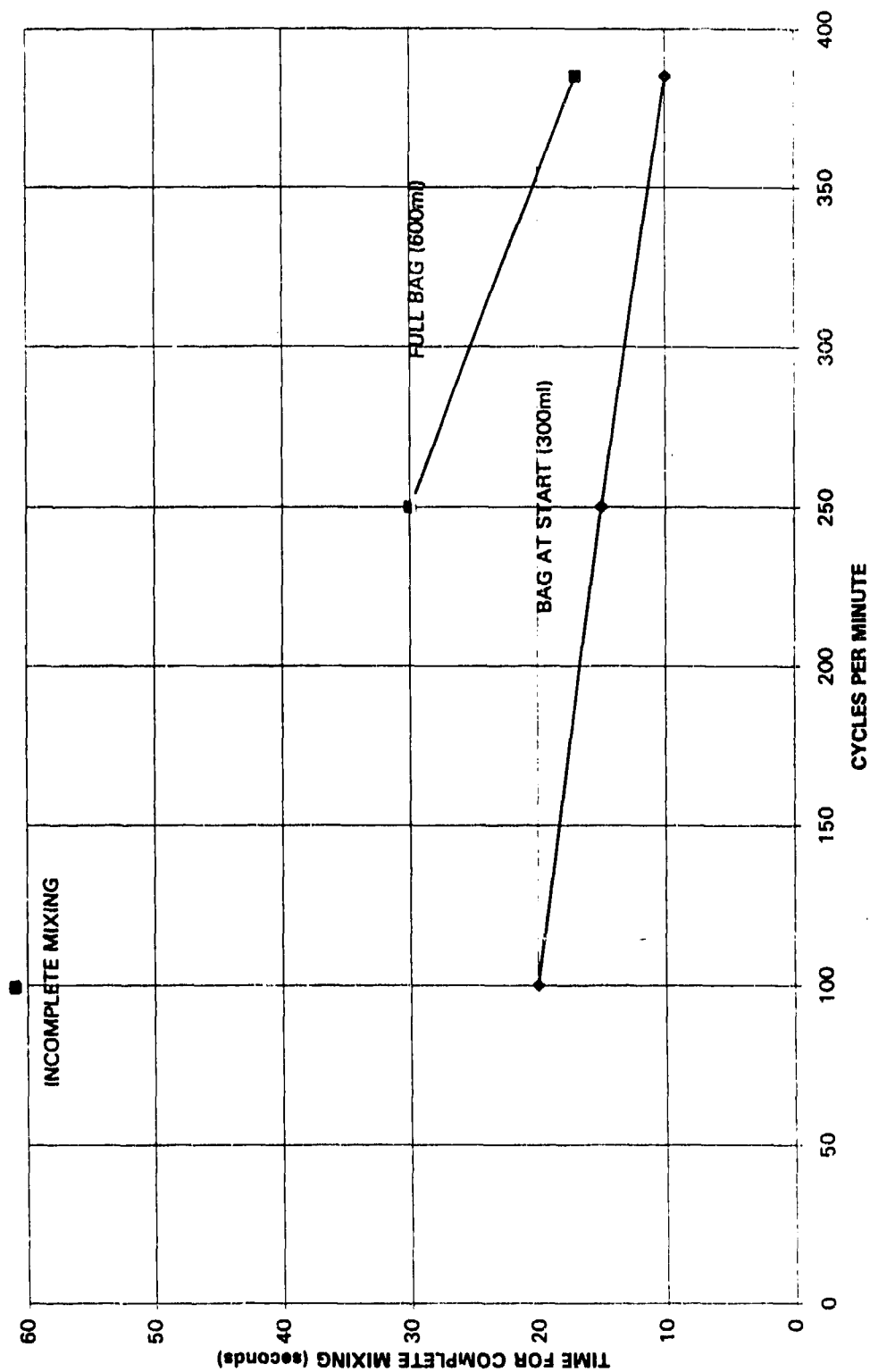
Fig. 7   MIXING TIME STUDIES FOR THE BLOOD-SALINE MIXING DEVICE

vi. The more gradual engagement and disengagement of pump rollers with the tubing as the larger pump head rotates, and the more optimal roller diameter and spacing for the tubing size used, results in lower blood damage, based on our previous roller pump development experience. Noise and vibration and fluid pressure variations are also reduced.

vii. The drive motor for each pump can be placed in a variety of locations to permit close spacing between pump heads and minimize control module size.

viii. Standard proven roller pump technology is used to ensure optimal pump function, high reliability, and long life.

A prototype of this roller pump was developed to demonstrate the characteristics and advantages of this concept. The conceptual design is shown in Fig. 8. Engineering layout, assembly, and detail designs were completed. Standard components were used wherever possible (sealed, lubricated ball bearing assemblies; belt drive and pulleys; brushless d.c. gearmotor and speed control; shaft coupler; snap rings; collars; roller pump rollers).

6.5 The Design, Fabrication, and Testing of Hollow Fiber Separators

Design
The hollow fiber separator was designed for the deglycerolization processes and system. A standard RAI cylindrical housing design for straight parallel hollow fiber bundles was used for ease of fabrication of prototype devices. The length of this separator was varied but the diameter of the housing remained constant, permitting hollow fiber length of 10 to 20 cm and effective surface areas from 0.2 m^2 to 1.0 m^2. A more optimal design of a 0.4 m^2 separator with a 15 cm fiber length was also made in order to simplify parts and assembly, reduce separator diameter, and reduce costs. This separator was designed to permit the use of both Akzo and Mitsubishi hydrophilic fibers, with standard urethane potting methods and end cap assembly using UV-cure adhesives. The end caps provide connections to tubing for blood entry and exit and manifolding with a small dead space and smooth flow path. A connection to tubing is provided at the side of the housing for waste flow removal.

Fabrication
Hollow fiber separators were fabricated specifically for blood deglycerolization. These separators were intended to meet the various requirements of the TBPS prototype. Two fibers meeting these requirements were evaluated for this purpose; one from Mitsubishi Rayon Company (MRC) and another from Akzo Faser AG. Our testing emphasized the MRC fibers because of our previous testing with these fibers for an autotransfusion application, with their demonstrated high waste flux capabilities and low hemolysis levels.
The fabrication of hollow fiber separators followed our standard design, materials, and construction methods. Our standard separator housings were modified to obtain the desired separator lengths. Table 9 summarizes the numbers, types, and characteristics of the separators fabricated for this project. This table also summarizes the dimensional characteristics of the hollow fibers used.

Testing
Initial tests were performed with separators having 0.8 m^2 surface area. The test data is summarized in Table 10. Bovine blood with plasma replaced by normal saline was used to simulate thawed frozen saline-diluted blood. This blood in a reservoir of fixed hematocrit (20% and 30%) is
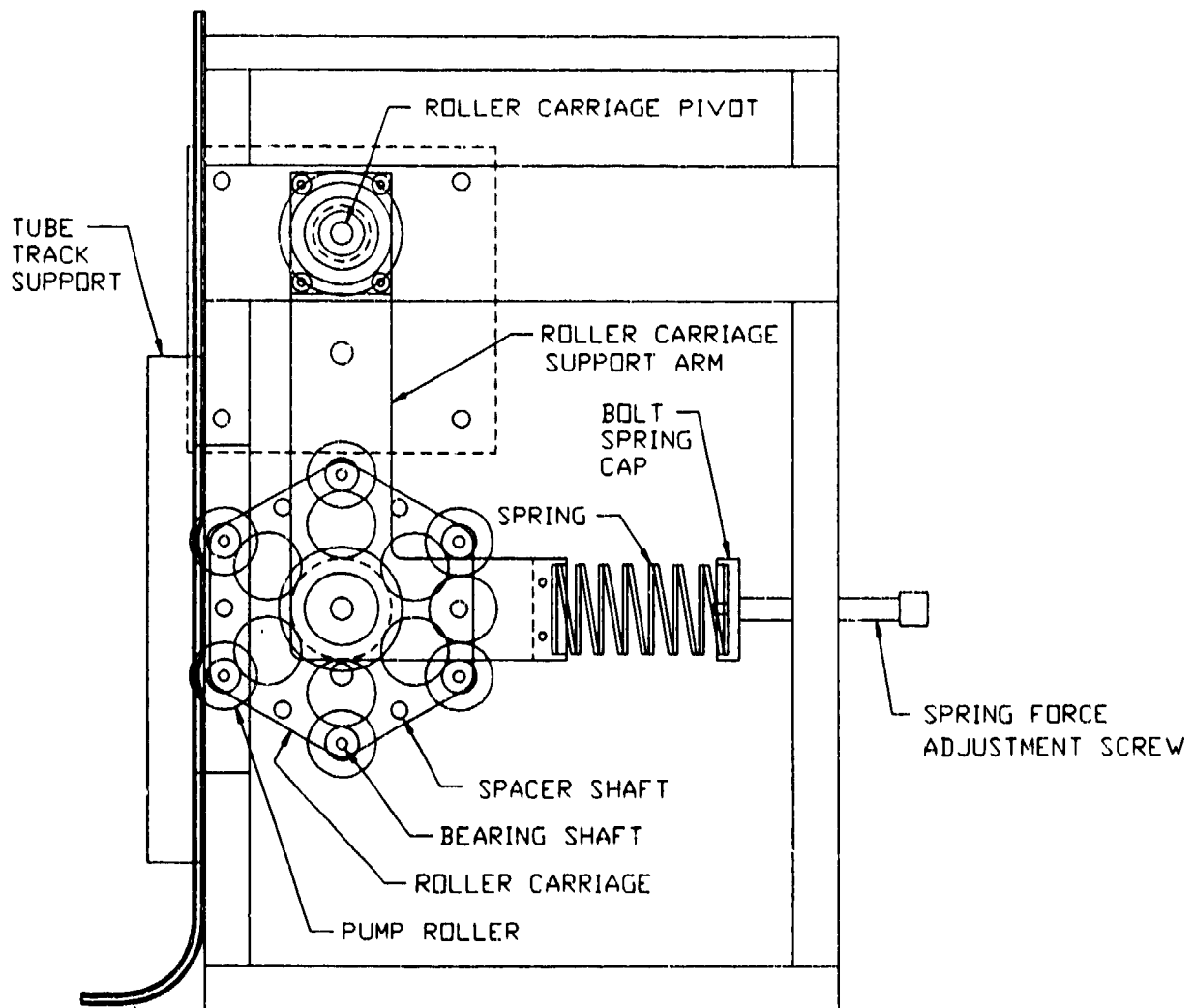
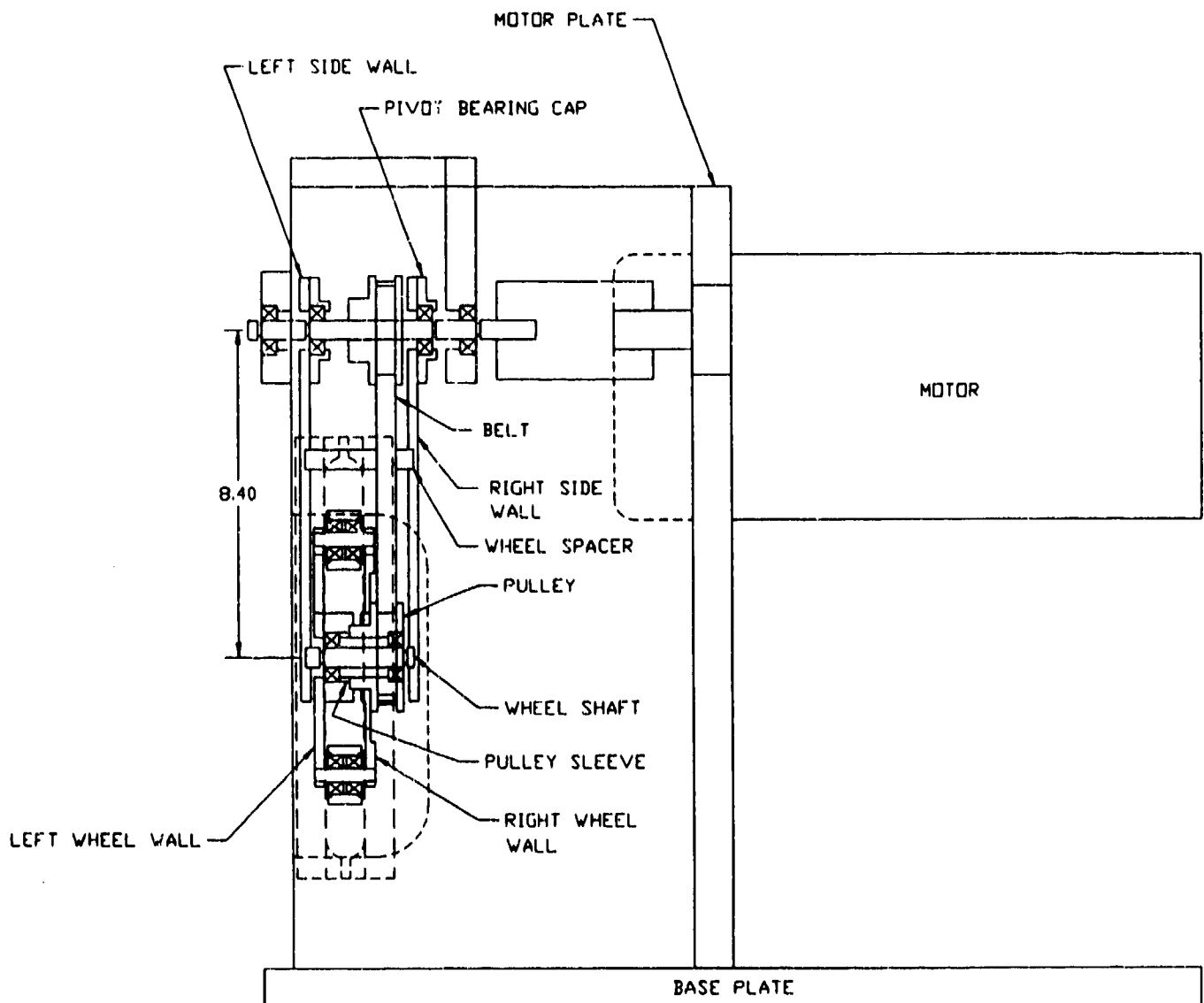Fig. 8A   MODIFIED ROLLER PUMP FOR CLOSE SPACING
OF THREE ROLLER CARRIAGES (Side View)

Fig. 8B   MODIFIED ROLLER PUMP FOR CLOSE SPACING OF THREE ROLLER CARRIAGES   (Front View)
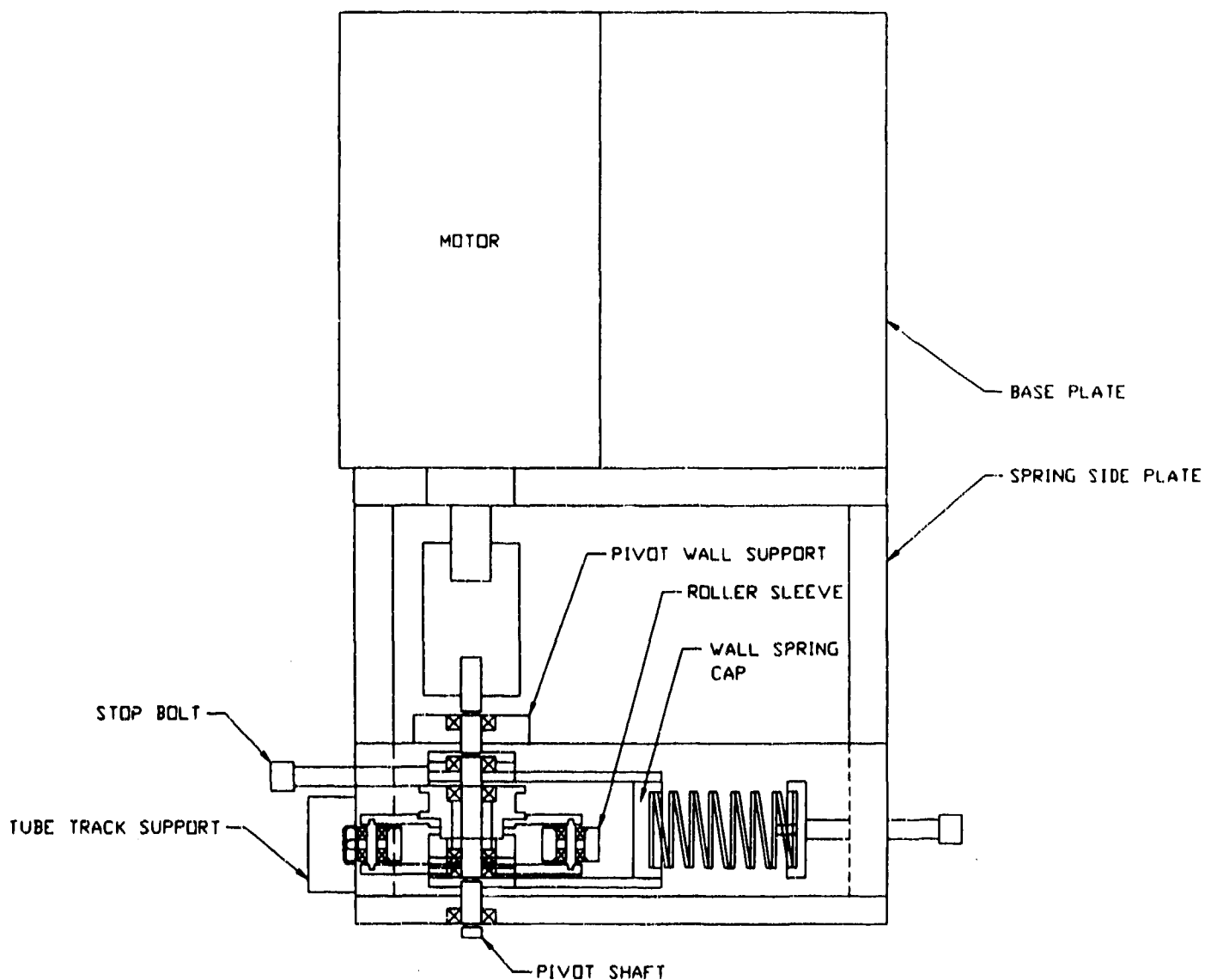
MOTOR

BASE PLATE

SPRING SIDE PLATE

PIVOT WALL SUPPORT

ROLLER SLEEVE

WALL SPRING
CAP

STOP BOLT

TUBE TRACK SUPPORT

PIVOT SHAFT

Fig. 8C   MODIFIED ROLLER PUMP FOR CLOSE SPACING
OF THREE ROLLER CARRIAGES   (Top View)

Table 9

HOLLOW FIBER SEPARATOR FABRICATION

Number Made	Fiber Source	Active Area m²	Number of Fibers	Overall Fiber Length cm
8	MRC	0.8	4500	24
9	MRC	0.4	3000	18
4	MRC	0.2	1500	18
2	Akzo	0.4	2785	18
3	Akzo	0.2	1480	18
MRC HOLLOW FIBER CHARACTERISTICS				
EX 270 Hydrophilic Fibers				
ID = 270 microns				
OD = 370 microns				
Pore Size = 0.2 microns				
AKZO HOLLOW FIBER CHARACTERISTICS				
Micro PES TF 10 Hydrophilic Fibers				
ID = 300 microns				
OD = 500 microns				
Pore Size = 0.22 microns				

Table 10

HOLLOW FIBER SEPARATOR TEST RESULTS

Fiber: MRC 270 HFE, 0.2 micron pore size / Blood: Bovine / Surface Area: 0.8 m²

| INLET BLOOD | | | OUTLET BLOOD | | WASTE | | TRANSMEMBRANE | COMMENTS |
Flow Rate ml/min	Hct %	PRESSURE mmHg	Hct %	PRESSURE mmHg	Flow Rate ml/min	PRESSURE mmHg	PRESSURE mmHg	
100	20	9	36	-2	45	-3	7	
		12	50	-1	60	-3	9	
		16	57	-1	65	-8	16	TMP rises 2/min.
200	20	25	38	2	95	0	14	
		28	49	2	118	-8	23	
		33	60	2	133	-25	43	TMP rises 8/min.
300	20	34	38	0	140	-5	22	
		40	48	0	176	-13	33	
		51	62	2	203	-30	57	TMP rises 8/min.
400	20	51	40	3	198	6	21	
		60	50	4	238	-11	43	
		74	61	6	266	-32	72	TMP rises 12/min.
100	30	23	39	4	23	8	7	
		27	50	4	40	8	8	
		33	60	6	50	8	12	
200	30	36	40	7	50	9	13	
		40	51	7	82	3	21	
		50	60	8	100	-12	41	TMP rises 8/min.
300	30	55	40	11	73	24	9	
		60	50	11	118	5	31	
		72	61	12	152	-14	56	TMP rises 6/min.
400	30	73	40	11	100	18	24	
		82	50	11	158	8	39	
		114	60	15	200	-10	75	TMP rises 8/min.

pumped at a fixed flow rate with a roller pump through the separator and into another reservoir. Waste fluid is pumped at a fixed flow rate with a roller pump into a waste container. Inlet blood flow rate was set at 100, 200, 300, and 400 ml/min. Waste flow rate was set and measured to achieve outlet hematocrits of 40, 50, and 60%.

No significant hemolysis occurred under most operating conditions. A free plasma hemoglobin (FPH) of about 50 to 100 mg/dL was observed after long run times at an outlet Hct of 60% or greater. The transmembrane pressure was above 200 mmHg when this occurred. This hemolysis did not occur at inlet blood flow rates below 200 ml/min or at transmembrane pressures below 200 mmHg for any of the tested inlet blood flow rates. Stable operation was achieved under all conditions, but transmembrane pressure increased slowly for outlet hematocrits of 60% and processing rates at or above 200 ml/min.

Higher outlet hematocrits (about 70%) were evaluated and substantial transmembrane pressures occurred due to red cell layering at relatively high waste flow rates. This effect was completely reversible. No membrane plugging occurred, as expected. Over 20 liters of blood were pumped through a single separator without performance degradation, excessive transmembrane pressures (for outlet hematocrits of 60% or below), membrane plugging or fouling, or any other problem. These tests defined satisfactory flow rates (blood and waste) and other operating conditions for this hollow fiber separator.

Testing was performed with bovine blood to evaluate the optimum hollow fiber separator area. Separators were tested having 0.4 m² and 0.2 m² surface area. The 0.4 m² unit was tested at 250, 300, and 400 ml/min blood inlet flow. 250 ml/min is the slowest processing rate that will perform the deglycerolization in under 20 minutes. Table 11 summarizes the data from this test. In all cases the transmembrane pressure remained constant for at least 20 minutes. No hemolysis was evident in any of these tests.

The 0.2 m² hollow fiber separator was tested at 250 ml/min blood flow. Transmembrane pressure exceeded 200 mmHg at the start of the test. Significant hemolysis was observed, therefore additional tests were not performed. The 0.4 m² separator was selected as the initial optimal separator design. A substantially shorter 0.2 m² separator with more fibers and a much lower pressure drop should also be evaluated.

The addition of long term storage solution requires the system to concentrate the deglycerolized red cells to a hematocrit of 75% ±5%. It is possible to concentrate RBCs to this level by reducing the inlet blood flow rate and waste flow rate to maintain low transmembrane pressures, resulting in very low levels of hemolysis. This concentration process is accomplished with multiple passes by recirculation and gradual concentration of the blood.

This process was tested and evaluated as a means to concentrate blood prior to adding a storage solution. A test was performed using a 0.4 m² hollow fiber separator and bovine blood at 36% hematocrit. The blood was preprocessed to remove the plasma which was replaced with normal saline. Table 12 shows that five passes were used to concentrate the blood from 36% Hct to 75% Hct. Waste flow was reduced for each pass to minimize transmembrane pressure. At a constant blood flow rate of 150 ml/min, 7 minutes were required for this concentration. No hemolysis occurred during this processing phase.

This test indicated that an effective concentration process was feasible in a short time with negligible hemolysis. When combined with the deglycerolization process, blood flow rates will be gradually decreased as outlet hematocrit increases instead of remaining at a low constant value. This will increase the efficiency of the deglycerolization process if saline is added to the separator output during the early steps of blood concentration, resulting in overall shorter deglycerolization plus concentration times.

Table 11

**BLOOD TESTS TO DETERMINE PRESSURE LEVELS, STABILITY OF
OPERATION AND HEMOLYSIS IN A 0.4 m² HOLLOW FIBER SEPARATOR**
Bovine Blood at 24°C
Inlet Blood Hct = 40% / Outlet Blood Hct = 55%

RUN #	PARAMETER	START	5 MINUTES	20 MINUTES
1.	Inlet Blood Flow, ml/min	400	400	400
	Blood In, mmHg	262	253	246
	Blood Out, mmHg	94	94	93
	Waste, mmHg	63	65	65
	Pressure Drop, mmHg	168	159	153
	Transmembrane Press., mmHg	115	109	104
2.	Inlet Blood Flow, ml/min	300	300	300
	Blood In, mmHg	197	193	191
	Blood Out, mmHg	76	75	75
	Waste, mmHg	55	55	54
	Pressure Drop, mmHg	121	118	116
	Transmembrane Press., mmHg	82	79	79
3.	Inlet Blood Flow, ml/min	250	250	250
	Blood In, mmHg	186	177	174
	Blood Out, mmHg	71	70	69
	Waste, mmHg	51	51	51
	Pressure Drop, mmHg	115	107	105
	Transmembrane Press., mmHg	78	72	70

Table 12

TESTS OF A PROCESS TO CONCENTRATE BLOOD TO HEMATOCRITS OVER 70%
Bovine Blood at 24°C, 300 ml at 36% Hct, 0.4 m^2 Hollow Fiber Separator

PASS #	BLOOD FLOW ml/min	WASTE FLOW ml/min	BLOOD OUT ml/min	HCT IN %	ACTUAL HCT OUT %	CALC HCT OUT %	INLET PRESS mmHg	WASTE PRESS mmHg	PRESS DROP mmHg	TMP mmHg
1	150	42	108	36	50	50	95	34	75	38
2	150	25	125	50	58	60	135	47	11	45
3	150	21	129	58	65	67	200	52	180	73
4	150	16	134	65	71	73	340	60	320	135
5	150	13	137	71	75	78	500	68	480	207

6.6 Construction of Prototype Systems

The purpose of this effort was to assemble a prototype system that was capable of performing all of the functions of the complete TBPS and which demonstrates by its testing all of the functional characteristics and advantages of this approach. Such a prototype system was assembled using standard components and some key components specially designed by RAI. The basic schematic of this system closely followed that of Fig. 1. Changes in the interconnections between the frozen blood bag, recirculation bag, and output blood bag were made to permit system testing using the two bag recirculation approach or alternatively the single bag recirculation approach.

Standard calibrated roller pumps were used (with 3 mm I.D. tubing) for the blood pump, saline pump, and waste pump. The flow rates through these pumps were manually controlled, as were all functions of this prototype. However, all automatic functions of the TBPS could be and were simulated and tested adequately using manual controls. The valving (ON-OFF flow controls) were performed using tubing clamps instead of solenoid-operated tubing pinch valves. Standard pressure transducers were used to monitor all system pressure levels, although the most important of these functionally were hollow fiber separator blood inlet, blood outlet, and waste pressures.

The recirculation bag was designed, constructed, and tested by RAI to permit efficient single-bag recirculation with blood entry from the separator (after saline addition) at the top and blood exit to the blood pump and separator at the bottom (Fig. 9). This blood bag provided a long folded blood path which prevented blood mixing within the bag and substantially increased glycerol removal efficiency. Tthis design avoided bag pinch-off and permitted effective and complete air and blood removal.

The blood-saline mixer was designed, constructed, and tested by RAI to achieve automatic predilutions of the thawed blood with saline. It was added to the system for the later series of blood tests.

Standard tubing, waste bags, 12% NaCl bags, 0.9% NaCl bags, and thawed blood bags, were the same as those used with the NBRL S.O.P. and the Haemonetics 115 centrifuge. Over 25 such disposable assemblies were constructed and tested.

6.7 Testing and Optimization of Prototype Systems

Preliminary testing of the prototype systems was done with saline. The roller pumps were calibrated using a stopwatch and graduate cylinder. Pressure transducers were calibrated against a mercury manometer. The system was analyzed for excessive pressure drops at 400 ml/min which is the maximum anticipated flow rate. All flow paths were checked for proper function. The hollow fiber separators were all first tested using saline to establish baseline pressure drops both along and through the fibers.

Initial testing of this system was performed with bovine blood at blood flow rates into the hollow fiber separator of 300, 400, 500, and 600 ml/min. The hollow fiber separators used for these tests had 0.8m² surface area, the high blood flow rates were used to find the upper limits of the separator performance. Transmembrane pressures, separator pressure drops, all fluid flow rates, and outlet hematocrits were measured for inlet hematocrits of 30, 35, and 40%. Operating saline and waste flow rates were selected to simulate glycerol final concentrations under 1% and separator outlet hematocrits in the range of 50% to 55%. Levels of free plasma hemoglobin were low in the output blood under all test conditions.

In preparation for human blood testing Tom Robinson was trained in the process of freezing, thawing, and deglycerolizing human blood according to the SOP during a visit with Dr. Valeri and his staff at the Naval Blood Research Laboratory in Boston. . The process and packaging requirements for shipping frozen blood to Irwin Memorial Blood Centers for RAI use and testing was established, as were the methods for taking and shipping appropriate samples to NBRL for their evaluation of the deglycerolization process (see Appendix).
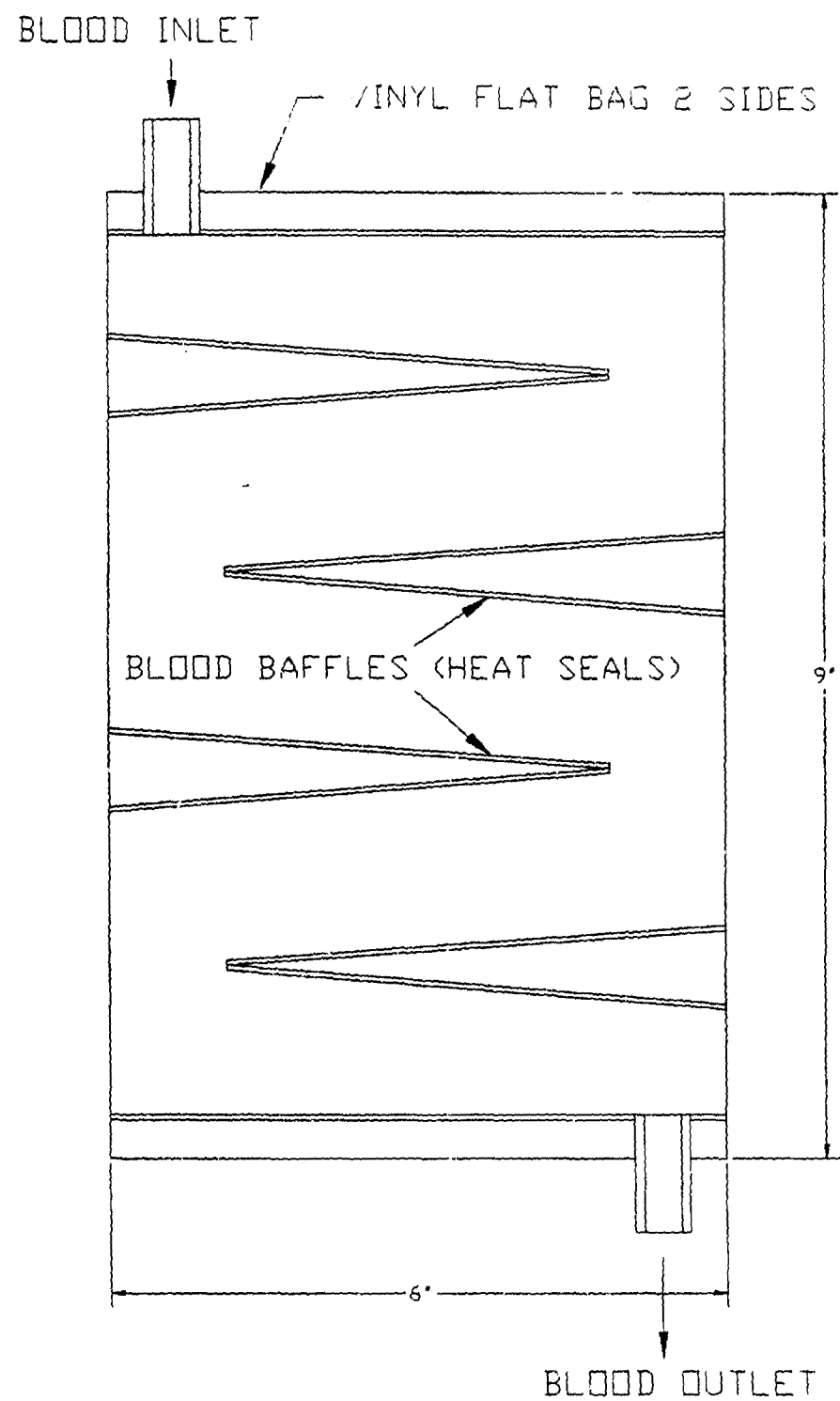
Fig. 9   BLOOD RECIRCULATION BAG DESIGN TO MINIMIZE BLOOD MIXING IN THE BAG

Initial tests (Series 1) were performed at the Irwin Memorial Blood Center in San Francisco. The mock-up system and its instrumentation were taken to Irwin Memorial and four units of frozen human blood obtained from the NBRL were thawed, predilutions manually performed, and deglycerolization processing done with the mock-up system. The test utilized the two-bag recirculation procedure.

The deglycerolization process was done at 400 ml/min blood inlet flow and took 12 to 13 minutes. Inlet hematocrit to the hollow fiber separator was 40% and the outlet hematocrit was 55%. The NBRL results show that this is inadequate time for glycerol to diffuse out of the red cell and into the saline solution; glycerol levels in the washed blood were 2 to 4% instead of the required 1% or below. Increased time and increased washing were expected to meet this requirement. The free plasma hemoglobin levels were somewhat high. This may have been due to inadequate washing or to inadequate control of the predilution process. Future predilutions and mixing were done more precisely and controllably. The red cell recovery was high, as expected with a hollow fiber device. This study helped prepare for better-run and more optimized procedures during the next testing phase.

Refinements were made to the prototype system to reduce dead space and the test protocol was modified to slow down the processing time. Four additional units of human frozen blood were processed at Irwin Memorial Blood Center using the two-bag recirculation procedure once again (Series 2). The results of these tests showed that the glycerol levels were below 1%. Recovery calculations based on pre wash and post wash samples was lower than expected due to retention of red cells in the hollow fiber separator and elsewhere in the system. Recovery was not specifically addressed in these tests and will increase as the separator size and system dead space are reduced and the red cell purge process is optimized. Each of these four units were processed in less than the 30 minutes maximum time required.

The last series of four human blood (Series 3) tests were performed using the single bag unmixed blood deglycerolization process. The thawed unit predilutions were performed using the vertical blood bag shaker. The blood bag shaker performed as expected. These tests show that all requirements for deglycerolization have been met, although the blood flow rate of 250 ml/min resulted in total deglycerolization times of 33 minutes instead of the required 30 minutes. Blood flow rates above 250 ml/min (and changes in other parameters) will ensure that this 30 minute requirement is met in future tests, as it was in nine out of twelve of these thawed frozen human blood deglycerolization processes. The free plasma hemoglobin level in the blood product was judged acceptable but was somewhat higher than the average observed with the NBRL S.O.P. This level can be significantly reduced with further optimizations, including decreasing hollow fiber separator pressure drops and transmembrane pressures.

The test results and the results of the NBRL analysis from blood samples provided to them from all 12 units processed at Irwin Memorial Blood Center have been summarized in Tables 13, 14, 15, 16, and 17.

Table 13

IN VITRO RECOVERY, SUPERNATANT HEMOGLOBIN, OSMOLALITY AND INTRACELLULAR AND EXTRACELLULAR SODIUM AND POTASSIUM MEASUREMENTS ON PREVIOUSLY FROZEN RED BLOOD CELLS WASHED USING THE RASOR PROTOTYPE DEGLYCEROLIZATION SYSTEM

Blood Samples received from Rasor Associates, Inc.
253 Humboldt Court, Sunnyvale, California, 94089 on 6/22/94

Report Date: 6/27/94   **Series 1**

    The Naval Blood Research Laboratory (NBRL) shipped 4 units of glycerolized red blood cells frozen at NBRL using the Primary Bag System to Rasor Associates, Inc. The red cells were thawed and deglycerolized using the Rasor prototype deglycerolization system. Seven samples from each unit were collected and shipped to the Naval Blood Research Laboratory along with the weights of the thawed units, waste collections and deglycerolized units.

    Hematocrit, hemoglobin and supernatant hemoglobin were measured on the samples from thawed units and supernatant hemoglobin was measured on the waste collections. Hematocrit, hemoglobin, supernatant hemogiobin, osmolality, intracellular and extracellular sodium and potassium were measured on the deglycerolized units. The freeze-thaw recovery and freeze-thaw-wash recovery of cellular hemoglobin were calculated.

RESULTS

    Table 1 shows the measurement values and calculated recoveries on the 4 units. The expected values from the NBRL deglycerolization method are also reported.

Thawed Units: The thawed recoveries were estimated from a diluted sample provided by Rasor and indicate that the units had acceptable freeze thaw recoveries and supernatant hemoglobin levels.

Waste Collections: Units 3 and 4 had greater waste volumes. The supernatant hemoglobin concentrations in each of the 4 units was <500 mg%, within an acceptable range.

Washed Units: In the final deglycerolized units the supernatant hemoglobin concentrations were relatively high compared to NBRL standards, especially in Units 1 and 2. The freeze-thaw-wash recoveries were 85% or more, with the higher recoveries in Units 3 and 4. The osmolality values were higher than acceptable (541 to 703 mOs/kg) indicating a less efficient glycerol removal in these units than in those washed using the NBRL method (300 to 360 mOs/kg). Units 3 and 4 had slightly better glycerol removal suggesting that the procedure used in these units may have resulted in a more efficient glycerol washout.

Technical Notes:
1.   Enclosure 1, as written, indicates that the sampling of the thawed unit takes place after the unit is weighed. The sampling of the unit must be done before the unit is weighed.
2.   A larger volume sample must be obtained from the thawed unit in order to provide 1 ml of supernatant following centrifugation at 250 x g. for 10 minutes.

(Retyped from report sent from NBRL on 6/27/94)

Table 14

**IN VITRO RECOVERY, SUPERNATANT HEMOGLOBIN, OSMOLALITY AND
INTRACELLULAR AND EXTRACELLULAR SODIUM AND POTASSIUM
MEASUREMENTS ON PREVIOUSLY FROZEN RED BLOOD CELLS WASHED USING
THE RASOR PROTOTYPE DEGLYCEROLIZATION SYSTEM**

Blood Samples received from Rasor Associates, Inc.
253 Humboldt Court, Sunnyvale, California, 94089 on 8/03/94

Report Date: 8/03/94   **Series 2**

The Naval Blood Research Laboratory (NBRL) shipped 4 units of glycerolized red blood cells frozen at NBRL using the Primary Bag System to Rasor Associates, Inc. The red cells were thawed and deglycerolized using the Rasor prototype deglycerolization system. Seven samples from each unit were collected and shipped to the Naval Blood Research Laboratory along with the weights of the thawed units, waste collections and deglycerolized units.

Hematocrit, hemoglobin and supernatant hemoglobin were measured on the samples from thawed units and supernatant hemoglobin was measured on the waste collections. Hematocrit, hemoglobin, supernatant hemoglobin, osmolality, MCV, MCH, MCHC and extracellular sodium and potassium were measured on the deglycerolized units. The freeze-thaw recovery and freeze-thaw-wash recovery of cellular hemoglobin were calculated.

RESULTS

Table 1 shows the measurement values and calculated recoveries on the 4 units. The expected values from the NBRL deglycerolization method ar also reported.

Thawed Units: The mean thaw recovery of 96% was within the expected range; however, Unit No. 5041525 was slightly low at 92%.

Waste Collections: The supernatant hemoglobin concentrations in the waste collections were <600 mg%, within an acceptable range.

Washed Units: The mean wash recovery of 71% was estimated from the pre wash and post wash samples and was lower than the expected range of 80 to 95%. In the final deglycerolized units the mean supernatant hemoglobin concentration of 273 mg/dl was higher than the NBRL range. The osmolality values in these units 2343 400 mOs/kg or less, indicating that the remaining glycerol was at or below 1%.

The mean corpuscular volume (MCV) of the red cells was 78, which was lower than expected. This small cell size is characteristic of a potassium leak. The red cell potassium levels were reduced and varied from 0.6 to 4.7 mEq/10^{12} RBC. The potassium expressed as mEq/liter of red blood cells did not appear as reduced because the size of the red blood cells was smaller than normal.

(Retyped from report sent from NBRL on 8/03/94)

Table 15

IN VITRO RECOVERY, SUPERNATANT HEMOGLOBIN, OSMOLALITY AND INTRACELLULAR AND EXTRACELLULAR SODIUM AND POTASSIUM MEASUREMENTS ON PREVIOUSLY FROZEN RED BLOOD CELLS WASHED USING THE RASOR PROTOTYPE DEGLYCEROLIZATION SYSTEM

Blood Samples received from Rasor Associates, Inc.
253 Humboldt Court, Sunnyvale, California, 94089 on 8/3/94

Report Date: 8/17/94   Series 3

The Naval Blood Research Laboratory (NBRL) shipped 4 units of glycerolized red blood cells frozen at NBRL using the Primary Bag System to Rasor Associates, Inc. The red cells were thawed and deglycerolized using the Rasor prototype deglycerolization system. Samples from each unit were collected and shipped to the Naval Blood Research Laboratory along with the weights of the thawed units, waste collections and deglycerolized units.

Hematocrit, hemoglobin and supernatant hemoglobin were measured on the samples from thawed units and supernatant hemoglobin was measured on the waste collections. Hematocrit, hemoglobin, supernatant hemoglobin, osmolality, MCV, MCH, MCHC and extracellular and red blood cell sodium and potassium were measured on the deglycerolized units. The free-thaw recovery and freeze-thaw-wash recovery of cellular hemoglobin were calculated.

RESULTS

Thawed Units: The mean thaw recovery was 98% and all 4 units were within the expected range.

Waste Collections: The supernatant hemoglobin concentrations in the waste collections were <400 mg%, within an acceptable range and slightly lower than the last 4 units tested on 6/27/94.

Washed Units: The mean freeze-thaw-wash recovery of 83.4% was estimated from the pre wash and post wash samples and was within the expected range of 80 to 95%. The recovery of hemoglobin in the waste gave a similar value of 86%. A mean of 45 grams of cellular hemoglobin was recovered in the washed units, compared to a mean of 37 grams in the test units of 6/27.

In the final deglycerolized units the mean supernatant hemoglobin concentration of 222 mg/dl was higher than the NBRL range of 25 to 165 mg/dl. The osmolality values in these units ranged from 359 to 404 mOs/kg H_2O, indicating that the remaining glycerol was at or below 1%.

The mean corpuscular volume (MCV) of the red cells was 85, which was slightly lower than expected, and the red cell potassium levels were slightly reduced with a mean of 5 mEq/10^2RBC.

The hemoglobin recovery, supernatant hemoglobin, MCV, potassium and osmolality were similar in the 4 units showing consistency in the product.

(Retyped from report sent from NBRL on 8/17/94)

Table 16

THAWED HUMAN BLOOD TESTS AT IRWIN MEMORIAL (Summary)

RUN #	UNIT ID NUMBER	FROZEN BLOOD BAG START (gm)	FROZEN BLOOD BAG EMPTY (gm)	FROZEN BLOOD BAG BEFORE GLYS REMOVAL (gm)	SOLUTION ADDED (ml)	SALINE/GLUCOSE BAG START 0.9% (gm)	SALINE/GLUCOSE BAG END 0.9% (gm)	SALINE/GLUCOSE USED 0.9% (gm)	SALINE BAG START 12% (gm)	SALINE BAG END 12% (gm)	SALINE USED 12% (gm)	WASTE BAG START (gm)	WASTE BAG END (gm)	WASTE PRODUCED (gm)	PROCESSED BLOOD BAG START (gm)	PROCESSED BLOOD BAG END (gm)	PROCESSED BLOOD PRODUCED (gm)	DEGLYS TIME (min) (includes 8 min for prediutions)	PROCESSING RATE (ml/min)	AVERAGE TMP (mmHg)
1-A	NA	393	57	678	285	2141	119	2022	NA	NA	NA	207	2113	1906	NA	NA	430	23.9	400	8
1-B	NA	410	59	705	295	2137	58	2079	NA	NA	NA	207	2234	2027	NA	NA	364	24.4	400	15
1-C	NA	389	63	700	311	2137	141	1996	NA	NA	NA	207	2165	1958	NA	NA	393	23.6	400	13
1-D	NA	384	71	703	319	2119	219	1900	NA	NA	NA	207	2210	2003	NA	NA	188	22.8	400	14
2-A	5041525	393	57	678	285	2141	119	2022	155	98	57	207	2113	1906	NA	NA	430	28.8	300	14
2-B	5703982	410	59	705	295	2137	58	2079	183	128	55	207	2234	2027	NA	NA	364	29.5	300	19
2-C	4323712	389	63	700	311	2137	141	1996	101	31	70	207	2165	1958	NA	NA	393	28.5	300	18
2-D	3303751	384	71	703	319	2119	219	1900	171	106	65	207	2210	2003	NA	NA	188	27.3	300	20
3-A	2528031	434	57	756	322	2142	100	2042	173	107	66	206	2199	1993	31	406	375	33.1	250	NA
3-B	5703983	393	57	687	294	2137	54	2083	95	42	53	208	2247	2039	30	336	306	33.8	250	NA
3-C	2203282	393	57	686	303	2122	78	2044	167	111	56	222	2217	1995	33	424	391	33.2	250	NA
3-D	2527426	483	59	786	303	2164	79	2085	198	146	52	235	2291	2056	34	425	391	29.5	300	NA

WEIGHTS & VOLUMES

Table 17A

Table 17A
MEASUREMENTS OF DEGLYCEROLIZED THAWED HUMAN BLOOD BY NBRL

TEST ID #	BAG ID #	THAWED UNIT				WASTE			WASHED UNIT				
		NET WEIGHT (g)	HCT v %	HB (g/dl)	SUP HB (mg/dl)	NET WEIGHT (g)	UNSPUN HB (mg/dl)	HB (g)	NET WEIGHT (g)	HCT v %	HB (g/dl)	SUP HB (mg/dl)	OSMOLALITY (mOs/kg)
1-A		286	62.0	18.3	NA	1271	257		292	38.0	12.2	599	595
1-B		360	57.0	18.0	631	1241	471		354	37.5	12.0	599	703
1-C		381	56.0	17.4	289	1648	278		349	45.0	14.9	246	494
1-D		425	53.0	16.0	245	1605	300		411	43.0	13.9	353	541
2-A	5041525	336	59.0	51.0	3467	1908	514	9.6	430	25.0	34.0	310	381
2-B	5703982	351	59.0	54.0	1584	2027	321	6.5	384	39.0	44.0	171	360
2-C	4323712	326	60.0	48.0	1862	1958	375	7.3	393	37.0	44.0	225	400
2-D	3303751	313	61.0	48.0	856	2003	460	9.2	188	48.0	26.0	385	376
3-A	2528031	377	57.0	54.0	631	1998	300	6.0	375	42.0	45.0	225	404
3-B	5703983	336	57.0	50.0	1712	2039	385	7.9	306	43.0	39.0	246	359
3-C	2203282	336	60.0	51.0	678	1995	278	5.5	391	40.0	46.0	182	390
3-D	2527426	424	56.0	56.0	792	2056	364	7.5	391	42.0	49.0	235	404
EXPECTED VALUES FROM S.O.P.							200-600			32-47	25-165		300-360

Table 17B
MEASUREMENTS OF DEGLYCEROLIZED THAWED HUMAN BLOOD BY NBRL

TEST ID #	K + EXTRA-CELL (mEq/L)	K + INTRA-CELL (mEq/L)	K + RED CELL (mEq/RBC)	Na + EXTRA-CELL (mEq/L)	Na + INTRA-CELL (mEq/L)	MCV (cu. microns)	FRZ-THAW (%)	FRZ-THAW WASH WASTE(%)	FRZ-THAW WASH PRE/POST HB (%)
	K +			**Na +**		**MCV**	**RECOVERY**		
1-A	1.0	41.7		177	93.3		99.5		
1-B	1.2	66.5		174	43.6		95.0	86.0	
1-C	0.5	51.6		167	49.9		97.6	91.0	
1-D	0.6	60.9		163	39.0		97.3	91.0	
2-A	0.1	58	2.2	165	37.0	67	92.3	75.9	61.3
2-B	0.0	8	0.6	161	7.0	82	96.4	86.4	79.2
2-C	0.0	57	4.5	166	40.0	76	95.8	84.9	88.2
2-D	0.0	53	4.7	163	48.0	87	98.1	73.1	53.5
3-A	0.0	59	5.2	167	47.0	88	98.3	87.5	82.5
3-B	0.0	53	4.5	162	52.0	82	95.8	82.4	75.0
3-C	0.0	63	5.5	168	43.0	84	98.7	88.6	90.0
3-D	0.0	51	4.7	165	57.0	89	97.5	86.8	86.0
EXPECTED									
VALUES	.5-2	50-70	5.5-7.5	140	25-40	88-110	94-99	80-95	80-95

7.0 CONCLUSIONS

The objectives of this Phase I program were to demonstrate the feasibility and potential advantages of a new technical approach for the deglycerolization of thawed frozen human blood. This approach uses a hollow fiber separator to remove glycerol and saline wash solution from red cells in a recirculation process. It also uses a specially designed blood-saline mixing device, a unique non-mixing recirculation bag, and modified roller pumps to meet system requirements. The completely automatic operation of the system is achieved with microprocessor-controlled electronics, control logic implemented by software, and sensors and monitors to achieve consistent, reliable operation.

All of the proposed Phase I tasks were successfully completed, as described in detail above. All of the objectives, requirements, and design goals for this Phase I program have been achieved or were shown to be feasible (Table 1). Specific estimates and demonstrations of feasibility are given below with reference to the objectives, requirements, and design goals for Phase I given in Table 1, and the more recently defined TBPS requirements of Table 2.

a. Testing of the TBPS manually-operated prototype simulating automatic operation and control was performed with thawed frozen human red cells, following to the extent feasible the NBRL S.O.P. for deglycerolization.

b. The requirements for glycerol removal to below 1% in the washed red cell product, with acceptable levels of hemolysis (free plasma hemoglobin) and of intra-cellular potassium, were achieved. The data indicates that this procedure does not "harm the cells" any more than the NBRL S.O.P. implemented with the Haemonetics 115. However, future work should be able to decrease and minimize red cell damage and hemolysis, improve the quality of red cells washed, and meet the 21-day storage requirement for washed red cells in an appropriate additive solution.

c. The prototype testing achieved the deglycerolization requirements in under the 30 minutes objective, including the initial dilutions with 12% and 0.9% saline solutions following the NBRL S.O.P. with respect to flow rates, volumes, mixing, and equilibration times. The entire process will very likely be achieved in under 30 minutes per unit of blood, including all operator involvement (not just set-up) time. Future work should address optimizing the process to decrease time.

d. System testing and analysis demonstrated that safe, effective, fully automatic operation was achievable using control and monitoring methods evaluated during this program. The automatic control approach is very similar to that successfully implemented by RAI with an autotransfusion system using hollow fiber separation.

e. Operator interactions are minimized to simple, easy, rapid, user-friendly operations. No system control functions are performed by the operator except to initiate the process. It may be necessary for the operator to manually expel a small quantity of air from the final blood product in the blood output bag.

f. The disposable module of this system is made closed and sterile by proven methods likely to be accepted by the FDA for long-term (21 day) storage of washed red cells. The ability of the red cells to retain function and have acceptable levels of hemolysis for 21 days depends in large part on the storage solution used. The evaluations of washed red cells in storage solutions was not a part of this Phase I effort but should be addressed in future work. Storage solutions are successfully used to achieve long term RBC

storage for red cells which have not been frozen. Such FDA-approved solutions are expected to provide long-term storage for frozen, thawed, washed red cells as well.

g.   The current methods to wash thawed frozen platelets can also be implemented with the TBPS. Such a process may require a different disposable and different processing parameters. A separate software program may be developed and implemented for platelet washing. Such a program may be selected manually or may be selected automatically by using a bar code on the platelet washing disposable to select the appropriate software. This should be evaluated in future work.

h.   All of the key components of the system have been tested, requirements have been met, and their feasibility has been demonstrated. This includes the pressure sensors, control valves, blood-saline mixer (vertical blood bag shaker), hollow fiber separator, and the non-mixing recirculation bag.

i.   The inherent simplicity, high reliability, and low maintenance of the key components of this system have been proven in other applications, except for the bag shaker. Its simplicity and conservative design suggest that it, too, will be highly reliable, long-lived, and require little or no maintenance. The entire TBPS system can be expected to be rugged and reliable, with very minimal maintenance and with easy modularized removal, repair, or replacement of components.

j.   All of the weight and size objectives for the system were demonstrated by system design and analysis, and system prototype assembly.

k.   The environmental, electrical power, and electrical safety requirements can all readily be met.

l.   The disposables module and the control module have been shown to be less expensive than current deglycerolization disposables and hardware. The disposables cost is likely to be under $30, but this may be dependent on what disposable sensors are used for hematocrit, hemolysis, and glycerol concentration measurements.

m.   The monitoring of hematocrit, hemolysis, and glycerol concentration in the washed red cells is recommended to verify and ensure consistent high quality. The evaluation, development, and/or adaptation of technologies or existing devices for this purpose is recommended as future work.

In summary, the Phase I program has demonstrated the feasibility of this technical approach and has shown that all of the objectives, requirements, and design goals for the desired TBPS have been met or have a very high probability and low risk of being met in a future TBPS product.

8.0 REFERENCES

1. Richard M. Berkman, et al, "Development and Fabrication of an Aseptic Fluid Transfer System (AFTS)," Final Report #5040-7, Jet Propulsion Laboratory, Pasadena, California, National Heart and Lung Institute. September 20, 1974, #PB-250 787.

2. F. Castino, et al, "Plasma Separation by Membrane Filtration," Plasma Exchange Therapy, 1981, pp. 11-19.

3. Clark K. Colton, "Analysis of Membrane Processes for Blood Purification," 4th Annual Meeting of the Int. Society of Blood Purification, Osaka, November 6, 1986, pp. 202-251.

4. J.P. Crowley and C.R. Valeri, "The Purification of Red Cells for Transfusion by Freeze-Preservation and Washing," Transfusion, Nov-Dec 1974, Vol. 14, No. 6, pp. 590-594.

5. P.C. Farrell, K. Schindhelm and C.G. Roberts, "Membrane Plasma Separation," from the Center for Biomedical Engineering, University of New South Wales, Kensington, pp. 37-44.

6. Eric J. Fogt, "Continuous Ex-Vivo and In-Vivo Monitoring with Chemical Sensors," Clin. Chem., Vol. 36, No. 8, 1990, pp. 1573-1580.

7. John W. Gilbert, MD, et al, "Hematocrit Monitor," Critical Care Medicine, Vol. 17, No. 9, 1989, pp. 929-933.

8. Toshikazu Ishihara, et al, "Continuous Hematocrit Monitoring Method in an Extracorporeal Circulation System and Its Application for Automatic Control of Blood Volume during Artificial Kidney Treatment," Artificial Organs, Vol. 17, No. 8, pp. 708-716.

9. S.R. Jurtz, et al, "Leukocyte-Poor Red Blood Cells Prepared by the Addition and Removal of Glycerol from Red Blood Cell Concentrates Stored at 4 C," Transfusion, July-August 1981, Vol. 21, No. 4, pp. 435-442.

10. A.J.P.F. Lombarts, et al, "Basic Principles and Problems of Haemocytometry," Ann. Clin. Biochem, Vol. 23, 1986, pp. 390-404.

11. Yitzhak Mendelson, Ph.D., John J. Galvin, and Yi Wang, "In-Vitro Evaluation of a Dual Oxygen Saturation/Hematocrit Intravascular Fiberoptic Catheter," Biomedical Instrumentation and Technology, May/June 1990, pp. 199-206.

12. H.T. Meryman and M. Hornblower, "Quality Control for Deglyceroiized Red Blood Cells," Transfusion, May-June 1981, Vol. 21, No. 3, pp. 235-240.

13. Harold T. Meryman, M.D., et al, "Extending the Storage of Red Cells at 4°C," Transplantation Department, Jerome H. Holland Laboratory for the Biomedical Sciences.

14. Martin S. Meyer and Gary L. Easley, "Optical Fiber Refractometer," Rev. Sci. Instrum. Vol. 58, No. 11, November 1987, pp. 2047-2048.

15. Gerald L. Moore, "Long-Term Storage and Preservation of Red Blood Cells," Chapter 2, pp. 31-46.

16. G.L. Moore, J.R. Hess, and M.E. Ledford, "In Vivo Viability Studies of Two Additive Solutions in the Postthaw Preservation of Red Cells Held for 3 Weeks at 4°C", Transfusion, Vol. 33, No. 9, 1993, pp. 709-712.

17. J.M. Radovich, et al, "Evaluation of Membrane Systems for Washing/Deglycerolizing Packed Red Blood Cells," Final Report, January 5, 1989, U.S. Army Medical Research and Development Command, Ft. Detrick, Frederick, MD, Contract No. DAMD17-86-C-6142.

18. Michael S. Rosenblatt, MD, Erwin F. Hirsch, MD, and C. Robert Valeri, MD, "Frozen Red Blood Cells in Combat Casualty Care: Clinical and Logistic Considerations," Department of Surgery and Naval Blood Research Laboratory, Boston University School of Medicine, Boston, MA.

19. D.G. Ross, W.A.L. Heaton, and S. Holme, "Additive Solution for the Suspension and Storage of Deglycerolized Red Blood Cells," Vox Sang 1989; Vol. 56, pp. 75-79.

20. B.A. Solomon, "Membrane Separations: Technological Principles and Issues," Trans Am Soc Artif Intern Organs, Vol. XXVII, 1981, pp. 345-350.

21. K. Sprenger and H.E. Franz, "Membrane Plasma Separation: Procedural Recommendations," Proc. EDTA, Vol. 17, 1980, pp. 353-356.

22. Tova A. Stepner and Edward F. Leonard, "Evaluation of Plasmapheresis Membranes, Journal of Membrane Science, Vol. 23, 1985, pp. 285-302.

23. Capt. C.R. Valeri, et al, "Freeze-Preserved Baboon Red Blood Cells: Effects of Biochemical Modification and Perfusion in Vitro," Am. J. Vet. Res, 1981, Vol. 42, No. 9, pp. 1590-1594.

24. C.R. Valeri, et al, "An Integrated Liquid-Frozen Blood Banking System for Operational Facilities," Office of Naval Research, Contract NO0014-79-C-0168, January 12, 1982.

25. C.R. Valeri, "Preservation of Red Blood Cells and Platelets: Freezing, Storage, Recovery, and Quality," Blood Separation and Plasma Fractionation, 1991, pp. 127-153.

26. C.R. Valeri, "Simplification of the Methods for Adding and Removing Glycerol During Freeze-Preservation of Human Red Blood Cells with the High or Low Glycerol Methods: Biochemical Modification Prior to Freezing," Transfusion, Vol. 15, No. 3, May-June 1975, pp. 195-218.

27. C.R. Valeri, et al, "Freezing in the Primary Polyvinylchloride Plastic Collection Bag: A New System for Preparing and Freezing Nonrejuvenated and Rejuvenated Red Blood Cells," Transfusion, March-April 1981, Vol. 21, No. 2, pp. 138-149.

28. C.R. Valeri, "The U.S. Navy's Experience with resuscitation of Wounded Servicemen in

Vietnam Using Frozen Washed Red Blood Cells - 1966-1973; Developments from This Experience," Office of Naval Research, Contract NO0014-79-C-0168, Naval Blood Research Laboratory, Boston, MA, February 18, 1982.

29. C.R. Valeri, "Blood Banking and the Use of Frozen Blood Products," Naval Blood Research Laboratory, Chelsea, MA, CRC Press, Inc., pp. 247-290.

30. C.R. Valeri, "Standard Operating Procedure for Preparation, Freezing, Transportation, Thawing, Washing and Quality Control of Human Platelets Isolated by Mechanical Cell Separating Systems and Frozen with Dimethylsulfoxide," Naval Blood Research Laboratory, Boston, MA, December 20, 1993.

31. C.R. Valeri, et al, "The Safety and Therapeutic Effectiveness of Human Red Cells Stored at - 80°C for as Long as 21 Years," Transfusion, 1989, Vol. 29, No. 5, pp 429-437.

32. D.J. Webb, et al, "A Novel Interferometric Liquid Refractometer," Rev. Sci. Instrum, Vol. 60, No. 10, October 1989, pp. 3347-3348.

33. A.A. Zaidi, "Simple Laser Refractometer for Liquids," Rev. Sci. Instrum., Vol. 58, No. 7, July 1988, pp. 1153-1155.

34. A.A. Zaidi, et al, "Accurate Method for the Determination of the Refractive Index of Liquids Using a Laser," Rev. Sci. Instrum., Vol. 60, No. 4, April 1989, pp. 803-805.

35. Andrew L. Zydney and Clark K. Colton, "Fundamental Studies and Design Analyses for Cross-Flow Membrane Plasmapheresis," Proc. Int Symposium on Artificial Organs, Biomedical Engineering and Transplantation, Salt Lake City, Utah, January 20-23, 1986, pp. 343-358.

36. Andrew Lawrence Zydney, "Cross-Flow Membrane Plasmapheresis: An Analysis of Flux and Hemolysis," Partial Fulfillment of the Requirements for the PhD. at MIT, February, 1985.

37. Andrew L. Zydney and Clark K. Colton, "A Red Cell Deformation Model for Hemolysis in Cross Flow Membrane Plasmapheresis," Chem. Eng. Commun., Vol. 30, 1984, pp. 191-207.

38. A.L. Zydney and C.K. Colton, "Continuous Flow Membrane Plasmapheresis: Theoretical Models for Flux and Hemolysis Prediction," Trans Am Soc Artif Intern Organs, Vol. XXVIII, 1982, pp. 408-412.

39. Andrew L. Zydney and Clark K. Colton, "A Concentration Polarization Model for the Filtrate Flux in Cross-Flow Microfiltration of Particulate Suspensions," Dept. of Chemical Engineering, MIT, Cambridge, MA, December 17, 1985.

40. Andrew L. Zydney and Clark K. Colton, "Augmented Solute Transport in the Shear Flow of a Concentrated Suspension," PCH Phsico Chemical Hydrodynamics, Vol. 10, No. 1, 1988, pp. 77-96.

41. Standard Operating Procedure, Naval Blood Research Laboratory, Boston University School of Medicine, Boston, MA, Revised 4/1992.

42. Personal communication with Jean Stanley, MT(ASCP)SBB, Technical Director, Blood Bank of the Alameda-Contra Costa Medical Association, Oakland, CA.

APPENDIX

BLOOD SAMPLES FOR NBRL MEASUREMENTS

1.0 PURPOSE

The Naval Blood Research Laboratory (NBRL) in Boston will be supplying frozen blood and performing analyses of blood, waste, and supernatant samples before and after deglycerolization. The weight of the thawed glycerolized red blood cells, the weight of the deglycerolized red blood cells, and the weight of the waste solution must be provided to the NBRL in order to calculate the *in vitro* recovery of the red blood cells.

2.0 SAMPLING

2.1 Thawed Unit (predilutions, prewash)
- A. Preblood: 5 ml mixed blood sample.
- B. Presup: Supernatant sample. Put 10 ml blood into a tube. Centrifuge at 3000 rpm (hard spin). Remove supernatant into another tube for shipment.
- C. Pre-RBC: RBC remaining from Step 2.1(B).
- D. Weight of the thawed glycerolized red blood cells.

2.2 Washed Unit
- A. Weight of the washed deglycerolized red blood cells.
- B. Postblood: 5 ml blood sample.
- C. Postsup: Supernatant sample. Hard spin of 10 ml blood sample. Remove supernatant to another tube for shipment.
- D. Postrbc: RBC remaining from Step 2.2(C).

2.3 Waste (after all dilutions and washings)
- A. Weight of the waste solution.
- B. Waste: 5 ml mixed waste sample.

3.0 SHIPPING PROCEDURES

There will be seven (7) samples (tubes) sent to NBRL for each unit of frozen blood, together with the weights of the thawed, glycerolized red blood cells, the washed-deglycerolized red blood cells, and the waste solution. The tubes should be unbreakable (not glass) and should be capped and sealed (leak-tight). Each tube should be labelled with the test number and type (Preblood, Presup, etc.). All tubes should be packaged in wet ice within a container that is sealed (leak-tight) and placed in another container which provides good thermal and mechanical insulation. This container is sent by Federal Express One-Day delivery to:

> Naval Blood Research Laboratory
> Boston University School of Medicine
> 615 Albany Street
> Boston, MA 02118
> (Ph: 617-638-4950)
> Attn: Ms. Marilyn Leavy

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)   25 Jan 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 written for the following Awards.

            DAMD17-91-C-1105   ADB206577
            DAMD17-93-C-3081   ADB202797
            DAMD17-94-C-4049   ADB190895
            DAMD17-95-C-5033   ADB206103
            DAMD17-95-C-5035   ADB231001
            DAMD17-95-C-5036   ADB208050

Request the limited distribution statement for Accession Document
Numbers 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