CHAPTER 1

BACKGROUND AND CORD BLOOD BANK (CBB) ORGANIZATION

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1.1 OVERVIEW OF THE CORD BLOOD TRANSPLANTATION STUDY

Bone marrow transplantation (BMT) from HLA-identical sibling donors has been successfully utilized in the treatment of high-risk or recurrent hematological malignancies, bone marrow failure syndromes and selected hereditary immunodeficiency states and metabolic disorders. Use of allogeneic BMT has been limited both by the lack of suitable donors, and because of the risk of life-threatening complications that arise when donor and recipient are not immunologically identical, namely, graft failure and graft-versus-host disease (GVHD).

In an attempt to increase the availability of suitable donors and reduce the morbidity and mortality associated with allogeneic bone marrow transplantation, clinical investigators worldwide have evaluated placental and umbilical cord blood as an alternate source of hematopoietic stem and progenitor cells for transplantation. Early successes with the transplantation of umbilical cord blood have prompted considerable investigation in this stem cell source. Numerous laboratory investigators have subsequently confirmed the high frequency of primitive hematopoietic progenitors and have begun to describe the functional capacities of the neonatal immune system. As a result of these clinical and laboratory observations, large scale banking of umbilical cord blood for clinical transplantation has been initiated in the U.S. and Europe.

As of December 1996, umbilical cord blood from sibling and unrelated donors has been used to reconstitute hematopoiesis in more than 375 patients with malignant and non-malignant disorders treated with myeloablative therapy. Reports from individual institutions and the International Cord Blood Transplant Registry (ICBTR) suggest that umbilical cord blood contains sufficient numbers of hematopoietic stem and progenitor cells for both early and late engraftment at least in recipients weighing less than 40 kilograms. Moreover, limited comparisons with young patients transplanted with bone marrow from sibling donors suggest that the risk of severe acute graft-versus-host disease (GVHD) may be lower in those transplanted with umbilical cord blood. To date, too few patients have been transplanted to know what are the true risks and benefits of this stem cell source.

1.1.1 Unrelated Donor Umbilical Cord Blood Transplantation

As a result of the early successes with umbilical cord blood from sibling donors, pilot programs for the banking of unrelated donor umbilical cord blood have been proposed in many countries worldwide and initiated in New York, Milan, Dusseldorf, Paris and London. Potential benefits of banked umbilical cord blood include: 1) absence of donor attrition, 2) rapid availability, and 3) minimal risk or inconvenience to the donor. Additional advantages which remain to be determined include: 1) low risk of transmissible infectious diseases, such as cytomegalovirus and Epstein-Barr virus, 2) lower risk of acute and chronic GVHD as compared to unrelated-donor marrow transplants, and 3) ability to tolerate HLA mismatched transplants.

1.1.2 **Summary**

The aim of this research is to establish Cord Blood Banks (CBB) and develop standardized collection, processing, and cryopreservation procedures. These banks will collect, process and store up to 15,000 cord blood units which can be used in unrelated hematopoietic stem cell transplantation. In addition to the CBBs, transplant centers will participate in this research project. The purpose of the transplant study is to accurately describe 180-day survival and other events after umbilical cord blood transplantation.

1.2 **OVERVIEW OF CBB ORGANIZATION**

The participating investigators in the Cord Blood Transplantation Study (COBLT) collaborate through an organization designed to maintain a continuity of operations and to facilitate effective communication and cooperation among the units. The National Heart, Lung and Blood Institute (NHLBI) Project Officer, the NHLBI-appointed Chairperson, the Principal Investigators from the Transplant Centers (TC), the Cord Blood Banks (CBB), and the Medical Coordinating Center (MCC) comprise the Steering Committee, which is responsible for the design, execution, and analysis of the study.

The success of a multi-center endeavor depends on the cooperation of the staff in all participating units to perform their tasks and responsibilities in an efficient, effective, and timely manner. The participating units in the COBLT study (i.e., Transplant Centers, Cord Blood Banks, MCC, and Program Office) are shown in Exhibit 1-1. The Transplant Centers' approach to treatment administration is defined by the COBLT protocol. An independent Manual of Procedures describes the study organization, the transplant center data forms, and special transplant center study procedures.

This CBB Standard Operating Procedures (SOP) manual describes the study organization, study procedures, and data forms for the Cord Blood Banks. This chapter will provide a detailed description of the CBB organizational structure as well as define the roles and purposes of the collaborating CBBs.

1.2.1 **Organization**

UCLA and the Carolinas' Cord Blood Bank at Duke University are the two NHLBI-funded Cord Blood Banks in the COBLT study. Cord Blood Banks are responsible for collecting, screening, testing, freezing and shipping all cord blood units, and for collecting all clinical, laboratory, demographic, and other data pertaining to the cord blood units. They are also responsible for ensuring donor confidentiality and maintaining linkage for all donor units. Exhibit 1-2 shows the organizational structure for the Cord Blood Banks.

Each Cord Blood Bank is led by a Principal Investigator who is responsible for ensuring that all aspects of the COBLT Standard Operating Procedures are followed. Other key Cord Blood Bank staff include the Medical Director, processing coordinator, collection/distribution coordinator, laboratory technicians and assistants, and administrative personnel. The responsibilities of the Principal Investigator, Processing Coordinator and Collection/Distribution Coordinator are further defined below.

EXHIBIT 1-1
COBLT Study Participating Units

Transplant Centers

Dana-Farber Cancer Institute
Duke University
Fred Hutchinson Cancer Research Center
Indiana University
University of Minnesota
University of California - Los Angeles
Children's Hospital of Los Angeles
Additional Centers (TBA)

Cord Blood Banks

Carolinas' Cord Blood Bank
University of California - Los Angeles

HLA Reference Laboratories

University of California, Los Angeles
University of California, San Francisco
Navy Medical Research Institute

Program Office

NHLBI

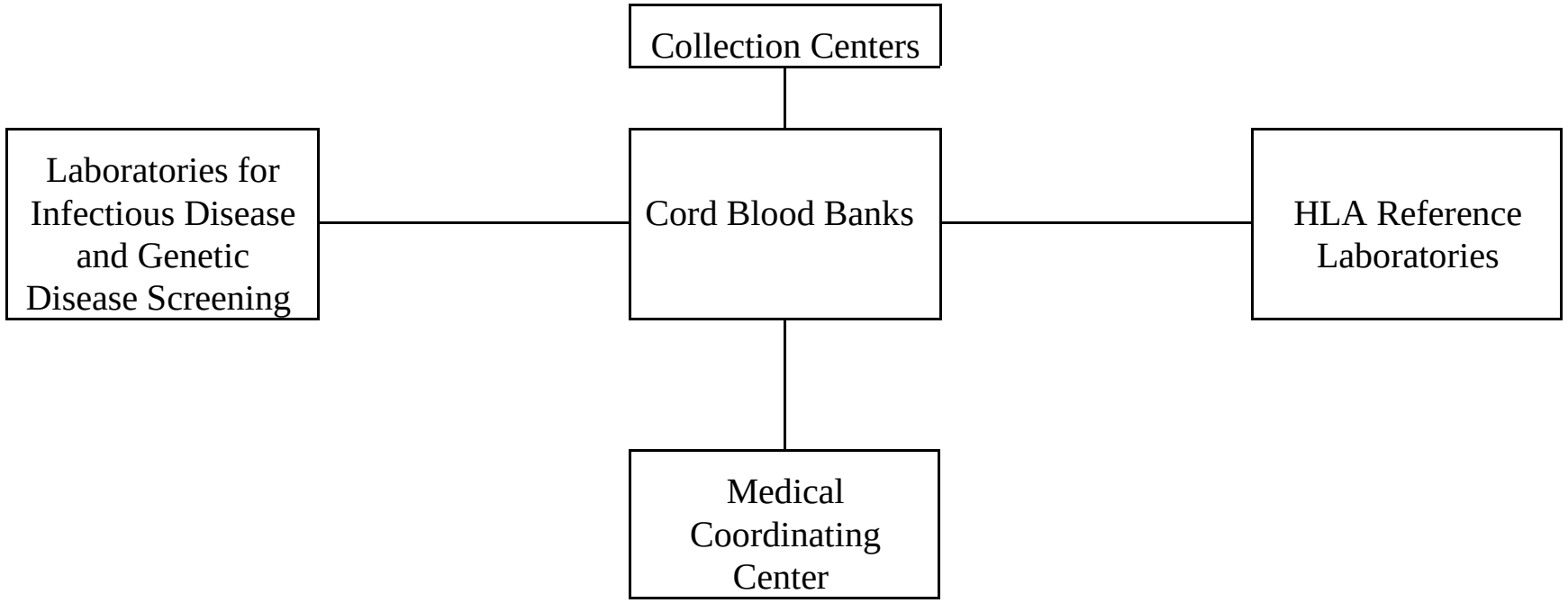
Division of Blood Diseases and Resources Program Office

Office of Biostatistics Research

Medical Coordinating Center

The EMMES Corporation

EXHIBIT 1-2
Cord Blood Banks Organizational Chart



1.2.2 **Functions of the Principal Investigator**

The primary responsibility of the Principal Investigator (PI) is to direct the activities of COBLT personnel in the Cord Blood Bank. The PI is responsible for maintaining communications with all collaborating laboratories and collection centers. Other duties of the PI include the following:

- Represent the Cord Blood Bank at meetings of the Steering Committee and Technical Subcommittees
- Coordinate the scientific and administrative operations of the Cord Blood Bank
- Ensure adherence by Cord Blood Bank personnel to the procedures described in and required by the COBLT Standard Operating Procedures
- Spend sufficient time in the Cord Blood Bank to adequately observe study procedures
- Ensure that personnel performing COBLT procedures are properly trained and certified
- Communicate with the Medical Director regarding the release of CBUs from quarantine and other relevant clinical issues
- Ensure the confidentiality of all donors while maintaining linkage between cord blood units (CBU) and donors.
- Ensure a confidential file of all notification records associated with donors testing positive for an infectious disease or genetic screening test.
- Assure the Cord Blood Bank's fiscal responsibility in the disposition of COBLT funds

1.2.3 **Functions of the Processing Coordinator**

The Processing Coordinator is responsible for supervising daily operations in the Cord Blood Bank and serves as a primary contact for the Medical Coordinating Center (MCC). The duties of the Processing Coordinator include:

- Ensure the accuracy, completeness, and consistency of reported data
- Ensure compliance with the COBLT Standard Operating Procedures, with particular concern to all aspects of CBU processing
- Notify the MCC of changes or impending changes in the Cord Blood Bank personnel, address(es), or telephone number(s)
- Maintain a file of correspondence with the MCC
- Maintain an up-to-date CBB Standard Operating Procedures

- Check completed data forms for accuracy and completeness
- Ensure that donor names, social security numbers, and any other personal identifiers are removed from all materials sent to the MCC
- Submit complete data to the MCC in a timely manner
- Communicate with the MCC regarding data processing matters concerning study forms and edit messages as appropriate
- Report irregularities or problems that can affect the data quality to the PI and the Protocol Monitor
- Track every cord blood unit received by the processing laboratory and its disposition
- Ensure that specimens for HLA typing, infectious disease screening, and genetic disease screening are collected and shipped to appropriate laboratories
- Participate in regularly scheduled, structured telephone calls with the Protocol Monitor from the MCC
- Other duties as defined by the Steering Committee, Technical Subcommittees, or Data and Safety Monitoring Board (DSMB)

1.2.4 **Functions of the Collection/Distribution Coordinator**

The Collection/Distribution Coordinator is responsible for supervising daily operations in the Collection Centers. The Collection/Distribution Coordinator regularly communicates with the Processing Coordinator and PI regarding issues related to the collection of the cord blood units, obtaining informed consent and medical chart data collection. The duties of the Collection/Distribution Coordinator include:

- Ensure that potential Cord Blood donors receive appropriate information about the study, including the Informed Consent statements
- Ensure that Informed Consent is obtained in a timely manner in accordance with CBB procedures
- Collect necessary maternal history and samples
- Collect CBUs according to the procedures specified in the CBB - SOP
- Supervise the transport of CBUs to the CBB and may also coordinate release and transport of CBUs to transplant centers

Each Processing and Collection/Distribution Coordinator will be given a copy of the CBB Standard Operating Procedures.

1.3 **FUNCTIONS OF THE MEDICAL COORDINATING CENTER**

The Medical Coordinating Center will collect data from the CBBs, distribute HLA types and performs searches. Other responsibilities are further defined in the COBLT Transplant Center - Manual of Procedures.

1.4 RECRUITING ADEQUATE NUMBERS OF PATIENTS

A critical task for all clinical studies is the enrollment of adequate numbers of patients, a task that often proves to be more difficult than anticipated. A recruitment goal has been established for each Transplant Center based on each Principal Investigator's assessment of the number of patients available. Each Transplant Center should develop a plan for tracking transplants and transplant candidates to ensure meeting this recruitment goal and review this plan continually throughout recruitment in order to determine its effectiveness. The plan must outline methods to identify and enroll minorities and women, in strict adherence to National Institutes of Health (NIH) and Department of Health and Human Services (DHHS) policies, as originally stated in the Request for Proposals for the COBLT Study. If the Transplant Center is not achieving its recruitment goal in a timely fashion, the plan will be modified.

1.4.1 Anticipated Accrual of Minority and Female Subjects

Minority Donors. Racial and ethnic groups vary in the diversity of their human leukocyte tissue antigens (HLA) haplotypes. In groups where many members have similar HLA types, not as many potential donors are needed. In groups with wide polymorphism among their HLA types, relatively more donors are needed. This may be mitigated somewhat by the ability to perform HLA-mismatched transplants.

By contract each cord blood bank must recruit a specific number of donors from minority populations. The number of units required from each group was calculated to enable potential transplant recipients from any group to have similar chances of finding a suitably matched cord blood unit from within the study's cord blood banks. This approach will maximize the number of minority transplant patients enrolled in the study. The targets for the cord blood bank are 43% Caucasian, 30% African-American, 17% Hispanic, and 10% Asian-American cord blood units. It is anticipated that approximately 51% of units will come from male donors and 49% from female donors, reflecting the proportion of births.

Minority Transplant Recipients. The population of patients eligible for this trial is restricted to patients who are able to find an unrelated cord blood donor matched or slightly mismatched for their HLA type from the study's cord blood banks. Previous studies in unrelated donor marrow transplantation have not indicated that outcomes are related to race or ethnicity.

The sample size for this study is approximately 400 patients. Based on the ethnicity of the first 156 unrelated cord blood transplants performed by the seven participating transplants centers, we estimate that the study will comprise the following numbers of patients:

African American	Asian/Pacific Is.	Caucasian	Hispanic	Native American
51	13	302	31	2

Exploratory analysis of engraftment and disease-free survival will be conducted to determine if there is evidence of a minority group effect in this study.

The number of unrelated cord blood transplants is increasing each year. The number of minority cord blood transplants is expected to increase faster than the number of Caucasian cord blood transplants.

This is due to the emphasis and contract requirements placed on the COBLT blood banks to recruit specified numbers of donors from all the minority groups.

If the results of this study show that cord blood contains sufficient numbers of cells to reconstitute adult size patients without an unacceptable increase in graft failure and relapse, it is likely that cord blood transplants will become more common and the number of transplants for minority patients will increase. Thus, despite the relatively small number of minority patients expected to enroll in this trial, the study may have a significant impact on the future of transplants for minority patients.

Based on previous studies in similar patient populations, we expect to enroll approximately 60% male patients and 40% female patients. Comparison of engraftment and disease-free survival by gender will be conducted in this study to determine if there is evidence of a gender effect on outcome.

CHAPTER 2

MATERNAL/DONOR ISSUES

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MATERNAL/DONOR ISSUES

2.1 EDUCATION

Principle

Education will be provided to a potential mother/donor of an umbilical cord blood unit so that she can make an informed choice on donation of her infant's cord blood to the Cord Blood Transplantation Study. At the very least, information will be given at the time informed consent is requested. However, attempts will be made to provide education to potential donors and to the community at large, using the guidelines listed below.

Guidelines

1. Brochures (Appendix A) may be placed in OB/delivering physician's offices. The brochure describes the donation and the COBLT study in generally understandable terms and includes a phone number to call for further information.

2. Brochures can be translated into several different languages, including, but not limited to English, Spanish, Chinese, Japanese, Vietnamese and Korean.

3. In-service sessions may be provided for the OB's/delivering physicians/nurse midwives to educate them about the purpose of the project and to give them the information they need to answer their patients' questions when they arise.

4. Public service announcements describing the Cord Blood Transplantation Study may be aired on local television and radio stations. Both local and nationally recognized celebrity spokespersons from various ethnic groups may be recruited to make these announcements.

5. Presentations before community groups can provide further education to potential donors. These presentations may include an appearance by celebrity spokespersons from various ethnic groups, with an instructional presentation and question-answer session given by the medical and technical staff of the Cord Blood Banks.

6. Booths may be set up at community events to promote the Cord Blood Transplantation Study and to provide information to the public regarding the program. Appearances by celebrity spokespersons may be arranged, with the booths manned by the medical and technical staff of the Cord Blood Banks and trained volunteers.

7. A phone line may be set up to facilitate responding to a potential donor's questions. If established, the phone line could have a recorded message, available in several languages, which could provide the caller with basic information and allow them to leave their name and phone number so that their call may be returned.

2.2 **OBTAINING INFORMED CONSENT**

Principle

Informed consent is a process that begins with information, encompasses a dialogue, and culminates with a written, signed document. The informed consent process must begin prior to the start of active labor and be obtained from every mother/donor, as detailed below. Consent will not be obtained during active labor or while the mother is under the influence of sedation or mood altering medications. Active labor should be determined at each collection center after consultation with the mother/donor's medical providers. Mothers/donors who do not receive information about the study prior to the start of active labor will not be considered eligible to participate in the study.

Obtaining Informed Consent

1. The informed consent process may start after the 22nd week of pregnancy and must be initiated prior to the start of active labor.

2. If written consent is obtained prior to delivery, verbal affirmation will be obtained in the hospital prior to administration of the maternal history questionnaire and drawing of maternal blood.

3. If written informed consent is not obtained prior to the start of active labor, verbal consent or a completed preliminary informed consent document, as well as documentation that the informed consent process started prior to the start of active labor, must be obtained prior to CBU and maternal sample collection. Consent must be reaffirmed using the full informed consent document following CBU collection and before hospital discharge.

4. Three signed copies of the informed consent document will be obtained. One copy will be placed in the mother/donor medical chart and one copy in the confidential CBB file. The third copy will be given to the mother/donor. No study bar code labels should be placed on the informed consent document in the mother/donor medical chart or given to the mother/donor. A study bar code label may be placed on the consent document for the confidential CBB file.

5. The Collection/Distribution Coordinator will alert the laboratory at the earliest possible time to discard the unit if consent is not given for donation of the cord blood unit, or if the medical history indicates that the unit should not be kept.

2.3 **OBTAINING DONOR MEDICAL HISTORY AND DELIVERY INFORMATION**

Principle

Obtaining a detailed medical history from mothers/donors provides a method of screening cord blood units for hereditary and infectious diseases which could potentially be transmitted to a CBU recipient. The Medical History Form is a questionnaire designed to review the past and current medical history of the mother/donor. The questionnaire is completed by trained personnel during an interview with the mother/donor following delivery and informed consent.

Obtaining information about the delivery of the donor's infant provides a method of screening cord blood units. The Donor and Delivery Information Form should be completed from a review of the medical chart after an informed consent has been signed.

Materials

Volunteer Cord Blood Donor Information Form
Informed consent document
Medical History Form
Donor and Delivery Information Form
Confidential manila envelope
Ink Pen
Bar code reader (*optional)*

Procedure

Obtaining Medical History

1. Obtain or reaffirm informed consent to participate in the study and complete the Volunteer Cord Blood Donor Information Form. Place one copy of the informed consent for the CBB confidential file in the confidential manila envelope.

2. Take the Medical History Form from the collection kit paperwork and verify that the bar code number on each page matches the bar code number on the Volunteer Cord Blood Donor Information Form. Place the Volunteer Cord Blood Donor Information Form in the confidential manila envelope.

3. Read each question on the Medical History Form to the mother/donor and record the answers using an ink pen. The interview should be conducted with the maximum amount of privacy that is possible. Document items as indicated on the form. Corrections to the form must be made at the time of the interview. Corrections must be made by drawing a single line through the incorrect information, recording the correct information, and dating and initializing the change.

4. The interview may be stopped if an exclusion criterion is met or if the mother/donor decides not to continue. If the interview is not completed, document the reason(s) for not completing the

interview on the 'Comment' line at the end of the form. As soon as possible, the processing laboratory should be notified to inform them that the unit is now unavailable so that unnecessary testing and processing can be avoided.

5. When all questions have been asked, review each page and make corrections or additions as necessary before finishing the interview.

6. Sign and date the form. Place the completed Medical History Form with the confidential manila envelope.

Obtaining Delivery Information

1. Remove the Volunteer Cord Blood Donor Information Form from the confidential manila envelope and verify that the hospital ID and mother/donor name matches the medical record. Verify that the bar code number of the Donor and Delivery Information Form matches the bar code number on the Volunteer Cord Blood Donor Information Form.

2. Return the Volunteer Cord Blood Donor Information Form to the confidential manila envelope. If the maternal blood samples have already been obtained, then seal the confidential manila envelope and sign in ink across the seal.

3. Complete the Donor and Delivery Information Form using the medical chart.

4. Sign and date the form. Place the form with the confidential manila envelope for return to the Cord Blood Bank.

Quality Control

1. The bar code numbers will be checked before each interview.

2. The questionnaire will be conducted by trained personnel.

3. The questionnaire will be reviewed, at minimum, by the person administering the questionnaire at the time of the interview for completeness and accuracy.

4. Linkage of the unit to the mother/donor will be maintained using the Volunteer Cord Blood Donor Information Form. Confidentiality will be maintained by placing confidential forms (VCBDI and one copy of the informed consent) in the confidential manila envelope, sealing the envelope, and placing a signature across the seal.

5. Cord Blood Banks will have procedures in place to allow the mother/donor to notify confidentially the interviewers of changes to the Medical History Form.

2.4 **OBTAINING MATERNAL BLOOD SAMPLES**

Principle

Samples for infectious disease testing will be obtained from the mother/donor of the umbilical cord blood unit. These samples will be collected and handled according to the following procedure so that samples are obtained as efficiently and with as little risk of error in identification as possible.

Policy

This sample collection procedure will be performed either by employees of the collection site or employees of the Cord Blood Bank, as determined by the policies of the individual collection sites. Samples will be obtained at the time consent is given or reaffirmed and after medical history is taken following delivery.

Materials

Two 7 ml red-top (serum-clot) Vacutainer tubes
One 7 ml lavender-top (EDTA) Vacutainer tube
Mother's hospital labels
Volunteer Cord Blood Donor Identification Form
Maternal Sample Form
Biohazard specimen bag
Manila envelope containing mother's sample bar code labels

Procedure

When consent is completed, the Clinical Research Nurse (CRN) either draws the blood samples or requests the collection site's phlebotomist to draw the mother's samples, based on the policies of the individual collection sites. The appropriate procedure given below will be followed, based on the identity of collecting personnel.

<u>For Samples Drawn by Cord Blood Bank CRN</u>

1. The CRN verifies the bar code label from the manila envelope matches that on the Volunteer Cord Blood Donor Identification Form and then labels two 7ml red top and one 7ml lavender top Vacutainer tubes with the bar code labels.

2. The CRN verifies the mother's hospital label on the Volunteer Cord Blood Donor Identification Form matches the mother's wrist band.

3. The CRN draws the blood samples from the mother's arm into the supplied tubes.

4. The CRN places the samples and the extra mother's sample bar code labels in a biohazard specimen bag for transport to the Cord Blood Bank.

5. The CRN verifies that the bar code label on the Volunteer Cord Blood Donor Identification Form matches the bar code labels on the Maternal Sample Form and collection tubes. The CRN records on the Maternal Sample Form the date and time when the samples were drawn, then signs the form, and adds the study ID number.

6. The completed Maternal Sample Form is placed with the confidential manila envelope for return to the Cord Blood Bank.

For Samples Drawn by the Collection Site's Phlebotomist

1. After consent is obtained, the Clinical Research Nurse (CRN) requests the collection site's phlebotomist to draw the mother's samples. The CRN labels two 7ml red top and one 7ml lavender top Vacutainer tubes with the mother's hospital labels. The phlebotomist draws the blood samples from the mother's arm into the supplied tubes.

2. Immediately after the samples are drawn, they are given to the CRN who verifies the labels on the tubes match the hospital label on the Volunteer Cord Blood Donor Identification Form.

3. After the consent and medical history interview are completed, the CRN verifies the bar code label from the manila envelope matches the bar code label on the Volunteer Cord Blood Donor Identification Form, removes the hospital labels from the sample tubes and relabels the tubes with the bar code labels.

4. The CRN places the samples and the extra mother's sample bar code labels in a biohazard specimen bag for transport to the cord blood bank.

5. The CRN verifies that the bar code label on the Volunteer Cord Blood Donor Identification Form matches the bar code labels on the Maternal Sample Form and collection tubes. The CRN records on the Maternal Sample Form the date and time when the samples were drawn, then signs the form, and adds the study ID number.

6. The completed Maternal Sample Form is placed with the confidential manila envelope for return to the Cord Blood Bank.

Quality Control

For Samples Drawn by Cord Blood Bank CRN

1. The CRN verifies the hospital label on the Volunteer Cord Blood Donor Identification Form matches the mother's wrist band and signs the form.

2. The CRN cross checks the bar code labels from the manila envelope with the Volunteer Cord Blood Donor Identification Form before labeling the tubes into which the samples are drawn.

3. Only one set of forms, labels, and tubes are to be brought into the room at one time. If other interviews are to be conducted immediately before or after, the forms, labels, and tubes for

other donors are to be left in a secure location outside the room.

For Samples Drawn by the Collection Site's Phlebotomist

1. The CRN and the phlebotomist verify the patient's arm band with the hospital labels on the tubes and sign the Volunteer Cord Blood Donor Identification Form.

2. The CRN verifies the hospital labels on the tubes with the Volunteer Cord Blood Donor Identification Form, and cross checks the bar code labels from the manila envelope with the Volunteer Cord Blood Donor Identification Form before relabeling the tubes into which the samples have been drawn.

3. Only one set of forms, labels, and tubes are to be brought into the room at one time. If other interviews are to be conducted immediately before or after, the forms, labels, and tubes for other donors are to be left in a secure location outside the room.

2.4.1 **Samples for HLA Typing**

Principle

Blood samples obtained from the mother/donor following consent will be processed and stored for HLA typing at the time a match for a potential recipient is identified for the corresponding CBU.

Specimen

One 7 ml lavender (EDTA) top Vacutainer tube containing the mother's blood sample. Samples for HLA typing will be delivered to the Cord Blood Bank processing laboratory within 24 hours of collection of the sample. Upon arrival in the laboratory, the date and time of receipt will be recorded on the Maternal Sample Form and the samples will be stored at 4°C prior to processing.

Equipment

Bar code scanner
Computer

Materials

Three cryovials Nunc Plastics
Transfer pipette
Mother's bar code labels

Procedure

1. Three ml of blood are removed from the Vacutainer tube with a transfer pipette, and divided equally between the three cryovials, labeled with the mother's sample bar code label.

2. Store the tubes in designated quarantine space in a mechanical freezer at ≤ -20° C until requested for HLA typing. Record the fact that processing for HLA typing is complete, as well as the location of quarantine storage, on the Maternal Sample Form and maternal sample database.

3. The Vacutainer tube with the remaining blood sample is set aside to be processed according to SOP 2.4.3 for infectious disease amplification and testing.

Quality Control

1. Only one set of mother's samples will be processed at a time.

2. Bar code numbers on the labels and the tubes will be verified against the original tube at each addition of a new label to a tube.

2.4.2 **Samples for Infectious Disease Testing**

Principle

Blood samples obtained from the mother/donor following consent will be processed prior to testing for infectious diseases. A serum sample will be stored pending results of the infectious disease testing for confirmatory testing if required. Each of the participating cord blood banks must determine the sample requirements for the laboratories performing their infectious disease testing. If the testing laboratory uses manual methods, the serum samples may be stored at or below -20°C prior to shipment. If the testing laboratory uses automated methods, they may require that the serum samples be stored at 4°C prior to shipment within 24-48 hours.

Specimen

Two 7 ml red top Vacutainer tubes, labeled with the mother's sample bar code labels and containing the mother's blood samples will be delivered to the Cord Blood Bank processing laboratory within 24 hours of collection of the sample. Upon arrival in the laboratory, the date and time of receipt will be recorded on the Maternal Sample Form and the samples will be stored at 4°C prior to processing. Samples must be processed and frozen within 5 days of collection.

Materials

Seven cryovials
Mother/donor bar code labels
Transfer pipette
Small manila envelope containing a set of bar code labels with unique number followed by mother's sample identifier (the letter M)

Procedure

1. Centrifuge the red-top Vacutainer at 1500g for 15 minutes.

2. Using the transfer pipette, divide the serum equally among the cryovials.

3. If samples are to be shipped frozen to the testing laboratory, all tubes may be stored at ≤ -20°C prior to shipment. If the testing facility requires that the samples not be frozen, store six of the tubes at 4°C prior to shipment. Store the seventh tube in designated quarantine space in a mechanical freezer at ≤ -20° C for future testing if needed.

4. Record the fact that processing for ID samples is complete, as well as the location of quarantine storage for the seventh sample, on the Maternal Sample Form and maternal sample database.

Quality Control

1. Only one set of mother's samples will be processed at a time.

2. Bar code numbers on the labels and the tubes will be verified against the original tube at each addition of a new label to a tube.

2.4.3 **Samples for Infectious Disease Amplification Study**

Principle

Blood samples obtained from the mother/donor following consent will be processed prior to testing for infectious diseases. Upon arrival in the laboratory, the date and time of receipt will be recorded on the Maternal Sample Form and the samples will be stored at 4°C prior to processing. Plasma samples will be frozen and sent in batches to Gen-Probe for infectious disease amplification and testing.

Specimen

One 7ml lavender-top Vacutainer tube with approximately 4ml of blood remaining, following processing as in SOP 2.4.1.

Materials

Transfer pipette
One cryovial

Procedure

1. The Vacutainer tube is centrifuged at 1500 x g for 15 minutes.

2. The plasma is removed to the cryovial, using the transfer pipette.

3. Tubes are stored at ≤ -20°C prior to shipment to Gen-Probe for infectious disease amplification and testing.

Quality Control

1. Only one set of mother's samples will be processed at a time.

2. Bar code numbers on the labels and the tubes will be verified against the original tube at each addition of a new label to a tube.

2.5 **DONOR CONFIDENTIALITY AND LINKAGE**

Principle

Protection of the identity of the mother and infant donors of umbilical cord blood units while at the same time maintaining linkage of a particular unit to its mother/donor is of the highest priority. The following procedures and guidelines will be followed to ensure that linkage and donor confidentiality are preserved and protected:

Materials

Set of bar code labels
Mother's hospital label
Volunteer Cord Blood Donor Identification Form

Procedure

Establishment of Linkage and Confidentiality at the Time of Cord Blood Harvest and Consent Process

1. Initial linkage of the mother/donor to the CBU will be established prior to the infant's birth when the cord blood collector places both a unique bar code label and the mother's hospital label on a Volunteer Cord Blood Donor Identification Form. This form will be placed with the basin.

2. Prior to delivery, the cord blood collector places a unique bar code label on the basin in which the placenta will be placed. This basin will be placed in the delivery room with the Volunteer Cord Blood Donor Information Form.

3. Following the delivery of the placenta, a member of the delivery staff or Clinical Research Nurse (CRN) will verify that the patient's name and hospital number on the patient's wristband matches the hospital number and name on the Volunteer Cord Blood Donor Identification Form, and will verify that the bar code number on the basin matches the bar code number on the Volunteer Cord Blood Donor Information Form. The staff member or CRN will sign the Verification of Placental Donor Identification statement on the back of the Volunteer Cord Blood Donor Identification Form, and hand the basin containing the placenta and the Volunteer Cord Blood Donor Information Form to the cord blood collector.

4. The cord blood collector will verify that the bar code number on the basin matches the bar code number on the Volunteer Cord Blood Donor Identification Form and on the collection bag and labels in the kit and sign the Cord Blood Collector's Verification Statement.

5. The Volunteer Cord Blood Donor Identification Form with the attached bar code and hospital labels will be placed in the confidential manila envelope with the collection kit in a secured location until the Cord Blood Bank CRN removes it at the time medical history, maternal blood samples, and delivery information are obtained or until he/she is notified by collection or processing personnel that the unit was not suitable for collection or storage.

Maintenance of Linkage and Confidentiality Following Consent

1. It is the duty of the Director/Principal Investigator (PI) of the Cord Blood Bank, and his/her designees to protect the identity of the mother/donors. Access to information linking the mother/donor to the unique bar coded unit number for a particular umbilical cord blood unit will be limited to key personnel authorized by the Director/PI of the Cord Blood Bank.

2. Computer records containing linkage and confidential information will be maintained. Access to these records will be controlled by a multi-level security system requiring passwords and available only to key personnel authorized by the Director/PI of the Cord Blood Bank. Passwords will be changed at a minimum of every three months.

3. Computer records will be maintained as encrypted files which will be backed up nightly. A weekly backup for off-site storage will also be performed.

4. Hard copies of records and computer backup files containing linkage and confidential information will be stored in locked file cabinets in a locked room in a secure area. Access to those records will only be given to key personnel authorized by the Director/PI of the Cord Blood Bank.

Quality Control

At the Time of Cord Blood Harvest and Consent Process

1. Only one Volunteer Cord Blood Donor Identification Form and one labeled basin at a time are to be brought into the delivery room. **Only one set of forms, labels, and tubes are to be available in the mother's room while data and blood samples are being collected.** If other interviews are to be conducted immediately before or after, the forms, labels, and tubes for other donors are to be left in a secure location outside the room.

2. The donor identification sheet containing linkage between the mother/donor and the unique bar coded unit number will be accessible only to the Cord Blood Bank phlebotomist and/or the research nurse, members of the labor and delivery staff, and personnel authorized by the CBB Director/PI.

3. Signatures of verification will be obtained when the placenta is given to the cord blood collector and when the maternal blood samples are obtained by the phlebotomist. These signatures will attest to verification of the mother/donor identity by examination of the mother's wrist band and comparison of the hospital number with the hospital label on the Volunteer Cord Blood Donor Identification Form and of the study bar code number on the Volunteer Cord Blood Donor Information Form and the basin.

4. All Cord Blood Bank and Cord Blood Transplant Center employees will sign confidentiality statements protecting the identities and other medical and personal information obtained from the mother/infant donors participating in this project. The importance of patient confidentiality will be emphasized to Cord Blood Bank and Cord Blood Transplant Center employees at the

time of their orientation to the project.

Following Consent

1. Access to all confidential records including computer records and hard copies will be limited to key personnel authorized by the Director/PI of the Cord Blood Bank.

2. A computer security system with passwords and limited access only to key personnel will protect computer records, which will be stored as encrypted files to further limit accessibility. Backup files with storage off-site will be performed to further protect vital information.

3. Hard copies of records and computer backup files containing linkage and confidential information will be stored in locked file cabinets in a locked room in a secure area. Access to those records will only be given to key personnel as authorized by the Director/PI of the Cord Blood Bank.

2.6 NOTIFYING DONORS OF POSITIVE INFECTIOUS AND GENETIC DISEASE TEST RESULTS

Principle

Donor notification is a sensitive area as it relates to donor health, product safety, and prevention of possible disease transmission. This procedure serves as a guide in the donor notification process.

Materials

Record of Donor Notification
Confidential donor file
Notification letter(s) for positive infectious disease tests and genetic screening, as appropriate

General Notification Procedures

1. The donor notification process should be initiated within seven working days from receipt of confirmed positive test results.

2. Compliance with state or local regulations is required.

Methods of Notification

1. Letters are an acceptable method of donor contact for all situations described in this procedure that require notification. Some test results also require donor counseling services.

2. Use notification letters such as those in Appendix C for each specific blood test or health history finding. No further donor contact is required unless counseling is indicated, a donor has questions, or a letter was returned as undeliverable.

3. Include appropriate fact sheet(s) with each notification letter.

4. If state or local laws/regulations require reporting of individuals found to be positive for certain laboratory tests, maintain a written copy of such law(s) in your files. Include the following statement in the notification letter:

   We are required by law to report the results of this test to the *insert state or local name here* Department of Health. It is possible that a health department official may communicate with you further on this matter.

5. Mail first notification letters for HIV in a plain, white envelope via certified mail. Mail will automatically be forwarded by the postal service to a current address. Send all other notification letters via first class mail.

6. If the donor does not contact the CBB in reference to the first HIV notification letter, the CBB should attempt to contact the donor by telephone as detailed below. If, after two telephone

attempts, the CBB fails to contact the donor, the second letter for HIV notification should be sent in a white envelope via certified mail.

7. All notification letters returned and marked undeliverable should be followed up in order to locate the donor's current address.

    a. Try to contact the donor at home by telephone.

    b. If unable to contact the donor at home, the "emergency" telephone listed on the Volunteer Cord Blood Donor Information Form should be attempted.

    c. If unable to contact the donor at the above two numbers, and a work telephone number is available, attempt to contact the donor at work only to obtain a correct mailing address. Speak only with the donor and specify that an updated mailing address is required. Do not discuss any blood test information on the telephone.

    d. If unable to obtain the donor's address after completion of the above attempts, document specific attempts that were made in the notification cross record log or in a donor notification form. At this time, the case is considered closed for notification purposes.

8. A log and/or records must be kept of all mailed notification letters (see example of Notification Log in Appendix D).

Information Release Form

1. Send an information release form (see example in Appendix D) to donors who request test results be sent to their physician.

    a. When a signed and completed Information Release Request is returned, a copy of the mother/donor's test results must be mailed within 10 working days to the physician identified in the release request.

    b. When mailing test results to the donor's physician, first class mail can be used for all test results. Stamp envelopes "Confidential" when mailing confirmed positive HIV test results via first class mail.

    c. Copies of signed information release forms must be retained by the laboratory.

Counseling Guidelines

1. It is important to refer or to counsel donors who have confirmed positive HIV and certain genetic disease results. Provision of counseling services for other infectious disease tests and genetic screening results is optional.

2. Prior to any discussion of test results, the counselor must request and verify donor

identification. Donor identification is confirmed by the donor stating a social security number and date of birth or by presenting a federal or state picture ID.

3. Discuss the following:

   a. Test results.

   b. Information from the appropriate fact sheet.

   c. The cord blood unit donation that tested positive was destroyed.

   d. Blood donor eligibility status.

   e. Refer donor to personal physician for further medical evaluation and follow-up, if indicated.

   f. For donors being counseled for confirmed positive infectious disease test results, inquire about previous blood donations within the last five years. Record where and when previous donations occurred (to the best of the donor's recollection) on a separate authorization for release of test result information form (see example in Appendix D).

4. Document counseling.

   a. Document on a Donor Counseling Worksheet (see example in Appendix D) that the above information was discussed during counseling. Include the signature of the counselor, date of counseling, and the fact that written materials were given to the donor.

   b. Do not document further health history information (i.e., high risk behaviors) volunteered by the donor on a donor counseling worksheet. Any record of this information must be kept in the donor notification file but is not recorded on this worksheet. Keep this worksheet with the record of notification in the confidential donor notification file.

5. Mothers and infants should be referred to their physicians for further medical counseling. Clearly indicate that the mother/donor physician may call upon the CBB Medical Director for further information.

6. At the time the mother is informed of test results, provide written materials (appropriate letters, pamphlets, or fact sheets in attachments) regarding the test results as well as any applicable local support resources.

Donor Notification Records

1. The Principal Investigator of each CBB, or appropriate designee, is responsible for maintenance of a confidential file of all records associated with donor notification.

a. Notification records may be kept in log format (see Appendix D) for hepatitis-related test results and confirmed positive syphilis test results.

At a minimum, the following information must be recorded: CBU bar code number, test results requiring notification, date of donation, date letter was sent, if letter was returned due to incorrect address, steps taken to find a correct address, and final disposition of notification, i.e., letter was sent to new address or was unable to obtain a current address.

b. A Record of Donor Notification must be used to document all **test results for HIV**.

Confidentiality

1. Test results are considered confidential information and must be handled accordingly. Access to this information must be limited and disseminated only on a need-to-know basis.

2. Employees who have access to donor names and related test results must sign a confidentiality statement. This confidentiality agreement must be kept in the personnel files.

3. All notification records should be kept in a locked file with limited access. Cord Blood Bank procedures must state, by position title, who has access to these records.

CHAPTER 3

COLLECTION PROCEDURES

Chapter 3

COLLECTION PROCEDURES

3.1 COLLECTION KIT

Principle

The cord blood collection kit will be carefully assembled by the Cord Blood Bank (CBB) supervisor or his/her designate according to the following procedure. Kits will be distributed to the cord blood collector(s) prior to collection so that collection and processing of the umbilical cord blood unit, blood samples and paperwork may proceed as efficiently and with as little risk of error as possible. The contents and assembly of collection kits may be customized to suit individual collection center needs. Customized kits must be fully documented in the CBB's internal SOPs.

Materials

Large zipper-locked bag, 8½" x 11"
Set of COBLT study bar code labels
CPD cord blood collection bag
Volunteer Cord Blood Donor Identification Form
CBU Collection and Receipt Form
Medical History Form (optional)
Donor and Delivery Information Form
Maternal Sample Form
Zipper-locked bag, 8" x 10", with 2 pieces of gauze
Small manila envelope
Tubes for mother's samples (optional)
2 large manila envelopes (1 for bar code labels, one for mother/donor paperwork)

Procedure

1. Inspect the CPD cord blood collection bag to be sure it is intact. Place the bag in foil pouch (optional) and record the appropriate expiration date.

2. Place a study bar code label on all pages of each study form and on both large manila envelopes. Place all forms in one of the large manila envelopes and place the envelope in the zipper-locked bag.

3. Place a maternal study bar code label on the small manila envelope. Remove the rest of the maternal bar code labels from the study bar code label sheet and place them inside the small manila envelope.

4. Place the remaining study bar code labels in the second large manila envelope. Place the

envelope in the zipper-locked bag.

5. Place the collection bag, zipper-locked bag with gauze, and small manila envelope in the large zipper-locked bag.

   NOTE: As each item is placed in the bag, inspect the bar code label to be sure it matches the bar code number on the label sheet.

6. Close the zipper-locked bag. The kit is now ready for distribution to the cord blood collectors at the collection sites.

Quality Control

1. One kit is to be assembled at a time, to eliminate the risk of mixing of label sets.

2. Numbers on the kit components are verified against those remaining on the label sheet.

3. Bar code label numbers for each collection kit will be recorded on the Label Release Log.

3.2 **LABEL CONTROL**

Principle

Bar coded labels provide an easy and accurate method for tracking and identifying CBUs and samples. The Cord Blood Transplantation Study uses labels bar coded with the standardized ISBT-128 system for CBUs, sample processing and data collection. Study labels are printed in sets with each set having a unique bar code number. Labels within the set are identically coded and are sized to fit on laboratory tubes, blood bags, and data forms. The COBLT Study bar code label set is illustrated in Figure 3.2.1.

Label sets are distributed to each Cord Blood Bank (CBB). The CBB supervisor or his/her designate distributes labels in collection kits. Labels are tracked using the Shipping Log and the Label Release Log.

Materials

COBLT Study Bar Code Label Set
Label Release Log

Procedures

Tracking Labels at the Cord Blood Bank

1. Ensure that all bar codes in the set are legible and that each set contains a complete set of labels (Figure 3.2.1). Remove from distribution incomplete sets or sets with illegible bar codes.

2. To release a set of labels, scan the bar code and complete the label release information on the Label Release Log in the COBLT Internet Data Entry System. Give the set of labels to the COBLT staff member responsible for assembling the collection kit.

3. At regular intervals defined by institutional procedures, use the label report utility in the study data system to print a list of released but unreturned labels. Labels not returned within 4 weeks of the release date should be located.

Tracking Labels at the Collection Center

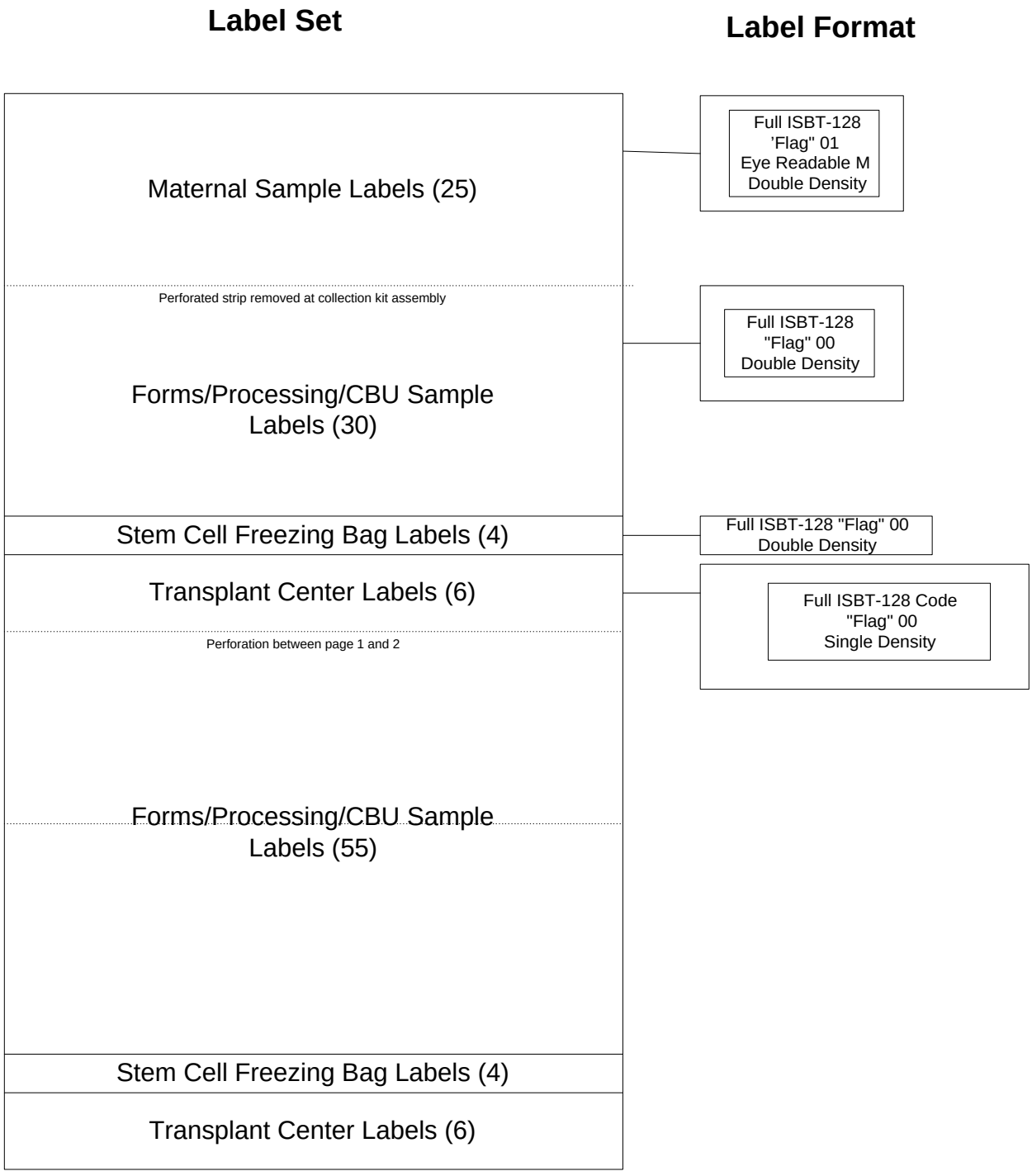
All opened collection kits are to be returned to the CBB. If a kit is opened but not used and returned to the CBB using the Shipping Log, record the reason in the specify section of this log.

Quality Control

1. Labels will only be issued by the CBB supervisor or his/her designate.

2. All labels issued will be accounted for on appropriate logs and forms.

Figure 3.2.1
COBLT Study Bar Code Label Set



3.3 **COLLECTION OF PLACENTAL BLOOD**

Principle

Placental or umbilical cord blood contains hematopoietic stem and progenitor cells that can substitute for bone marrow in human bone marrow transplants. Placental or umbilical cord blood can be cryopreserved in the laboratory for later use.

Specimen

Placenta in collection container

Equipment

Collection Stand	
Orbital Rotator (optional)	e.g. Nutator, Clay Adams
Metal and Plastic Hemostats	
Scale/Balance	
Hand Sealer with Clips	e.g. Fenwal Baxter
or Heat Sealer	e.g. Sebra

Reagents

70% Isopropyl Alcohol

Supplies

CPD Placental/Umbilical Cord Blood Stem Cell Collection Set	Pall Medical Corporation (Figure 3.3.1)
Collection Kit	
Chux Pad	Hospital Storeroom
Alcohol Wipes	Hospital Storeroom
Iodophor-PVP Scrub and Wipes	Hospital Storeroom
Nonsterile Gloves	Hospital Storeroom
Sterile Gauze Pads	Hospital Storeroom
Disposable Mask with Plastic Eye Shield (optional)	Hospital Storeroom
Disposable Booties and Gowns (as required by collection facility)	Hospital Storeroom

Procedure

1. Before the delivery, ensure equipment and supplies are organized and complete.

2. Glove, then wait until the placenta is placed in the container and handed out from the delivery

room.

3. Verify that the label on the container holding the placenta matches the label on the collection bag. Have Labor and Delivery personel sign the verification box on the reverse of the VDIF.

4. After double gloving, place the placenta fetal side down into the Chux pad which has been attached to the collection stand. Punch a hole in the middle of the Chux, then pull the cord through. Inspect the collection bag to be sure that it appears intact and close the blue clamps located on the tubing. Place the collection bag (Bag #1) of the CPD Placental/Umbilical Cord Blood Stem Cell Collection Set (Figure 3.3.1) on the scale including the tubing and attached needles to obtain the pre-collection weight. Record this weight on the CBU collection form.

5. Optional: Place a metal hemostat on the cord near the cord clamp and below the planned puncture site. This will serve as a means to hold the cord taunt during the collection.

6. Rinse the blood off of the surface of the cord by squirting the cord with Isopropyl alcohol from a bottle.

7. Sterlize the cord according to institutional SOP. Do not touch the cord puncture site after it has been cleaned.

8. Remove the needle cap from one of the two donor needles attached to the collection bag (Bag #1, Figure 3.3.1). Hold the cord tight with the metal hemostat. Puncture the cord with the needle bevel turned away from you. (Optional: Use a small plastic hemostat to hold the needle in place by attaching the hemostat below the blue plastic grip site of the needle and the metal hemostat.) Open the blue clamp located on the tubing attached to the needle used to puncture the vein. Blood should flow through the tubing into the collection bag by gravity. Rotate the collection bag periodically to allow complete mixing of the CPD anticoagulant with the cord blood.

9. If necessary, rotate the placenta several times to get a better collection particularly when the cord is long; sometimes the placenta has to be raised out of the stand, but held over the Chux.

10. If blood flow ceases, but it appears that additional blood remains in the placenta, close the tubing attached to the first needle with the blue sliding clamp. Make a second puncture by selecting a site proximal to the placental surface. Clean the new site(s) using institutional SOP and proceed as in Step 8.

11. When the collection is completed (the cord will appear empty and mostly white-ish when all the blood has been removed), close off the tubing with the blue sliding clamp to prevent air from entering the bag. Vent the tubing by turning the white vent clamp clockwise. Strip any blood remaining in the tubing down into the collection bag. Heat seal the tubing at the site where the needles and tubing are joined. Cut off the needle(s), and place in a sharps container.

12. Weigh the collection bag and attached tubing containing the cord blood. Subtract the pre-

collection weight of the bag obtained in step #4 from the post collection weight to calculate the weight (Volume 1ml=1gm) of cord blood collected. Add 7.5 gms to adjust for the weight of the needles removed in step #11. Record the calculated weight/volume on the CBU Collection and Receipt Form in the collection kit.

Volume of CBU = (Post Collection weight +7.5 gm) - precollection weight

NOTE: The exact weight (in this example, 7.5 gm) to be added must be determined and validated at each bank.

13. Strip the tubing off the blood bag and sterility seal tubing directly underneath the Y-connection (the site where the tubing splits to the two collection needles). Cut away any excess tubing.

14. Compare the bar coded label on the bag with the Volunteer Cord Blood Donor Identification Form and the CBU Collection and Receipt Form. Complete all the collection information on the collection form.

15. Place the blood bag in the Collection Kit zipper-locked bag containing the gauze strips. Place this bag in the larger Collection Kit zipper-locked bag together with forms and bar code labels to be returned to the CBB.

16. Store the collection as indicated in SOP Section 3.4, Post Collection Storage of CBU Prior to Transport to CBB Processing Laboratory, or take directly to the processing laboratory.

Quality Control

1. To ensure that contamination does not occur, collection kits will be pre-assembled and shipped to the collection centers.

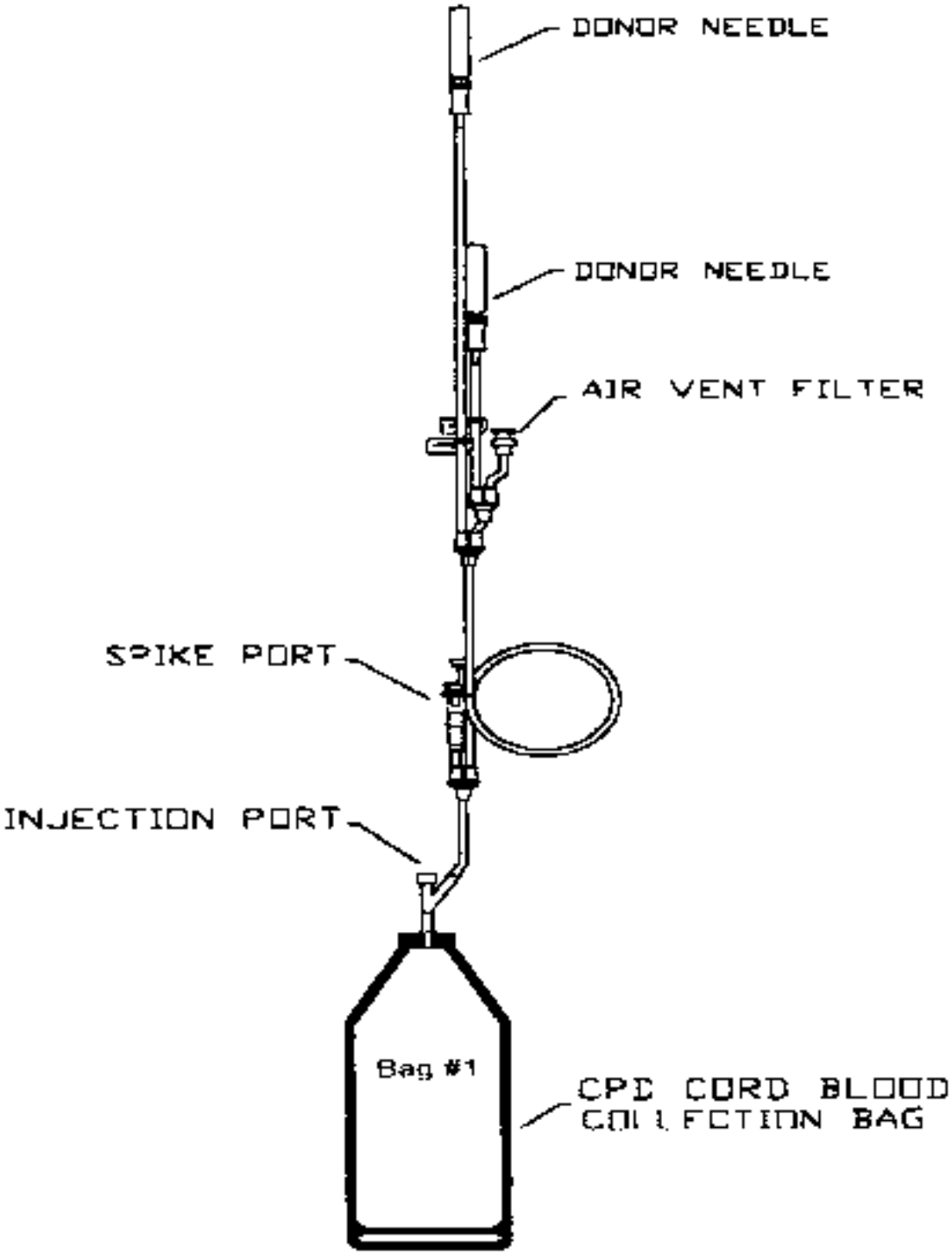
2. All personnel will be trained prior to placental blood collections.

References

New York's Mount Sinai Hospital and The New York Blood Center

Dr. Pablo Rubinstein

Figure 3.3.1
CPD Placental/Umbilical Cord Blood Stem Cell Collection Unit (Set #1)



3.4 POST COLLECTION STORAGE OF CBUS PRIOR TO TRANSPORT TO CBB PROCESSING LABORATORY

Principle

Collected umbilical cord blood has been found to maintain cell viability when temporarily stored at temperatures of 15° to 25° C. Insulated platelet shippers with temperature stabilizing gel packs successfully maintain units within this temperature range for several hours. All platelet shippers used to transport COBLT CBUs to the CBB will be monitored to verify that internal temperatures remain within acceptable ranges.

Specimen

Cord Blood Units collected after placental delivery into a collection bag containing CPD anticoagulant.

Equipment

Numbered Platelet Shipper	EnduroTherm® E-38
2-4 3-lb Non-toxic Temperature Stabilizing Packs	e.g. Polar Pack, Arctic Ice
Thermometer	

Supplies

Large Plastic Bag	
Plastic-backed Absorbent Pad	e.g. Underpads, Kimberly-Clark
Shipping Log	
Shipping Labels	
COBLT Study Bar Code Labels	

Procedures

<u>Preparing the Platelet Shipper</u>

1. a. For new shippers, attach a study-specific label to the platelet shipper. The label should include the name of the study, the shipper number, and the dates a min/max thermometer was used. The unique shipper number should be recorded using a permanent ink marker.

   b. For used shippers, check the outside of the platelet shipper for damage. Return damaged shippers to the CBB Processing Laboratory.

2. Check that the platelet shipper number is legible. Check that the following labels are securely attached and legible:
   STUDY-SPECIFIC LABEL (See Step 1a)

"CORD BLOOD BANK ADDRESS/IF SHIPMENT IS DELAYED ... NOTIFY"
"PERISHABLE"
"WARNING! DO NOT ICE"

Replace illegible, missing or loose identifiers.

3. Record the shipper number on the Shipping Log. Indicate on the study-specific label whether or not a min/max thermometer is used.

4. Place temperature stablizing packs (TSP) (conditioned to 15-25° C) in the shipping container, laid flat and squeezed side by side. Add absorbent sheet, plastic side down. Place the plastic bag, the remaining conditioned TSPs, the Shipping Log, and the min/max thermometer (if applicable, see Quality Control instructions) in the shipper.

5. Replace the lid and store the shipper in a secure location at ambient room temperature of 15° - 25° C.

Storing Umbilical CBUs Following Collection

1. Remove the Shipping Log, top two TSPs, and min/max thermometer (if applicable) from the shipper. Place a bar code label from the collection kit on the Shipping log.

2. Visually inspect zipper-locked bags containing CBUs to ensure that they are sealed. Seal if necessary. Compare each CBU bar code label with the bar code label on the log. Complete "packing information" on the log. Resolve any discrepancies. Record unresolved discrepancies on the log.

3. Place the zipper-locked bag containing the CBU in the large plastic bag in the shipper. Place two TSPs on top of the plastic bag, laid flat and squeezed side by side.

4. Return the thermometer (if applicable, see Quality Control instructions) and Shipping Log to the shipper and replace the lid.

Quality Control

1. Platelet shippers will be placed in secured areas with limited access.

2. Preparation and packing of the shipper will be performed by trained staff.

3. All platelet shippers must have their internal temperatures monitored. New platelet shippers must have a min/max thermometer included for the first ten shipments. The temperature must be monitored monthly thereafter. Monitoring information will be recorded on the study label and the Shipping Log.

4. When shipping in extreme temperatures (< -10° C or > 37° C), a min/max thermometer must be placed in the platelet shipper.

5. Platelet shippers may not be used for long-term storage. Each shipper has been validated to remain within acceptable temperature ranges for a specified time period. CBUs may not be stored in the shippers for longer than this time period.

References

George VM, Pringle TC, Kline L, Friedman LI. Development and evaluation of a shipping system for platelet components. **Transfusion** 1996, 36, 335-338.

CHAPTER 4

PROCESSING PROCEDURES

Chapter 4

PROCESSING PROCEDURES

4.1 SAMPLE RECEIPT AND LOG-IN

Principle

Correct assignment of a particular cord blood unit (CBU) and its corresponding paperwork and samples requires a carefully maintained data tracking pathway. One key point in this pathway is at the time of receipt of a CBU by the CBB processing facility. This SOP outlines the steps performed at this time to maintain data tracking and quality assurance.

Specimen

Cord blood aseptically collected after delivery into an CPD anticoagulant collection bag according to the Collection SOP, bar code labels and accompanying paperwork, transported to the Cord Blood Bank according to the SOP for Shipping CBUs from Collection Centers to CBB.

Equipment

Electronic Bar Code Scanner
Computer

Reagents

None

Supplies

Shipping Log	From Platelet Shipper
CBU Collection and Receipt Form	Cord Blood Bank Stationary
Manila Folder	Cord Blood Bank Stationary
COBLT Study Bar Code Labels	From collection kit(s) in platelet shipper

Procedure

1. Upon receipt of the CBU from the collection center, open the platelet shipper (if applicable), and record the date and time the shipper was opened, the total number of CBUs received, and the min/max temperature (if applicable) on the Shipping Log. Sign the Shipping Log and record the study ID number. Exclude CBU(s) if pre-processing storage temperature is $< 4^{\circ}C$ or $> 37^{\circ}C$.

2. For each CBU, locate the sheet of adhesive bar code labels and any paperwork included in the collection kit zipper-locked bag.

If more than one CBU is contained in a shipper, Steps 3 through 9 should be completed for one CBU at a time.

3. a. If the bar code labels are absent, discontinue log-in until they are located.

   b. Scan the bar code label on the collection bag and on the bar code label set in the collection kit to confirm identity.

   c. If identity is not confirmed, discard sample according to the Discarding CBUs SOP. Otherwise continue processing.

   d. Place an adhesive bar code label on a new manila folder to create a Cord Blood Unit Paperwork folder. Place the CBU Collection and Receipt Form and any other paperwork in this folder.

   e. If the Confidential Information manila envelope is included in the shipper, **DO NOT OPEN IT**. Give the unopened package to the COBLT staff member designated to process confidential information.

4. Record the identity of the Receipt Technologist and the date and the time of receipt on the CBU Collection and Receipt Form and/or CBU receipt database.

5. Check collection bag for leaks and label integrity. Record result on the CBU Collection and Receipt Form and in the database.

6. a. Open the CBU receipt database and enter the collection and receipt data recorded on the CBU Collection and Receipt Form.

   b. Calculate the volume of the cord blood without anticoagulant (see Procedure Note 1). If this volume is less than 40 ml, discard the CBU according to the Discarding CBUs SOP.

   c. Calculate elapsed time from collection. If more than 48 hours have elapsed, discard the sample according to the Discarding CBUs SOP. Otherwise continue processing.

   d. If the bag contains a leak or the label is unreadable discard the CBU according to the Discarding CBUs SOP.

7. Place the CBU, the adhesive labels, and the Paperwork Folder in the Processing in-box or institutional equivalent.

Quality Control

1. Quality control is maintained by electronic entry and confirmation of the CBU ID number through use of the adhesive bar code label and scanner.

2. All records are to be maintained both electronically (in the database) and in hard copy form (in the Paperwork Folder).

3. Only one CBU will be processed at a time by a trained staff member.

Procedure Notes

1. Calculation of Cord Blood Volume

Volume of Cord Blood = Volume of CBU - 25 ml Anticoagulant

(Volume of CBU = [Post Collection Weight + 7.5 gm] - Pre-collection Weight)

NOTE: The exact weight (in the example, 7.5 gm) to be added must be determined and validated at each bank.

4.2 **CBU SEPARATION AND SAMPLE PREPARATION**

Umbilical Cord Blood Volume Reduction and Red Cell Depletion Using Hydroxy Ethyl Starch (Hespan)

Principle

Umbilical cord blood unit (CBU) grafts indicate that umbilical cord blood is a useful source of hematopoietic stem cells for routine bone marrow reconstitution in both the unrelated and related donor setting.

In order to efficiently store a large number of CBUs most of the plasma and red blood cells are removed by differential centrifugation using the ability of Hespan to induce red blood cell agglutination. The final product is then brought to a standard volume in preparation for cryopreservation.

Specimen

Umbilical cord blood properly logged into the bank according to Sample Receipt and Log-In SOP.

Equipment

Laminar Flow Hood	
Centrifuge	
Heat Sealer	e.g. Sebra Model 2100
Plasma Expressor	
Bar code scanner	
Balance	
Tube stripper	
Sterile Docking Device	
Plastic centrifuge inserts (optional)	
RBC sensor (optional)	e.g. Melcor

Reagents

Hespan	Dupont Pharmaceuticals, Catalog #NDC 0056-0037-44/NSN 6505-01-281-1247

Supplies

Stem Cell Processing Kit (Set #2)	Pall Medical Corporation (Figure 4.2.2)
Hemostats	Hospital Store Room
18 Gauge Luer Lock Needles (or higher gauge, i.e., a smaller needle may be used)	Hospital Store Room
Alcohol Swabs	Hospital Store Room
Sterility Testing Tubes	

1ml Syringe (2)	Hospital Store Room
60 ml Syringe (opt)	Hospital Store Room
30ml Syringe (2)	Hospital Store Room
Zipper-locked bag	Hospital Store Room
Sample Tubes	Falcon Plasticware
CBU Collection and Receipt Form	Cord Blood Bank Stationary
CBU Processing Form	Cord Blood Bank Stationary
Cryovials (1-2ml)	Nunc Plastics
Six Microscope Slides	Hospital Store Room
Cord Blood Paperwork Folder	Receipt Technician
COBLT Study Bar Code Labels	

Procedure

1. Locate the sheet of adhesive COBLT Study bar code labels included in the collection kit. Place a bar code label on the two sterility testing tubes, the cryovials, microscope slides, sample tubes and other forms as required by institutional procedures.

2. a. Place a bar code label on the CBU Processing Form. Open processing database and enter the processing record for the CBU using the bar code scanner.

   b. Record the identity of the processing technologist, the date and the time in the database and on the CBU Processing Form. Data may be keyed directly into the database. Hereafter, reference to the CBU Processing Form or processing database will be generically termed “the worksheet”.

3. Strip any tubing attached to the bag with blood in it back into the bag and mix very thoroughly. Seal off the tubing close to the bag after stripping the blood back into the bag.

4. Obtain 0.5 ml of whole blood from the syringe port of the collection bag (Bag #1) using sterile technique as described below. See Figure 4.2.1, CPD Placental/Umbilical Cord Blood Stem Cell Collection Unit.

   a. After disinfecting the septum of injection port, use a 1 ml syringe and needle to mix the blood in the tubing between the injection site and the bag back into the bag.

   b. After mixing, remove 0.5 ml of whole blood from the collection bag (Bag #1) and transfer to a pre-labeled tube. Scan bag and tube to confirm label match.

   c. Perform automated nucleated cell count and manual viability count (using trypan blue according to institutional SOPs). Enter data on the worksheet.

   d. If the volume of CBU (cord blood plus anticoagulant) is between 65 and 85 ml, and the total nucleated cell count is less than 6×10^8 cells, discard the CBU according to the Discarding CBUs SOP. Calculate the volume of CBU using Procedure Note 1 if the

database is not available.

e. Prepare three slides for a manual differential count.

f. Place remainder of sample in "Unit Sample Testing In-Box" or institutional equivalent for ABO and Rh testing.

5. Add the Hespan to the collection bag (Bag #1) as follows.

a. Calculate the volume of Hespan to add to give the bag a final ratio of Hespan: CBU (blood+anticoagulant) of 1:5. The database will automatically calculate this volume or it can be done manually by using the formula in Procedure Note 2.

b. Record the lot number and expiration date of the Hespan on the worksheet.

c. Disinfect the outside of the septum of the sterile injection port and use a 30 or 60 ml syringe to add the correct volume of Hespan through the injection port.

d. Mix the blood and Hespan thoroughly by inverting the bag 4-5 times.

6. Sterile connect the Stem Cell Processing Kit (may be done now or after Step 10, according to institutional protocol) as follows. See Figures 4.2.1 and 4.2.2 for bag diagrams.

a. Use a sterile connection device to attach the tubing from the Stem Cell Processing Kit to the tubing of the collection bag (Bag #1).

b. Place a bar coded label on each bag (processing bag [Bag #2], plasma bag [Bag#3], stem cell freezing bag[Bag #4]), scan all labels on the bags, and compare with the bar coded label on the collection bag. Do not proceed if labels are not identical; resolve any discrepancy.

c. If sterile connection device is not available, connect the spike of the processing bag (Bag #2) to the spike entry port of the collection bag (Bag #1).

7. Place the collection bag (Bag #1) (and Stem Cell Processing Kit, if connected) into a centrifuge cup.

a. Add or adjust inserts to maintain the bag(s) in an upright position free of folds or creases.

b. If using customized Plexiglas inserts, make sure that the sealed bottom edge of the bag extends below the Plexiglas inserts, so that the lower level of blood in the bag is even with the bottom of the Plexiglas inserts. Use velcro straps to secure the blood bag assembly.

8. Balance the centrifuge.

9. Centrifuge at g force, rpm's, and time as calculated and validated by the bank to give a recovery of ≥60% of nucleated cells or ≥80% of mononuclear cells.

10. Carefully remove the entire centrifuge cup and place near the plasma expression. If Stem Cell Processing Kit has not been connected to the collection bag (Bag #1), then connect and label as detailed in Step 6 above.

11. Place collection bag (Bag #1) into plasma expressor and arrange processing bag (Bag #2) on scale so that only the weight of the bag (not the tubing, hemostats, or clamps) is being weighed. If using Plexiglas inserts, they may be left around the bag in the expressor. Tare the scale.

    NOTE: At this point in the procedure, it is recommended that the collection bag hang on the expressor stand for approximately 15-20 minutes to allow time for additional RBC sedimentation. This clarifies the interface between the leukocyte-rich plasma and the red blood cells before expression of the leukocyte-rich plasma into the processing bag.

12. Using a hemostat or roller clamp to control flow, slowly express supernatant into processing bag (Bag #2) in order to avoid turbulence.

    a. If a red cell sensor is used, manually express a few ml of plasma to clear RBCs from the tubing between the collection bag and the processing bag. Then turn on sensor and express leukocyte rich plasma until the sensor clamps the tubing. If expressing manually, as red cells start to enter connecting tubing, monitor scale to let the desired number of grams of red cells (validated at the bank) pass through to processing bag (Bag #2), then clamp bag closed. Record weight of the processing bag (actually the weight of the leukocyte-rich plasma) on worksheet and in the database.

    b. If mixing occurs, resulting in apparent retention of white cells or excessive transfer of red cells to processing bag (Bag #2), return all material to collection bag (Bag #1) and re-initiate procedure from step 7.

    c. Temporarily close off the collection bag (Bag #1). Do not permanently seal off this bag at this time to allow for poor end recoveries requiring a second spin.

13. Place Stem Cell Processing Kit in the centrifuge cup. Add inserts to make bag stand upright with no folds or creases. If using customized Plexiglas inserts, place the processing bag (Bag #2) between the Plexiglas inserts. Use velcro straps to secure the blood bag assembly.

14. Balance the centrifuge.

15. Centrifuge at g force, rpm's, and time as calculated and validated by each bank to give a recovery of ≥60% of viable nucleated cells or ≥80% of viable mononuclear cells.

16. Carefully remove the Stem Cell Processing Kit from the centrifuge cup and place in the plasma expressor.

    a. If using Plexiglas inserts, they may be kept attached to the processing bag (Bag #2) in the plasma expressor.

    b. Arrange plasma bag on scale with tubing and other bags stablized and not interfering with weight of the plasma.

    c. Tare the plasma bag.

17. Subtract institutional adjustment factor (e.g. 21.5 grams, see Note) from the actual weight of the leukocyte-rich plasma in the processing bag (Bag #2) from Step 12a and express this amount from processing bag (Bag #2) to plasma bag (Bag #3).

    NOTE: The exact weight (in this example, 21.5 grams) to be subtracted must be determined and validated at each bank. The volume retained in the processing bag must be 21.5 ml.

    a. If mixing occurs, resulting in excessive retention of plasma or excessive transfer of white cells to plasma bag, return all material to processing bag (Bag #2) and re-initiate procedure from step 13.

    b. Temporarily close off the plasma bag (Bag #3). Do not permanently seal off this bag at this time to allow for poor end recoveries requiring a second spin.

18. Remove a specimen for sampling.

    a. Under sterile conditions, use a 1 ml syringe to remove a test aliquot of leukocyte-rich plasma from the processing bag (Bag #2) and put into a sterile labeled tube. The test aliquot can be either a standard volume ranging from 450-500 µL or a calculated volume with a minimum cell count of $3x10^6$ nucleated cells.

    b. Remove enough sample from the sterile aliquot to perform an automated cell count and manual viability (using trypan blue according to institutional SOPs).

    c. Remove enough sample from the sterile aliquot to prepare three slides for manual differential count. An optional hematocrit may also be performed.

    d. Record the overall viability on the worksheet and in the database. If viability is < 90%, discard according to SOP.

    e. Record the total nucleated cell count and percent viability on the worksheet and in the database and calculate the recovery of viable nucleated cells either by computer database or manually.

f. If recovery is < 60% of viable nucleated cells and < 80% of viable mononuclear cells (if performed), and the total viable nucleated cell count is < 6 x 10^8 cells, reconstitute plasma and cells back to original product and re-process from Step 7. Do not spin the bag more than twice. If recovery is still not adequate after a second spin, discard CBU according to the SOP.

g. If recovery is adequate, seal off and remove the collection (Bag #1) and plasma (Bag #3) bags and proceed with completion of processing.

19. Remove 400 µL from the test aliquot for flow cytometry. Confirm label by scanning and place the aliquot in the flow cytometry in-box or institutional equivalent.

20. Use the viable nucleated cell count to calculate the volume required to obtain $0.5x10^5$ cells from the testing aliquot and remove this volume for colony assays (see Procedure Note 3). Confirm label by scanning and place the aliquot in the colony assay in-box or institutional equivalent.

21. Swab the sterile sampling site injection port of collection bag (Bag #1) with an alcohol wipe, and remove ≥ 7 ml of granulocyte/packed red cells. Add seven ≥ 1 ml aliquots to seven pre-labeled Cryovials. Scan to confirm labels and place vials in Granulocyte/Red Cell Storage freezer (≤ -20°C). Record location on worksheet.

22. Retrieve leukocyte-poor plasma bag (Bag #3), swab the sterile sampling site injection port with an alcohol wipe, and remove 15ml. Add 7.5ml of plasma to each of two pre-labeled sterility testing tubes for sterility testing (Aerobic and Anaerobic). Confirm labels by scanning.

23. Retrieve collection bag (Bag #1), swab the sterile sampling site injection port with an alcohol wipe, and remove 5ml of granulocyte/packed red cells. Add 2.5ml of red cells to each of the two sterility testing tubes referred to in Step 22.

24. Mix sterility testing tubes and place in Unit Sample Testing In-Box or institutional equivalent.

25. Swab the sterile sampling site injection port of plasma bag (Bag #3) with an alcohol wipe, and remove 7 ml of plasma. Add one 1ml aliquot to seven pre-labeled Cryovials. Scan to confirm labels and place vials in Plasma Storage freezer (≤ -20°C). Record location on worksheet.

Quality Control

The primary quality assurance tests are recovery of viable nucleated and/or mononuclear cells and sterility testing (post-processing). All samples in the Unit Sample Testing In-Box may be released for testing only after confirmation of receipt of informed consent.

Procedure Notes

1. Calculation of Volume of CBU

Volume of CBU = [Post Collection Weight + 7.5 gm] - Pre-collection Weight

NOTE: The exact weight (in the example, 7.5 gm) to be added must be determined and validated at each bank.

2. Calculation of Volume of Hespan to Add

Volume of CBU * 0.2

Volume of CBU = (Post Collection Weight + 7.5 gm) - pre-collection weight

NOTE: The exact weight (in the example, 7.5 gm) to be added must be determined and validated at each bank.

3. Calculation of Volume for Colony Assays

$$\frac{0.05 \times 10^6 \text{ cells}}{\text{viable nucleated cell count } (\times 10^6/\text{ml})}$$

Figure 4.2.1
CPD Placental/Umbilical Cord Blood Stem Cell Collection Unit (Set #1)

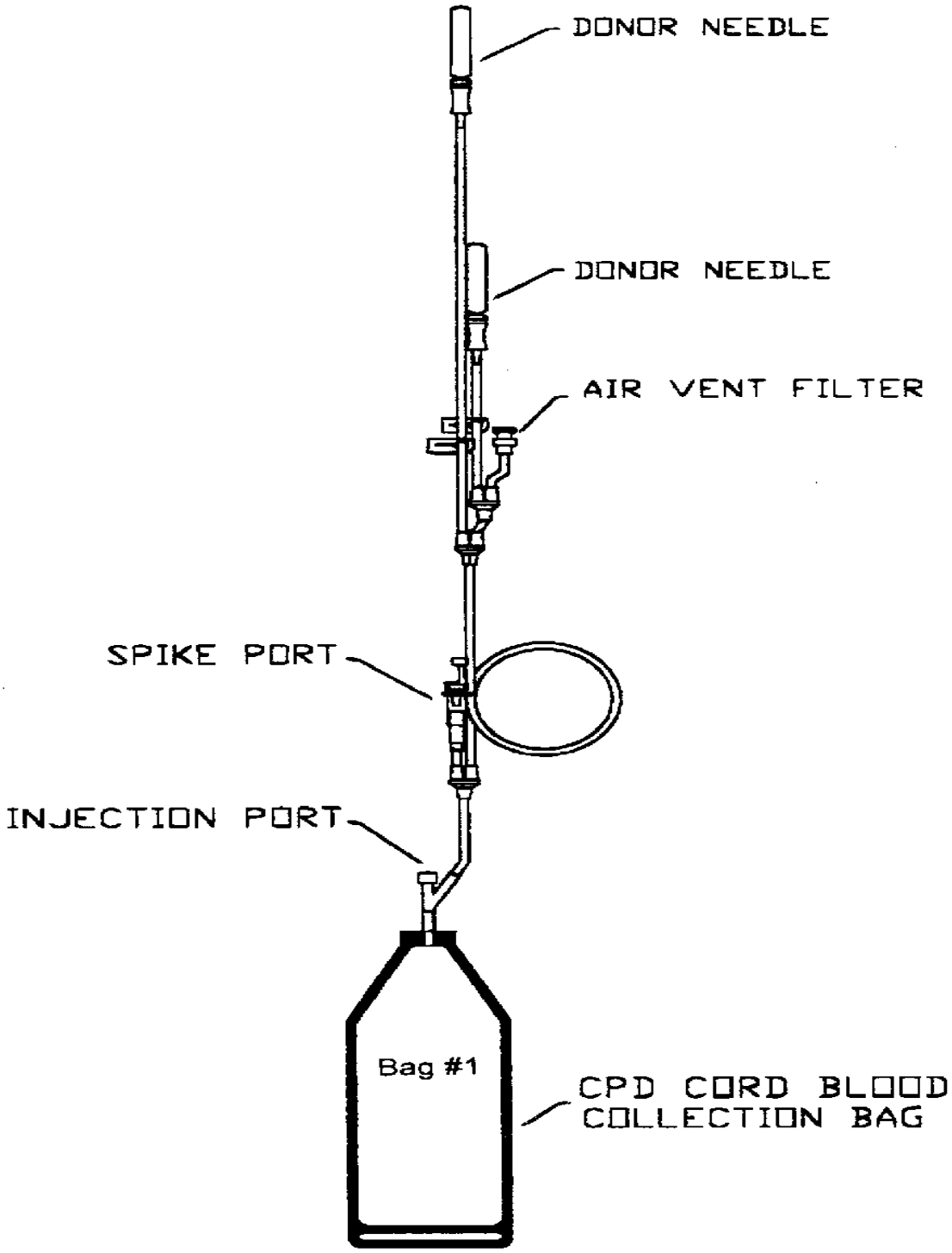
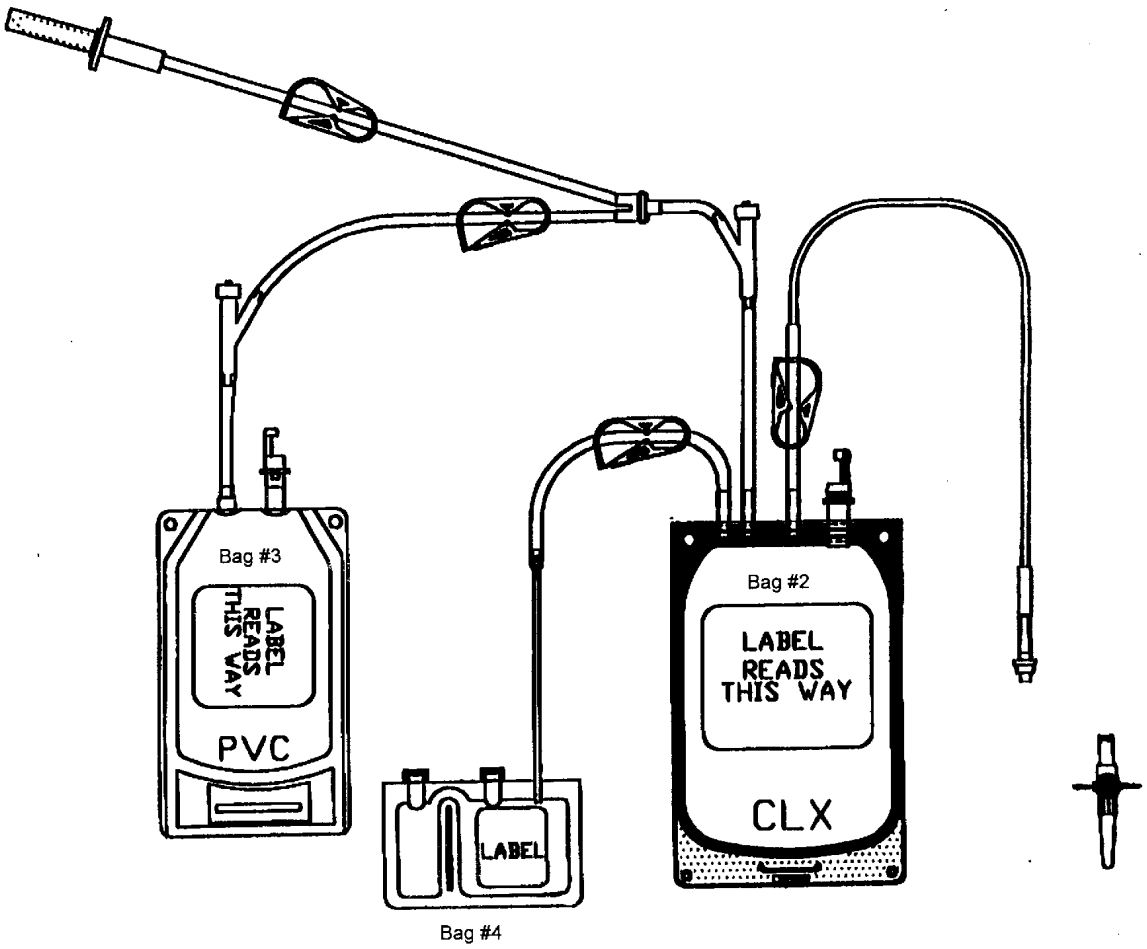


Figure 4.2.2
Stem Cell Processing Kit (Set #2)



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Areman E, Deeg HJ, Sacher RA. ***Bone Marrow and Stem Cell Processing: A Manual of Current Techniques***. F.A. Davis Company, Philadelphia, 1992.

Rubinstein P, Dobrila LI, Rosenfeld R, et al. Proc of the National Academy of Sciences USA, Volume 92, pp. 10119-10122, October 1995.

Zuck TF. The applicability of cGMP to cord blood cell banking. Journal of Hematotherapy 5:135-137, 1996.

4.3 **CRYOPRESERVATION OF CBU**

Cryopreservation of Umbilical Cord Blood Cells Using DMSO and Dextran 40 as Cryoprotectants

Principle

Results of umbilical cord blood unit (CBU) grafts suggest that umbilical cord blood is a useful source of hematopoietic stem cells for routine bone marrow reconstitution in both the unrelated and related HLA matched donor setting.

Maintenance of the transplantable hematopoietic cells in umbilical cord blood is achieved by storage in liquid nitrogen (temperature <-185°C). This protocol details steps involved in preparing cord blood for storage at this temperature with minimal loss of cell viability.

Specimen

Cord blood leukocyte-rich plasma (Bag #2) (Figure 4.2.2, SOP 4.2) in a volume of 21 ml following processing according to the SOP entitled "CBU Separation and Sample Preparation"

Equipment

Cryopreservation System

- BioArchive™ System (ThermoGenesis Corp.) which includes:
  - Robotic storage freezer
  - Two controlled rate freezer modules
  - Graphical User Interface (G.U.I.)
  - Bar code scanner and printer
  - Overwrap sealer
  - Magnetic stainless steel canisters
  - Insulated canister sleeves

OR

- Conventional System
  - Quarantine liquid nitrogen freezer
  - Controlled rate freezer
  - Liquid nitrogen storage freezer
  - Freezer gloves
  - Bar code scanner
  - Vacuum pump
  - Storage cassettes (i.e. Custom Biogenics)

Laminar Flow Hood
Controlled Rate Freezer
Bar code scanner
Heat Sealer e.g. Sebra Model 2100

Syringe pump OR
  Pall Medical positioning jig
Orbital Rotator or rocker
Cool Packs (0-4°C)
Freezer Gloves
Black marker pen
Balance
Ring stand with clamps (optional)
Large rubber band (optional)
Vacuum pump

Reagents

Cryoprotectant ambot containing 50% DMSO 5% Dextran 40 OR DMSO and Dextran 40	Pall Medical Corporation Must be approved for human use.

10% bleach (or equivalent non-corrosive disinfectant)

Supplies

Stem Cell Freezing Bag	Pall Medical Corporation, Stem Cell Processing Set (Set #2) (Figure 4.2.2, SOP 4.2)
Cryovials (2ml)	e.g. Nunc Plastics
1 ml Syringe (2)	Hospital Store Room
5 ml Syringe	Hospital Store Room
10 ml Syringe (2)	Hospital Store Room
30 ml Syringe	Hospital Store Room
CBU Cryopreservation Form	Cord Blood Bank Stationary
Alcohol Swabs	Hospital Store Room
Sterile Gauze Pads	Hospital Store Room
Cryopreservation Labels	Cord Blood Bank Stationary
Large rubber band	Hospital Store Room
Overwrap material	e.g. Dupont FEP-L overwrap bags (Thermogenesis Corp.)

COBLT Study Bar Code Labels

Procedure

1. a. Open cryopreservation database and create new cryopreservation record. Scan bar code label into new record.

   b. Record the identity of the responsible technologist, the date and the time in the database and on the CBU Cryopreservation Form (referred to hereafter as "the worksheet").

2. Apply adhesive bar code labels to Cryovials and storage cassette. Scan specimen and tubes to ensure match. Apply product labels to the stem cell freezing bag (Bag #4) (Figure 4.2.2, SOP 4.2). See Figure 4.3.1 for positioning COBLT label set bar code labels and product labels on the stem cell freezing bag (Bag #4). Labels produced by the BioArchive label printer should be attached as indicated in the freezer documentation.

3. If cryoprotectant ambot containing 50% DMSO and 5% Dextran 40 is available, then record the lot number and expiration date of the cryoprotectant on the worksheet and in the database.

   If cryoprotectant ambot is not available, then proceed with Steps 3a and 3b.

   a. Load a 10ml syringe with 5.25ml pre-cooled cryoprotectant (see Procedure Notes below on Preparation of Cryoprotectant).

   b. Record the lot number and expiration date of the DMSO and the Dextran on the worksheet and in the database.

4. If using frozen gel packs to pre-chill the CBU, then proceed with Steps 4a and 4b.

   a. Precool the processing bag (Bag #2) containing the CBU, the stem cell freezing bag (Bag #4), and the labeled cryovials for 15-25 minutes and a maximum of 6 hours.

   b. Retrieve the bag set and express the air from the stem cell freezing bag (Bag #4) into the processing bag (Bag #2) by opening the clamp on the tubing connecting the two bags, squeezing Bag #4, and reclamping the tubing.

   c. Surround the processing bag (Bag #2) with pre-cooled CoolPacks. Secure with a rubber band. Place the processing bag (Bag #2) in plastic dish on rocker and CoolPacks on the Orbital rotator and start rotation.

5. Initiate metered flow (0.25 ml/min) of DMSO into processing bag (Bag #2) of the stem cell processing set. Continue rotating the processing bag (Bag #2) to ensure adequate mixing of cells and cryoprotectant until addition of cryoprotectant is complete. A syringe pump or Pall Medical positioning jig may be used to add this solution.

6. After all cryoprotectant is added transfer contents of processing bag (Bag #2) into stem cell freezing bag (Bag #4).

   Note: It is expected that some of the contents of Bag #2 will remain in the tubing connecting Bag #2 and Bag #4. This material will be used to create segments (step 8) and to save cells for future testing (step 13).

7. Seal tubing to create 3 segments, starting from the proximal end of the tubing. Seals should be made at 90° to the coronal axis of the bag to allow tubing to bend parallel to the bag for proper storage (see diagram).

NOTE: Must seal the tubing first at the proximal end closest to the bag. Then seal out to end of the tubing to alleviate pressure buildup in the tubing. Then detach the processing bag (Bag #2) by cutting across the heat seal most distal to the stem cell freezing bag (Bag #4). Put processing bag (Bag #2) with attached tubing back on ice for removal of residual cells and processing of cryovials after completion of step 11. See step 12.

8. Wipe the surface of the stem cell freezing bag (Bag #4) with gauze soaked in 10% bleach or equivalent disinfectant. Wipe off excess. Overwrap the stem cell freezing bag (Bag #4) with overwrap material. Place the overwrapped bag into the storage cassette.

9. Controlled Rate Freezing

   BioArchive System: Insert the cassette into the controlled rate freezer (CRF) module and insert the loaded CRF into Port 1 or 2 as available. Use cursor of Graphical User Interface (GUI) to activate recommended CRF program. Each cord blood bank should empirically validate their BioArchive CRFs, beginning with the recommended CRF program 10°C/min to -3°C, 100% fan power to -15°C and 2°C/min to -50°C.

   OR

   Conventional System: Place the cassette into the controlled rate freezer, insert the temperature probe into the cassette, and initiate the freezing program. The precise program used is determined by the equipment and the number of CBUs being cryopreserved. Each cord blood bank should independently empirically optimize and validate its equipment and controlled rate freezing curves through measurement of recovery of viable colony-forming cells, beginning with a recommended freezing rate of 1°C/min to 2°C/min, starting at 4°C to -40°C and 10°C/min thereafter to -90°C.

10. After the CBU reaches the target temperature (e.g. -50°C in the BioArchive system, -90°C in the conventional system), place the CBU in the quarantine storage location and record the location on the CBU Cryopreservation Form.

    In the BioArchive system, the robotic arm will remove the CBU from the CRF and transfer it to a storage address in liquid nitrogen. In the conventional system, the CBU can be placed in a quarantine freezer in the vapor phase of liquid nitrogen.

11. Remove the printout of the freezer trace, apply an adhesive bar code label to the trace and place in the CBU's processing folder. For digitally recorded traces, record the trace ID number in the cryopreservation database and CBU Cryopreservation Form.

12. To prepare cryovials, remove residual cells from processing bag (Bag #2) and freeze in cryoprotectant as follows.

    a. Prepare 2 ml of 1x cryoprotectant by mixing 0.4 ml of residual 5x cryoprotectant with 1.6 ml of dextran in a 3 cc sterile syringe.

    b. Inject a total of 2 ml of 1x cryoprotectant formulated in 12a into processing bag (Bag #2).

c. Strip any remaining cells + cryoprotectant left in the tubing that had been attached to the cryo bag back into processing bag (Bag #2). Mix with 1x cryoprotectant.

d. Remove all residual fluid from processing bag (Bag #2). Divide equally into two, 1-2ml labelled cryovials and freeze in liquid nitrogen according to institutional practices.

13. Seal cryovials inside of tubing for safe storage of LN_2 (to avoid the possibility of contamination and explosion)(optional). Freeze cryovials to maintain cell viability. Place cryovials in the vapor phase of the quarantine liquid nitrogen storage freezer. Record location on the worksheet.

Quality Control

The trace of the controlled rate freezer showing the freezing curve/sample temperature must be within validation tolerances as specified below in Procedure Notes.

Procedure Notes

Batch Preparation of Cryoprotectant (If Required)

1. Cryoprotectant is to be prepared freshly each day with the lot numbers and expiration dates of each reagent noted and recorded on the container (a 150 ml glass bottle with a needle entrance port). Weigh the empty bottle for later calculations. Record the weight onto the bottle.

2. The volume of cryoprotectant prepared each day is dependent upon the expected workload. The basic CBU is approximately 105ml.

3. Using a syringe remove 55ml of DMSO and inject into the 150 ml glass bottle.

4. Weigh the bottle, subtract the weight of the empty bottle and divide the answer by 1.1 to determine the volume to dextran to add.

5. Using a second syringe remove the calculated volume of dextran from the container and add to the DMSO in the bottle.

6. Mix the cryoprotectant by inverting the bottle 4-5 times and place in the refrigerator to pre-cool.

Controlled Rate Freezer Program

The precise program used is determined by the equipment and the number of CBUs being cryopreserved. Each Cord Blood Bank will independently validate their equipment and program to provide freezing from 10°C/min starting at 4°C to -3°C, 100% fan power to -15°C and 2°C/min to -50°C for the BioArchive system, or 1°C/min to 2°C/min, starting at 4°C to -40°C and 10°C/minute thereafter to -90°C for the conventional system.

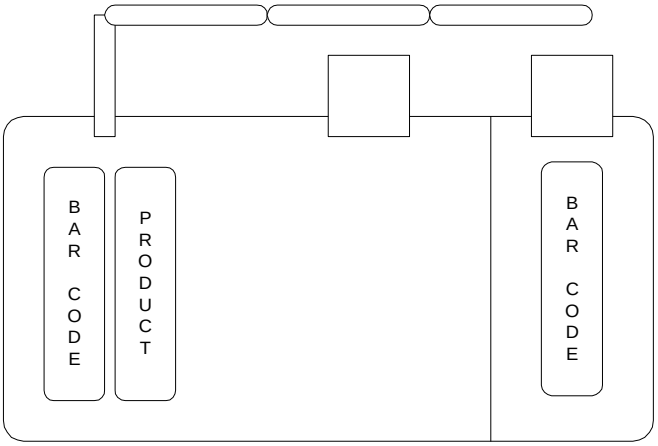
References

Areman E, Deeg HJ, Sacher RA. ***Bone Marrow and Stem Cell Processing: A Manual of Current Techniques***. F.A. Davis Company, Philadelphia, 1992.

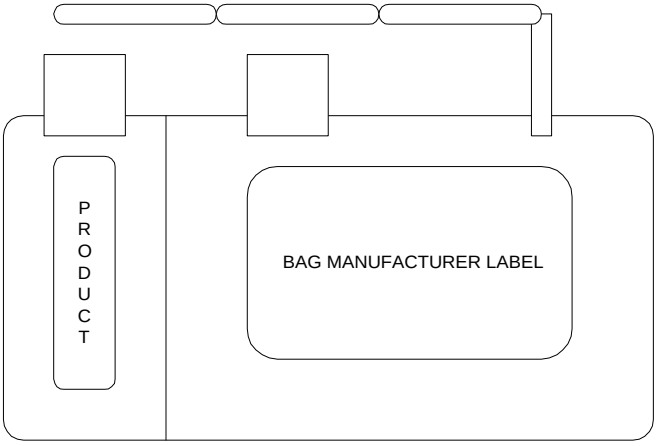
Rubinstein P, Dobrila LI, Rosenfeld R, et al. Proc of the National Academy of Sciences USA, Volume 92, pp. 10119-10122, October 1995.

Figure 4.3.1

POSITION OF COBLT BAR CODE LABELS
AND PRODUCT LABEL ON THE FRONT
OF THE STEM CELL FREEZING BAG



POSITION OF COBLT PRODUCT LABEL
ON THE BACK OF THE STEM CELL
FREEZING BAG



4.4 RELEASING CBU FROM QUARANTINE TO LONG TERM STORAGE

Principle

All umbilical cord blood units (CBU) will be kept in a quarantine storage location until the results of all exclusion criteria have been determined and found to be negative. All samples of the mother's peripheral blood or umbilical cord blood stored for future testing will be stored in long term storage freezers at appropriate temperatures. The following guidelines will apply for release from quarantine of the CBU and placement in long term storage.

Guidelines

1. No CBU will be released from quarantine until all exclusion criteria have been determined and found to be negative. This determination will be made by a designated CBB staff member AND the Director/PI of the Cord Blood Bank, or his/her designate, using the CBU Exclusion and Quarantine Release Form.

2. If one or more of the exclusion criteria are met at any point, the CBU will be discarded using the discard procedures in CBB SOP - Discarding CBUs. If the infectious disease testing results are positive for HIV-1, HIV-2, Hepatitis B, Hepatitis C or HTLV-I/II, the mother will be notified according to CBB SOP - Notifying Donors of Positive Infectious and Genetic Disease Test Results.

   CBUs will be excluded from long term storage and discarded if newborn hemoglobinopathy screenings indicate the presence of the following diseases:

   - β Thalassemia (homozygous)
   - Hemoglobin C (homozygous)
   - Hemoglobin E (homozygous)
   - Hemoglobin SS
   - Hemoglobin SC
   - Hemoglobin S/β Thalassemia
   - Hemoglobin S/ and other clinically relevant abnormal β gene

3. When all criteria for release into long term storage have been met, the CBUs will be transferred from the quarantine freezer (as documented in the CBB SOPs) into the long term storage freezer, where they will be stored in the liquid phase of liquid nitrogen. If the BioArchive system is used to store cryopreserved CBUs, the CBU storage address will remain unchanged.

4. Vials of the mother/donor's peripheral blood, designated for future HLA typing, will be placed in long term storage freezer at $\leq$ -20°C.

5. The aliquot of the mother/donor's serum, designated for future infectious disease testing, will be placed in long term storage freezer at $\leq$ -20°C.

6. The information on the CBU Exclusion and Quarantine Release Form and all other data regarding the CBU will be transmitted to the Medical Coordinating Center for entry into the cord blood bank registry.

4.5 **RELEASE FROM LONG TERM STORAGE**

Principle

Once a Transplant Center, in collaboration with the MCC, has designated a particular cord blood unit (CBU) for a particular recipient there are several steps which must be followed to ensure that the correct CBU is supplied to the Transplant Center in a timely fashion. Further, Cord Blood Bank Quality Control activities require that the Transplant Center provide feedback regarding the state of the CBU following thawing.

Specimen

Cryopreserved umbilical cord blood that has been designated for transplant into a particular recipient.

Equipment

Database computer

Reagents

None

Supplies

Release Checklist	CBB Stationary
Confirmation of Registration/ CBU Release Request	Transplant Center
Investigators Brochure	Medical Coordinating Center

Procedure

1. a. CBUs can be released by the COBLT CBB after receipt of a Confirmation of Registration/CBU Release Request from the Transplant Center and an Investigators Brochure from the MCC.

   b. The Transplant Center will provide the following information on the Confirmation of Registration/CBU Release Request:

      1. the requested CBU ID
      2. the COBLT Recipient ID
      3. name and contact address of Transplant Center staff receiving CBU
      4. proposed CBU shipment and transplant dates
      5. the signature of the responsible physician

      In addition, the Transplant Center must provide the delivery address of the Transplant

Center processing laboratory and, if possible, a map showing the precise location.

On receipt of the Confirmation of Registration/CBU Release Request, the CBB Distribution Coordinator (or authorized representative) will check the information and fax the completed request to the MCC.

c. The MCC will provide the Investigators Brochure with the following information:

- a packing information sheet
- a Transplant Center Feedback Sheet with the CBU ID and COBLT Recipient ID
- CBU receipt procedures
- COBLT CBU thawing procedures
- COBLT CBU infusion procedures
- product information

2. The CBB Distribution Coordinator (or authorized representative) will confirm that the CBU ID and Recipient ID from the Transplant Center and from the MCC match. Any discrepancy must be corrected before continuing. The CBB Coordinator will then confirm, using an internal checklist, the location of the CBU and of saved specimens, and recheck that all paperwork in the CBU folder is complete.

3. The Cord Blood Distribution Coordinator (or authorized representative) will contact the Transplant Center Coordinator to confirm receipt of the request and the proposed shipping date.

4. The Cord Blood Distribution Coordinator (or authorized representative) will contact Federal Express to schedule the pick-up and delivery of the CBU 'dry shipper' to the Transplant Center.

5. Once a preliminary schedule is obtained, the Cord Blood Distribution Coordinator (or authorized representative) will contact the Transplant Center Coordinator to confirm delivery. The Cord Blood Distribution Coordinator records the confirmed date of delivery on the Transplant Release Checklist, then signs and dates. The completed checklist must be sent to the Transplant Center Coordinator and CBB Laboratory Supervisor for review within 24 hours.

6. On the day of release of a CBU, the Laboratory Supervisor ensures that:

   a. The Dry Shipper is loaded with sufficient liquid nitrogen for maintenance of the CBU at < -150° C for at least 48 hours following the scheduled time of arrival at the Transplant Center.

   b. The Investigators Brochure and return shipping information to accompany the Dry Shipper are completed and packed with the shipper (see SOP 5.2).

7. The CBB Cord Blood Unit Paperwork Folder is located and a minimum of 8 bar code labels are removed and packed with the Investigators Brochure.

8. The cryopreserved CBU is removed from long term storage as documented in the CBB SOPs. The CBU identity is re-confirmed after retrieval from long term storage by the Laboratory Supervisor and at least one other staff person. Confirmation is obtained by one person reading the number aloud while the other confirms with the written request.

9. The CBU is then placed in the Dry Shipper, packaged, labeled, and handed to the courier for transport according to CBB SOP Section 5.2, Shipping Cryopreserved CBUs to Transplant Centers.

10. Following shipment, data on the CBU Disposition Form indicating the CBU was shipped for transplant are entered into the COBLT data system. The CBU Disposition Form is filed in the CBU Paperwork Folder.

Quality Control

1. All CBU paperwork, information, and infectious disease data are checked prior to release.

2. The identity of the CBU is confirmed by the Laboratory Supervisor and at least one other staff person. Confirmation is obtained by one person reading the number aloud while the other confirms with the written request.

3. The Transplant Center is provided with a feedback sheet to provide information on the following Quality Control variables:

    - Date and time of receipt
    - Weight of unpacked 'dry shipper'
    - Condition of CBU on receipt
    - Condition of shipper on receipt
    - Shipper temperature

4. Only one CBU will be released at a time if more than one CBU is scheduled to be shipped on the same day.

4.6 **DISCARDING CBUS**

Principle

A number of tests and standards are used to determine the potential suitability of a cord blood unit (CBU) for transplant. When a particular CBU fails to meet any one of these standards (as defined by the exclusion criteria on the CBU Exclusion and Quarantine Release Form) that CBU must be discarded. CBUs returned to the Cord Blood Bank (CBB) from a transplant center will also be discarded following the procedures below. Records associated with discarded CBUs must be updated accordingly.

Specimen

Umbilical cord blood at any stage of processing and storage that has failed to meet all tests and standards; CBUs returned to the CBB from a transplant center.

Equipment

Electronic Bar Code Scanner
Database computer

Reagents

None

Supplies

Cord Blood Unit Paperwork Folder	From Cord Blood Unit Records
Adhesive Bar Code Label Sheets	From Collection Kit
CBU Disposition Form	Cord Blood Bank Stationary

Procedure

1. Locate the paperwork folder for the CBU and the associated adhesive bar code labels. Scan labels, folder, and CBU to confirm identity.

2. a. Review and confirm the test result(s) that triggered the exclusion criterion.

   b. If the result is **not** confirmed take the CBU and all paperwork to the Laboratory Supervisor for second confirmation. If the CBU is found to be within acceptable standards it can be returned to the processing path. A Corrective Action form outlining the confirmation process must be filed in the CBU Paperwork Folder.

   c. If the result is confirmed, the laboratory supervisor or director/PI must be brought in to repeat confirmation. If the supervisor or director/PI confirms the result the CBU must be discarded according to the following procedures.

3. If any additional tests on the CBU are pending (e.g.: Flow Cytometry, Colony Assays, Infectious Disease Screening etc.) inform the laboratory supervisor or designee, who will then take appropriate steps to halt further testing.

4. Place an adhesive bar code label on the CBU Disposition Form. Open the CBU Disposition database and scan to confirm label identity. The CBU Disposition Form and database should both be completed following this procedure. Hereafter, they will be collectively termed the database.

5. Record on the database the reason for discard and the name of the responsible technologist and the laboratory supervisor or designee who confirmed the results. The Laboratory Technologist should sign the CBU Disposition Form at this time.

6. Open the database, locate the record for this CBU (using the scanner to enter the ID number) and record the date of discard, technologist ID, laboratory supervisor ID, and reason for discard.

7. a. The laboratory supervisor shall then determine whether the CBU can be used for Quality Assurance purposes. Note: If the reason for discard is failure to obtain consent the CBU must be discarded according to Step 9 below and cannot be used for Quality Assurance or research.

   b. If the CBU is deemed suitable for Quality Assurance testing this should be recorded in the database. Quarantined samples shall then be transferred to the QA section of the Quarantine Liquid Nitrogen Storage Freezer and the new location recorded in the database. Unprocessed CBUs will be processed according to the appropriate SOP and stored in the QA section of the Quarantine Liquid Nitrogen Storage Freezer.

8. If the CBU to be discarded is not required for Quality Assurance the Director or Medical Director may release it for ancillary research provided that consent for such use was obtained and that the researcher provides evidence of a currently approved, IRB reviewed, research protocol that allows for use of umbilical cord blood. The identity of the Principal Investigator of the research program, the IRB approval number, and the date of release will be recorded in the database.

9. If the CBU is unsuitable for research or is surplus to requirements, the sample can be discarded as Biohazardous Waste according to the standard practices of the host institution. The means and date of disposal must be recorded in the database.

Quality Control

The Laboratory Supervisor and the Responsible Technologist must both sign the CBU Disposition Form and their identities must be recorded in the database once both have confirmed that a CBU is

unsuitable for banking. The Laboratory Supervisor must also sign for the release of any CBU for Quality Assurance or research.

4.7 MANUAL DIFFERENTIAL CELL COUNTS

Principle

Manual differential cell counts will be required to calculate the leukocyte count and recovery of viable mononuclear cells before Hespan sedimentation and after plasma depletion/pre-cryopreservation.

Specimens

Blood smears on microscope slides will be made in triplicate from the pre-Hespan and post-plasma depletion/ pre-cryopreservation product. The amount of materials per slide may be estimated as follows:

a. Pre-Hespan: Three slides with one drop of unprocessed umbilical cord blood per slide.

b. Post-plasma depletion/pre-cryopreservation: Three slides with 20 µl of the leukocyte enriched fraction per slide.

Procedure

1. Proportions of leukocytes and nucleated erythrocytes will be determined from a 200 nucleated cell differential. Mononuclear cells (MNC) will be differentiated from other leukocytes and nucleated erythrocytes. The proportions of mononuclear cells and nucleated erythrocytes in 200 cells counted will be recorded on the CBU Processing Form. The proportion of leukocytes will be calculated by subtracting the nucleated erythrocytes from the total nucleated cell count.

2. Mononuclear (defined as blasts + monocytes + lymphocytes) cell counts, nucleated erythrocytes, and leukocyte (defined as all nucleated cells other than nucleated erythrocytes) cell counts will be determined by performing a manual differential of unprocessed umbilical cord blood (pre-Hespan sedimentation) and 20 µL of leukocyte enriched umbilical cord blood (post-plasma depletion/pre-cryopreservation). One slide of each triplicate will be stained using Wright-Giemsa. The method of staining may be automated or manual using manufacturer's instructions. The four remaining unstained slides will be stored for future use.

3. Total viable leukocyte count in the umbilical cord blood unit (CBU) before Hespan sedimentation and after plasma depletion/pre-cryopreservation will be determined by calculating the product of the total viable nucleated cell count and the percentage of leukocytes (i.e., excluding nucleated erythrocytes) in the manual differential.

Total viable leukocyte count = (total viable nucleated cell count) (100%-% nucleated erythrocytes)

4. The total viable mononuclear count (MNC) in the CBU before Hespan sedimentation and after plasma depletion/pre-cryopreservation will be determined by the following calculation and recorded on the CBU Processing Form:

Total viable MNC = (total viable nucleated cell count) (% mononuclear cells)

5. Viable mononuclear cell recovery after plasma depletion/pre-cryopreservation will be determined by the following calculation and recorded on the CBU Processing Form:

$$\% \text{ viable MNC recovered} = \frac{\text{total viable MNC}_{post} \times 100}{\text{total viable MNC}_{pre}}$$

Quality Control

1. Specimens from only one CBU will be made at any one time.

2. Bar code numbers on the slide label will be verified against the CBU before and after Hespan sedimentation.

3. Bar code numbers on CBU Processing Form will be verified against bar code numbers on the microscope slides.

4. Technologist's ability to perform manual differentials on umbilical cord blood specimens will be documented by in-service training and biannual recertification testing.

5. Filed unstained slides will be available for future analyses.

CHAPTER 5

SHIPPING PROCEDURES

Chapter 5

SHIPPING PROCEDURES

5.1 SHIPPING CBUS FROM COLLECTION CENTERS TO CORD BLOOD BANKS

Principle

Collected umbilical cord blood has been found to maintain cell viability when temporarily stored at 15° to 25°C. To ensure proper temperature maintenance during shipment, standard platelet shippers are used. In addition, shipments are tracked using a post-collection storage and shipment log. **This procedure should be followed in conjunction with SOP 3.4, Post-Collection Storage of CBU Prior to Transport to CBB Processing Laboratory.**

Specimen

Umbilical Cord Blood Units collected after placental delivery into a collection bag(s) containing CPD anticoagulant and packed in a numbered platelet shipper (see SOP 3.4).

Equipment

Packed Numbered Platelet Shipper

Supplies

Shipping Log
Adhesive Tape
Shipping Labels
Shipping Envelope

Procedure

1. Check the platelet shipper number against the number recorded on the Shipping Log.

2. Check the outside of the shipper for damage. If damage has occurred, transfer all packed items to a new shipper as described in SOP 3.4. Return the damaged shipper to the CBB Processing Laboratory.

3. Check the following labels are securely attached to the outside of the platelet shipper:
   STUDY-SPECIFIC LABEL (See SOP 3.4, Post Collection Storage of CBUs Prior to Transport to CBB Processing Laboratory)
   "CORD BLOOD BANK ADDRESS/IF SHIPMENT IS DELAYED ... NOTIFY"
   "PERISHABLE"
   "WARNING! DO NOT ICE"
   Replace missing and/or loose labels.

4. Visually inspect stored CBUs to ensure that all zipper-locked bags are securely sealed. Seal bags if necessary.

5. Check unit bar codes against the bar codes on the Shipping Log and record the total number of units in the shipper. Resolve any discrepancies. Record unresolved discrepancies on the log.

6. Check for evidence of blood spills. Record evidence of blood spills in the appropriate 'Comment' line on the Shipping Log. Do not discard or transfer any unit to a new shipper if a blood spill occurs. All collected units will be discarded by the Cord Blood Bank.

7. Repack the shipper (see SOP 3.4). If substantial space exists, and/or extended storage is anticipated, add additional conditioned TSPs.

8. Complete the Shipping Log by recording the number of units packed, the date and time packing was completed, and the packer's study ID number. Sign the log and place in the shipper.

9. Place the foam plug in the box and push until it is on top of the TSPs. Close the shipper lid and tape the lid securely to the base.

10. Transport the filled shipper to the CBB Processing Laboratory. If a courier service is used for this purpose, verify that the shipper will be transported and handled carefully.

Quality Control

1. The above steps are the responsibility of a trained staff member.

2. One staff member will prepare and verify each shipment.

3. Documentation of this responsibility is indicated by the name/initials of the staff member on the Shipping Log.

5.2 **SHIPPING CRYOPRESERVED CBUS TO TRANSPLANT CENTERS**

Principle

Shipping of cryopreserved umbilical cord blood units requires that the unit remains in a frozen state for at least 72 hours. "Dry" shipper containers allow shipment of cryopreserved units at liquid nitrogen temperatures without the risk of liquid nitrogen spillage in transit. A fully charged "dry" shipper is capable of holding a temperature of < -120°C for a period of 8 days. This SOP outlines the steps performed to prepare, pack, and ship cryopreserved units using a "dry" shipper.

Specimen

Labelled frozen umbilical cord blood unit, contained in a metal canister and stored in a liquid nitrogen storage freezer in the liquid phase of liquid nitrogen.

Equipment

Validated "Dry" Shipper e.g. MVE Cryo-Mini
Polystyrene Outer Container

Reagent

Liquid nitrogen

Supplies

Thermal Exposure Indicator e.g. Cryoguard M-120
Federal Express Airway bill forms
Sealable Plastic Bag
Investigators Brochure MCC
COBLT Bar Codes (minimum of 8) CBU File
Labels - "Biological Product", "Dry Shipper Containing Liquid Nitrogen", "Do Not X-Ray", and "Do Not Invert or Tip"
Gloves for cryogenic work
Heavy Duty Scale

Procedure

1. Confirm that a validated "dry" shipper is available. See Procedure Notes for initial and ongoing validation requirements.

2. - Confirm that a complete Investigators Brochure from the MCC and information from the Transplant Center have been received (SOP 4.5, Release from Long Term Storage). A complete Investigators Brochure has the following items: Transplant Center Feedback Sheet, Packing Information, Receipt Procedures, Procedure for Thawing Cryopreserved Cord Blood (CBU) for Transplant, Procedure for Infusing Thawed CBU,

and Product Information.

- Make arrangements with Federal Express and the receiving transplant center for pick up and delivery as detailed in the Release from Long Term Storage SOP 4.5. File air bill.

- Complete the CBB Shipper Number, Federal Express Tracking Number, and CBB contact information on the Packing Information in the Investigators Brochure and fax a copy of this sheet to the receiver at the Transplant Center prior to shipment.

3. Prepare the "dry" shipper 24 hours before shipment by filling at least halfway with liquid nitrogen. Replace the cover and allow to stand to allow the liquid nitrogen to soak into the sponge material.

4. Pour off any remaining free liquid nitrogen, weigh the shipper, and record the weight on the Transplant Center Feedback Sheet in the Investigators Brochure.

5. Place the released cryopreserved CBU and thermal exposure indicator in a plastic bag and seal the bag to prevent leakage. Place the unit in the "dry" shipping container and close the shipper.

6. Place the complete Investigators Brochure and COBLT Bar Code Labels in the top portion of the shipper. Close and lock the "dry" shipper top.

7. Label the shipping container as "Biological Product", "Dry Shipper Containing Liquid Nitrogen", "Do Not X-Ray", and "Do Not Invert or Tip".

8. Attach the following information:

   - Name and address of the receiving institution.

   - Name of receiving laboratory including the name, room number, and telephone number of the staff member responsible for receiving the shipment.

   - Description of contents.

   - Name and address, and telephone number of shipping institution, and name of the responsible person at the institution, as well as emergency notification instructions such as pager number for the responsible person.

9. Turn shipment over to Federal Express courier.

Quality Control

1. Validated "dry" shippers will be used to ship cryopreserved CBUs to transplant centers.

2. The shipping container will be charged to its full capacity. When fully charged, the shipper should maintain a temperature at < -120°C for 8 days, according to manufacturer's instructions.

Shipment will be by overnight courier.

3. Transplant Center staff will be notified of the expected arrival time, courier service and tracking numbers for the unit.

4. The unit's bar code number will be verified by two members of the Cord Blood Bank staff.

5. Transplant Center staff will notify Cord Blood Bank staff of arrival of the shipper.

Reference

Areman E, Deeg HJ, Sacher RA. Bone Marrow and Stem Cell Processing: A Manual of Current Techniques. F.A. Davis company, Philadelphia, 1992.

Procedure Notes

Validating "Dry" Shippers - Summary of Validation Requirements

- Initial Validation. Record daily temperature and weight of the charged "dry" shipper. Record for a total of 8 days from the time of charging.

- Quality Control. Upon return from transport, the "dry" shipper must be recharged and tested for temperature and weight for a period of 8 days from the time of the recharge. The results must be recorded.

- All "dry" shippers must be tested for temperature semi-annually (summer and winter).

Initial Validation

Upon receipt from the supplier or manufacturer, perform the following steps:

1. Remove the cover of the shipper.

2. Fill out the warranty activation card and return to the manufacturer.

3. Fill out the Vendor/Validation Sheet.

4. Obtain a liquid nitrogen supply tank. Attach an LN2 supply hose with phase separator to the LN2 tank.

   NOTE: Use insulated gloves and face shield while filling the shipper with liquid nitrogen. Follow established safety practices and procedures for transferring liquid nitrogen.

5. Fill the vapor shipper to approximately 3/4 full.

6. Replace the cover and let stand for 24 hours, allowing the liquid nitrogen to be absorbed by the

shipper.

7. At the end of the 24-hour period, refill the shipper according to the steps above and let stand for an additional 24 hours.

8. After the second 24-hour period, pour off any remaining liquid nitrogen and weigh the shipper. Record the weight. Remove the shipper cover and insert a type J/K thermocouple probe into the center of the shipper in a manner such that the other end of the thermocouple remains outside of the shipper. Replace the cover and let stand for 1 hour.

9. At the end of 1 hour, attach a type J/K thermometer to the exposed end of the thermocouple. Turn the thermometer on and wait until the digital temperature display stops fluctuating. Record the temperature on the Initial Validation Sheet.

   Acceptable temperature range: colder than -120°C.

10. Repeat steps 8 and 9 for 8 days. Calculate the evaporation rate by subtracting the weight of the shipper on the second day from the weight of the shipper on the first day. Record the rate.

    NOTE: If it is not possible to check the temperature and weight on all 8 days (due to lab closure, weekends, etc.), it is important to check the temperature and weight on the eighth day. This will determine if the shipper has held an acceptable temperature through the maximum amount of time recommended by the manufacturer.

11. Record any uncommon occurrences such as excess frosting or sweating along the outside of the shipper or excess evaporation.

12. If the temperature and weight tests pass validation, the shipper is cleared for use.

13. If the shipper fails the validation tests in any manner, the manufacturer or service dealer must be contacted for further instructions. In this case, the shipper must not be used for transportation until it passes the validation requirements.

Validation After Use

1. Upon return of the "dry" shipper from the transplant center, inspect the following:

   - Check the outer polystyrene container for damage (cracks, indentations or holes, etc.).
   - Remove the "dry" shipper from the outer container and check for damage (cracks, dents, leakage, etc.).

2. Follow steps 1 through 12 above and record results.

3. The shipper must pass the validation requirements for continued service.

4. If the shipper does not pass the validation requirements, it must be taken out of service and referred to the manufacturer or service specialist.

Semi-Annual Validation

1. The temperature holding capabilities of the "dry" shipper must be checked once during the winter and once during the summer. Checks performed as part of the initial validation or validation after return from the transplant center may be used for the semi-annual validation.

2. Follow steps 1 through 12 above for the temperature requirements only.

3. The "dry" shipper must pass the temperature requirements for continued service.

4. If the "dry" shipper does not pass the temperature requirements, it must be taken out of service and referred to the manufacturer or service specialist.

References

Manufacturers' Instruction Pamphlet

Code of Federal Regulations, 606.60, Title 21, 1996.

CHAPTER 6

SAMPLE TESTING AND CHARACTERIZATION

Chapter 6

SAMPLE TESTING AND CHARACTERIZATION

6.1 HLA Typing

6.1.1 Preliminary

Principle

Molecular HLA typing will be performed for every cord blood unit stored in the cord blood bank. The minimum level of typing will be low resolution (a level similar to serological HLA typing) for HLA-A and -B and intermediate resolution (corresponding to five or less alleles for most samples) to high resolution (corresponding to a single allele) for DRB1. The alleles which can be detected are listed in Appendix E. The types are defined according to the WHO Nomenclature Committee for Factors of the HLA System with the original list obtained as of December 1996. These will be updated semi-annually.

Specimen

The specimens for HLA typing will be frozen aliquots from the granulocyte/red cell-enriched pellets that remain after preparation of the cord blood unit. Cord blood banks will freeze 1 ml aliquots of the granulocyte/red cell-enriched pellet. Each week the cord blood bank will ship to each HLA Reference Laboratory (Dr. Terasaki's laboratory at the University of California - Los Angeles, Dr. Lee Ann Baxter-Lowe's laboratory at the University of California - San Francisco, and Dr. Jennifer Ng's laboratory at the Navy Medical Research Institute-NMRI) one vial per cord blood unit which has completed quarantine release requirements.

Materials and Reagents

Appropriate HLA typing reagents as described in the detailed protocols of each Laboratory including oligonucleotide sequences of PCR and sequencing primers.

Note: A list of these reagents will be maintained by the Laboratory in compliance with ASHI regulations. Reagents may be revised during the project as techniques and knowledge of HLA polymorphism improves. Historic records regarding reagents are maintained in accordance with ASHI regulations.

Procedure

1. Locate HLA Request Log included with each shipment. Unpack shipments of specimens stored on dry ice, confirming that the contents of the shipment correspond to the HLA Request Log. Consult with the Cord Blood Bank regarding any discrepancies between the log and contents of the shipment.

2. Log each sample into the Laboratory inventory using bar codes provided by the Cord Blood

Bank. An example of a log in sheet is provided in Appendix E.

3. Type samples according to the appropriate SOPs within the Laboratory. The final data will be interpreted with respect to a list of recognized HLA alleles that is maintained by the Medical Coordinating Center (MCC). This list will be updated semi-annually to include additional alleles that satisfy the following criteria:

   a. Recognized by the WHO Nomenclature Committee

   b. Sufficient length of sequence available to perform typing

   HLA typing protocols comply with ASHI regulations. These reagents and protocols may be updated during the project as techniques and knowledge of HLA polymorphism improve. Historic records regarding protocols and reagents are maintained in accordance with ASHI regulations.

4. Laboratories will store remaining frozen specimens (cord blood, blood, or and DNA) until further directions are received from the MCC, not to exceed the concluding date of the project.

Data Reporting

The last working day of each week the Laboratory will send to the MCC a report and an electronic data file containing the following information for typings completed during the week:

a. Specimen identification, including bar code label number
b. Assigned type
c. HLA alleles that are potentially present in the specimen
d. Special notation for samples with unusual linkage
e. Typing method
f. Data required by the MCC to update typing assignments as knowledge of HLA polymorphism improves

Electronic data may be sent via diskette or Internet.

6.1.2 **Confirmatory**

Principle

Confirmatory molecular HLA typing will be performed for every cord blood unit that is a potential candidate for transplant. The minimum level of typing resolution will be low (a level similar to serological HLA typing) for HLA-A and -B and high resolution (corresponding to a single allele for most samples) for DRB1. The alleles that will be detected are listed in Appendix E. The types are defined according to the WHO Nomenclature Committee for Factors of the HLA System with the original list obtained as of December 1996. These will be updated semi-annually. Supplemental typing of HLA-C and -DQB1 will be performed as clinically indicated for selection of cord blood units for transplant.

Specimen

The specimens for HLA typing will be frozen aliquots from the granulocyte/red cell-enriched pellets that remain after preparation of the cord blood unit. The MCC will request confirmatory typing of samples that satisfy the following criteria:

- satisfy the minimal match criteria for a transplant candidate
- no prior confirmatory typing at a level of resolution that is required for evaluation of the cord blood unit for the transplant candidate

Note: This may include retyping to detect alleles that were not detectable using reagents that were available at the time of prior confirmatory typing and/or HLA-C and -DQB1 typing as clinically indicated.

The NMRI laboratory will perform confirmatory typing for all CBUs which were not preliminary typed at their lab. The University of South Carolina will perform the typing for the remainder of the CBUs.

Materials and Reagents

See *Materials and Reagents* in Section 6.1.1

Procedure

See *Procedure* in Section 6.1.1

Data Reporting

See *Data Reporting* in Section 6.1.1

Quality Control

The MCC will compare the original typing and confirmatory typing. Concordance or discrepancy will be recorded. Concordant typings will be forwarded to the requesting Transplant Center. The HLA Laboratories will be contacted regarding discrepant typings. If review of available data and/or retyping resolves the discrepancy, the revised results will be sent to the MCC along with an explanation for the cause of the incorrect typing. These data will be reviewed by the MCC and Histocompatibility Subcommittee on a semi-annual basis to identify causes of error that can be corrected. If the discrepancy cannot be resolved by the two laboratories, the specimens will be sent to the third laboratory. The results of typing of the three samples will be forwarded to the MCC. All data will be reviewed by the MCC and Histocompatibility Subcommittee to resolve the typing discrepancy and to identify causes of error that can be corrected.

6.1.3 **Maternal Samples**

Principle

Molecular HLA typing will be performed for maternal samples when a CBU has been identified as a match for a potential recipient and for 200 CBUs collected by each CBB from ethnic minorities. The minimum level of typing will be low resolution (similar to serological HLA typing) for HLA-A and -B and DRB1, unless higher level resolution is useful to establish HLA haplotypes. Alleles which can be detected are listed in Appendix E. The types are defined according to the WHO Nomenclature Committee for Factors of the HLA System with the original list obtained as of December 1996. These will be updated semi-annually.

Specimen

Cord blood banks will freeze 1-ml aliquots of maternal blood samples. As requested, the cord blood bank will ship to a designated Laboratory (Dr. Terasaki's laboratory at the University of California - Los Angeles, Dr. Lee Ann Baxter-Lowe's laboratory at the University of California - San Francisco, or Dr. Jennifer Ng's laboratory at the Navy Medical Research Institute-NMRI) one vial per maternal blood sample requested. The Coordinating Center will also provide typing of the associated cord blood unit for use in determining the appropriate level of resolution and deducing haplotypes.

Materials and Reagents

See *Materials and Reagents* in Section 6.1.1

Procedure

See *Procedure* in Section 6.1.1

Data Reporting

Each Laboratory will send to the Coordinating Center a report containing the following information for:

a. Specimen identification
b. Assigned types
c. Typing method
d. Haplotypes (or indication that haplotypes cannot be deduced from maternal typing)

6.1.4 **Retrospective**

Principle

Retrospective molecular HLA typing will be performed for donor cord blood units and recipients. The typing will be high resolution (corresponding to a single allele for most samples) for HLA-A, -B, -C, DRB1, and DQB1. The types are defined according to the WHO Nomenclature Committee for Factors

of the HLA System with the original list obtained as of December 1996. These will be updated semi-annually. Supplemental typing of HLA-DQA, DPB, and DPA may be determined at a later date.

Specimen

The specimens for HLA typing will be:

- frozen aliquots from the granulocyte/red cell-enriched pellets that remain after preparation of the cord blood unit.

  Cord blood banks will freeze 1-ml aliquots of the granulocyte/red cell-enriched pellet. As requested, the cord blood bank will ship to a designated Laboratory (Dr. Terasaki's laboratory at the University of California - Los Angeles or Dr. Lee Ann Baxter-Lowe's laboratory at the University of California - San Francisco) one vial per cord blood unit requested for retrospective typing by the Coordinating Center.

- blood samples from the recipient.

Materials and Reagents

See *Materials and Reagents* in Section 6.1.1

Procedure

See *Procedure* in Section 6.1.1

Data Reporting

Reporting of retrospective typings completed during each month will be reported on the last working day of each month. Each Laboratory will send to the Coordinating Center a report containing the information specified in *Data Reporting* in Section 6.1.1.

6.1.5 **Shipment of HLA Samples**

Each shipment should contain bar code labeled specimens, packing lists with bar code labels, and a minimum of 10 specimens. Specimens should be shipped on dry ice using a local courier or an overnight shipper as appropriate.

For preliminary typing, specimens should be sent after the CBU has cleared quarantine or with the Cord Blood Bank Director's approval.

6.2 **SCREENING FOR GENETIC DISEASES**

Principle

Transmission of heritable disorders through hematopoietic stem cells is a risk associated with umbilical cord blood transplantation. Attempts to minimize this risk will be accomplished by 1) restricting collections of cord blood to those mother/infant pairs that have uncomplicated pregnancies and deliveries, normal physical examinations, and family histories unremarkable for genetic diseases; 2) obtaining results of state screening programs for thalassemia and sickle cell disease.

Viable mononuclear cells, plasma and DNA from the umbilical cord blood graft will be stored for future genetic disease screening. Specimens will be available for genetic disease screening: 1) to evaluate the donor for presence of a specific genetic disease, 2) to determine carrier status for a specific genetic disease, or 3) to evaluate new strategies for genetic disease screening as they become available.

Specimens

0.5 ml aliquots of leukocyte enriched umbilical cord blood cells ($\geq 3 \times 10^6$/vial) from the leukocyte pellet prior to cryopreservation; aliquots of plasma (1 mL/vial) from the leukocyte poor plasma fraction; aliquots of the granulocyte/red cell-enriched pellet.

Procedure

An umbilical cord blood unit that has been identified for a patient with a specific enzyme deficiency should be screened for the same enzyme deficiency found in the potential transplant recipient. When feasible, the sample should be screened prior to transplantation. Cryopreserved nucleated cells, plasma and/or DNA will be made available for this purpose. Samples may be sent to the following laboratories:

DISEASE	ENZYME ASSAY	SAMPLE
Hurler syndrome	a-L-iduronidase	Cells
Maroteaux-Lamy syndrome	aryl sulfatase B	Cells
Adrenoleukodystrophy	co-A ligase	Plasma
Metachromatic Leukodystrophy	aryl sulfatase A	Cells
Globoid Cell Leukodystrophy	galactocerebrosidase	Cells
Mannosidosis	mannosidase	Cells
Fucosidosis	fucosidase	Cells
Wolman syndrome	acid lipase	Cells
Lesch-Nyhan syndrome	HGPRT	Cells
Type III Gaucher disease	glucocerebrosidase	Cells

All listed diseases can be evaluated using DNA based methods should cells or plasma not be available.

Quality Control

1. The quality control assurance tests will be performed by the reference laboratories.

2. Bar code labels on the plasma or cell specimens shipped to the reference laboratories will be verified with the bar code labels on the TEST RESULTS DATASHEETS that will have accompanied the specimen. Test results will be handwritten by the reference laboratory.

Notes

The Medical Director of the CBB will be notified of positive test results. Donor units identified as indicative of complete enzyme deficiency will be discarded and the donor/donor's mother will be notified of positive test result. Donor units identified as indicative of partial enzyme deficiency (carrier status), will not be discarded. Use of units with partial enzyme deficiency are only contraindicated for patients being treated for the same enzyme deficiency. Donor/donor's mother will <u>not</u> be notified of a positive test results unless: 1) the donor is a female carrier of co-A lipase deficiency (adrenoleukodystrophy) or 2) the donor is a carrier of glucocerebrosidase deficiency (Gaucher disease).

6.3 **MATERNAL INFECTIOUS DISEASE SCREENING**

Principle

Maternal serum samples will be tested for the following infectious disease markers:

- Cytomegalovirus (CMV) IgM antibody
- Anti-HBc (antibody to hepatitis B core antigen)
- Anti-HCV (antibody to hepatitis C virus)
- HbsAg (hepatitis B surface antigen)
- HIV-1/2
- HIV p-24 antigen
- HTLV-I/II
- Syphilis

Each cord blood bank must determine if the facility performing their infectious disease testing can utilize frozen, batched samples, or if the samples must be stored and shipped at 4°C within a certain time span. For facilities performing tests manually, frozen samples may be batched for shipment to the testing facility.

Specimen

Cryovials containing maternal serum, stored frozen at or below -20°C, or at 4°C, depending on the requirements of the testing facility.

Equipment

Electronic Bar Code Scanner
Computer

Materials

Serum samples
Infectious Disease Sample log
Small biohazard specimen bags
Small zipper-locked bags
Temperature indicator strips

Procedure

1. A set of samples will be removed from the freezer. The bar code number will be scanned into the Maternal Sample database and infectious disease log (if applicable), indicating that the samples have been sent for testing.

2. Each set of samples will be placed in an individual biohazard specimen bag.

3. Batched, frozen samples will be packed in dry ice in a styrofoam shipping container and sent to the testing facility. Samples stored and shipped at 4°C will be packed in a styrofoam container with sufficient frozen cold packs to maintain the temperature during transport. A temperature indicator strip will be sealed in a small zipper-locked bag and placed on top of the sample shipment.

Quality Control

1. One set of maternal samples at a time will be prepared for shipping.

2. The scanned number will be compared by visual inspection to the number on the serum tubes.

3. Samples will be handled and transferred as quickly as possible from storage to the shipping container, to maintain the appropriate sample temperature.

4. Sample tubes will be packed in sufficient dry ice or frozen cold packs to maintain the sample temperature until they arrive at the testing laboratory. Testing facilities will be instructed to note the temperature visible on the temperature indicator strip at the time of sample arrival.

6.4 COLONY-FORMING ASSAYS: CFU-GM, CFU-GEMM AND BFU-E

Principle

One surrogate measure of the hematopoietic cell number and engraftment potential of a sample of cord blood is its content of hematopoietic colony forming cells. These cells are enumerated in a culture-based assay that allows growth of a population of immature hematopoietic cells into colonies of mature hematopoietic cells over approximately two weeks.

This assay involves the growth of colony forming units-granulocyte-macrophage (CFU-GM), colony forming units-granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM), and burst forming units erythroid (BFU-E) under specified conditions. By counting the number of these elements formed at 14-16 days the concentration of progenitor cells that are present in the cord blood sample can be calculated. Each colony or burst results from one proliferating progenitor cell. Using methylcellulose, a semisolid media, the progeny of each precursor remain localized so that a visible colony is distinguishable.

Specimen

Umbilical cord blood that has been red-cell and volume-depleted according to the Processing CBU SOP. Sample to be provided with nucleated cell count and adhesive bar code labels in the Colony Assay 'In-Box' or institutional equivalent in accordance with the Processing CBU SOP.

Equipment

Bar Code Scanner
Inverted Microscope
CO_2 Incubator
Automatic Micro Pipetter
Vortex Mixer

Reagents

Methocult Medium	StemCell Technologies (aliquoted and stored according to Procedure Note)
Iscove's Modified Dulbecco's Medium	GIBCO
Fetal Calf Serum (FCS) (Pre-tested and Filtered)	StemCell Technologies, HCC-6100
Sterile Water	

Supplies

3 ml syringes (2)	Hospital Supply Room
17 gauge x 1½ inch blunt-ended needles or as recommended by StemCell Technologies	

24-well tissue culture plate	
Sterile Pipette Tips	Fisher Cat #21-197-8H
Study bar code labels	
Laboratory Worksheet	Laboratory Stationary
Graft Characterization Form	Cord Blood Bank Stationary

Procedure

Note: Procedures 1-12 are to be performed in the tissue culture hood under sterile conditions and using sterile technique.

1. Prepare the laboratory worksheet by placing an adhesive bar code label on the sheet. Scan this label and the label on the sample tube to confirm identity.

2. Record the identity of the responsible technologist, the date and the time, the lot numbers of the MethoCult medium and Iscove's medium, and the cell concentration of the sample (provided by the processing technologist) on the worksheet.

3. Label the tissue culture dish with an adhesive bar code label and the date.

4. Thaw an aliquot of MethoCult medium. Dilute cells to 0.5 ml in 2% FCS (2 ml FCS per 98 ml Iscove's medium). Use the following numbers of nucleated cells for each umbilical cord blood unit:

   Umbilical cord blood: 0.5×10^5/tube or 1.25×10^4/well

5. Add the 0.5 ml of cell suspension containing 0.5×10^5 cells to the MethoCult medium tube.

6. Mix each tube by vortexing twice for 3 seconds each time.

7. Allow the tubes to sit at room temperature for approximately 5 minutes. This allows MethoCult to drain down the side of the tube.

8. Label rows of a 24-well tissue culture plate with the bar code label, date, and cell concentration.

9. Using a blunt ended needle and a 3 ml syringe, draw up 1.5 ml of mix. **Avoid forming bubbles.**

10. Place .5 ml into each of 3 wells of a tissue culture plate. Replace lids and swirl gently to cover the bottom of the dish.

11. Fill the fourth well of a tissue culture plate with 1.5 ml sterile water. Tissue culture plates may be used for different patient samples. If cells are placed in position A-C on one row with sterile water in D, cells should be placed in B-D on the next row, with sterile water in A. Sterile water should also be placed in wells A-D of the first and sixth rows. This will allow the sterile water and humidity to be evenly distributed on the plate. (See diagram below.)

	1	2	3	4	5	6	
A	⊠	⊠	O	⊠	O	⊠	⊠ = sterile water
B	⊠	O	O	O	O	⊠	
C	⊠	O	O	O	O	⊠	
D	⊠	O	⊠	O	⊠	⊠	

12. Place the tissue culture plate into a 37°C incubator with 5% CO_2 for 14-16 days.

13. At the end of culture, remove the tissue culture plate from the incubator. Recover the laboratory worksheet and scan the label on the datasheet and the plate to confirm identity. Record the date and the identity of the technologist responsible for scoring on the datasheet.

14. Colony growth is enumerated using an inverted microscope with scoring according to the following criteria (and using the handbook provided by Stem Cell Technologies).

CFU-GM:
- colorless, sometimes granular or foamy cells
- ≥ 30 cells/colony
- cells often disbursed from a center
- each focal center is counted as a distinct colony
- macrophage colonies may be dispersed and have no well defined center, but are still counted as one colony

CFU-GEMM:
- "fried egg" appearance
- compact, spherical hemoglobinized area at center or at one side of flat lawn of nonhemoglobinized translucent cells (granulocytes, macrophages, and/or megakaryocytes)
- 40 or more cells
- can be mistaken for pure erythroid colonies if not examined under high power

BFU-E:
- bright red
- ≥ 50 cells/burst
- cells in each portion of a burst are tightly packed
- a multicentric burst is counted as a single entity
- bursts generally appear to look as though they would fit back together (e.g. "continental drift")
- cells from different individual centers of a burst that are closest to the center of mass of the whole BFU-E tend to be in the same focal plane as those from adjoining centers

If the colony count is greater than 100 colonies per plate, score the plate > 100.

15. Record the counts for each colony type on the datasheet. Using the mean count from each

triplicate plate, calculate the total CFU-GM x 10^5, CFU-GEMM x 10^5, and BFU-E x 10^5.

16. Open the Graft Characterization database and scan in the bar code identifier. Transcribe the calculated total CFU-GM, CFU-GEMM, and BFU-E from the worksheet into the database and onto the form.

Quality Control

1. The colony count for each plate should be within 15% of the others in the triplicate.

2. Each week the laboratory supervisor will select colony assay plates for blinded counting by each qualified lab staff member to determine reproducibility. Any staff person whose counts are outside 15% of the average will undergo retraining.

3. Each month the laboratory supervisor will select a cord blood sample for parallel assay by each qualified lab staff member to determine reproducibility. Any staff person whose assays are outside 15% of the average will undergo retraining.

Procedure Notes

1. Preparation of MethoCult medium: Each bottle comes from the manufacturer in a volume of 100 ml. Thaw the medium by soaking in a 32-35°C waterbath for 30-45 minutes and protected from light. Label sixty-six 17 x 20 mm snap cap tubes with the medium's lot number, expiration date, date aliquoted, volume, and technician's initials.

2. Using a 3 ml syringe with a blunt needle, aspirate 1.5 ml medium into each snap cap tube. Place the tubes in the -20°C freezer to use.

3. Volume required (ml) = $\frac{\text{Cell Number Required}}{\text{Cells/ml}}$

References

Colony Assays of Hematopoietic Cells Using Methylcellulose Medium-An Introductory Manual, Stem Cell Technologies, Vancouver, Canada, 1992.

Areman E, Deeg HJ, Sacher R. Peripheral Blood Stem Cell Processing Protocol. In: *Bone Marrow and Stem Cell Processing: Manual of Current Techniques.* F.A. Davis Company, Philadelphia, 1992.

6.5 FLOW CYTOMETRY FOR PRE-FREEZE GRAFT CHARACTERIZATION

Principle/Purpose

Flow cytometry uses immunostaining to differentiate antigens on immature hematopoietic cells (HPC). These antigens are a potential predictor of time to engraftment. Flow cytometry allows rapid acquisition (separation and identification) of a significant number of these low frequency antigens/cells. This procedure briefly outlines the steps needed to stain different aliquots of cells, to pass them through the flow cytometer (acquisition), and to analyze the resulting computer acquisition files.

Specimen

Umbilical cord blood that has been red cell- and volume-depleted according to the Processing CBU SOP. Sample to be provided with cell count in the Flow Cytometry 'In-Box' or institutional equivalent in accordance with the CBU Separation and Sample Preparation SOP.

Equipment

Repeating Pipetter
Automatic Muro Pipetter (or equivalent)
20, 200, and 1000 μl Pipetters
Flow Cytometer and Analysis Section with CD34 Analysis Template (Procount)
Becton-Dickinson (or equivalent)
Electronic Bar Code Scanner
12x75 mm Test Tube Holder
Refrigerator

Reagents

Tube	Marker	Stains	Supplier
1	Procount (or equivalent)	Nucleic Acid Dye/34/45	Becton-Dickinson
2	Procount Control (or equivalent)	Nucleic Acid Dye/Gamma 1/45	Becton-Dickinson
3	34+/61+ (optional)	CD61 FITC / CD34 PE / CD45 (Per-CP or equivalent)	Becton-Dickinson
4	34+/90+ (optional)	CD90 FITC / CD34 PE / CD45 (Per-CP or equivalent)	Becton-Dickinson
5	34+/38- (optional)	CD38 FITC / CD34 PE / CD45 (Per-CP or equivalent)	Becton-Dickinson
6	34+/Control (optional)	IgG1 FITC Control / CD34 PE / CD45 (Per-CP or equivalent)	Becton-Dickinson
7	19+/16+ & 56+	CD3 FITC / CD16 & 56 PE / CD19 APC / CD45 Per-CP (or equivalent)	Becton-Dickinson
8	3+/8+/4+	CD3 FITC / CD8 PE / CD4 APC / CD45 Per-CP (or equivalent)	Becton-Dickinson

FACS Lysis Buffer	Becton-Dickinson
FACS Buffer	Procedure Note 1
PBS Buffer	

Supplies

TruCount Tubes (or equivalent)
12x75 mm Polystyrene Tubes
Eppendorf 1.7 ml Microcentrifuge Tubes (or equivalent)
10 ml Pipettes
200 μl Pipette Tips
1000 μl Pipette Tips
Automatic Pipetter Tips
Graft Characterization Form

Procedures

1. Check the sample ID.

    a. Prepare the Graft Characterization Form by placing an adhesive bar code label on the sheet. Scan this label and the label on the sample tube to confirm identity. If identity is not confirmed, i.e. identical, do not proceed until discrepancy is resolved.

    b. Record the identity of the Responsible Technologist, the date and the time, the lot number and expiration date of the wet reagents (antibodies, buffer, lysis solution, and fixative), and the volume and cell concentration of the sample (provided by the Processing Technologist) on the worksheet and, if applicable, in the computer database.

2. Immunostain the cells.

    a. Obtain the 400 μl of post-processed cells.

        Note to ProCOUNT users: If automated cell count exceeds cell concentration of $45x10^3/\mu l$, dilute sample and record dilution factor on the Graft Characterization Form.

    b. Label 2 TruCount tubes with the CBU number and numbers 1 and 2.

    c. Label additional clear polystyrene 12x75 mm tubes with the CBU number and tube number.

    d. If CD34+ subset analysis performed, aliquot 50 μl of cells to tubes 3-6.

    e. Add 10 μl of stain from each antibody pre-mix (3-6) to each corresponding sample tube (3-6), and incubate for 20 minutes in the dark at 4°C.

f. Add 20 μl of ProCount antibody to tube 1, add 20 μl of ProCount Control antibody stain to tube 2, and 50 μl of cells to both. Add 10 μl of Multi-Test antibody stain (or equivalent) and 50 μl of cells to tubes 7 and 8. Incubate tubes in the dark at room temperature for 20 minutes.

g. Add 1 ml of FACS lysing solution to tubes 3-7 and incubate in the dark at room temperature for 5 minutes (or institutional equivalent).

h. If CD34+ subset analysis performed, wash cells in tubes 3-6 with 1 ml of FACS buffer each, decant supernatant, add 250 μl of FACS lysis solution to each tube, and store at 4°C.

i. Add 450 μl of FACS lysis buffer to tubes 1, 2, 7, and 8, and incubate for 30 minutes in the dark at room temperature (or institutional equivalent).

j. Wrap tubes 1-8 in aluminum foil, mark the bundle of tubes with the date of collection and the bar coded unit number, and store in the dark at 4°C.

k. Perform acquisition of samples within 96 hours.

3. Acquisition:

a. Acquire sample data according to manufacturer's instructions for Becton Dickinson Flow Cytometer (or equivalent).

b. To enhance the reliability for the determination of CD34+ cell subpopulations, efforts should be made to acquire at least 1,000 CD34+ events for tubes 3, 4, 5, and 6.

c. For Procount (or equivalent) tubes, acquire a minimum of 300 CD34+ cells. For Multi-Test (or equivalent) tubes, acquire a minimum of 10,000 lymphocytes (45 bright low-side scatter).

4. Analysis:

a. Access the ProCount file (or equivalent).

b. Initiate appropriate program for tubes 1 and 2 to analyze the acquired data.

c. As appropriate, record the number of dye positive events, the number of nucleated cells per microliter, total number of CD45+ events, number of CD45+ cells per microliter, percent of lymphocytes of CD45+ cells, number of CD34+ events, the number of reference beads, and total number of beads added to each TruCount tube. The data system will calculate the % of CD45+ events, the number of CD34+ cells per microliter, and the total CD34+ cells in the collection.

d. If CD34+ subset analysis is performed, analyze tubes 3-6 using Analysis of CD34+ Sub-

Pops template (or equivalent).

e. Adjust all gates to include the appropriate cell populations, adjust marker M1 to include 0.50% of control cells exhibiting the most flourescence, and adjust M2 to include 85% of control cells of least flourescence.

Note: Adjustment may be necessary for the M1 marker of tube 3 (CD61+CD34+) if the 0.50% setting excludes part of a clearly defined positive population.

f. Copy markers M1 and M2, paste them into the sample histogram, adjust the markers to include the appropriate cell populations if necessary, and for tubes 3-6 record the percentage of CD 34++ cells that fall within the M1 or the M2 boundary as it applies to the stains exhibited.

g. Record the number of CD34+events acquired and the percent of CD34+ events expressing the second marker. The data system will calculate the number of CD34 subset cells per microliter and the total number of CD34 subsets in the collection.

h. Analyze tube 8 using the T-cell Analysis template (or equivalent).

i. Adjust all gates and quadrant settings to encompass all appropriate cell populations.

j. Record the total number of CD45+ events acquired (if applicable), the number of lymphocytes, and the number of CD3+ events acquired. The data system will calculate the percent of CD45+ events, the number of CD3+ cells per microliter, and the total CD3+ cells in the collection.

k. Record the percentage of CD4+, CD8+, and CD4+/CD8+ cells. The data system will calculate the total CD4+, CD8+, and CD4-/CD8- cells in the collection.

l. Analyze tube 7 using the B-cell and Natural Killer Cell Analysis template (or equivalent) and select file sample tube 7 for all plots in the screen set up.

m. Adjust all gates and quadrant settings to include the appropriate cell populations.

n. In the lymphocyte gate, calculate and record the percent of cells that are CD19+/CD16-CD56-, the percent of cells that are CD19-/CD16+/56+, and the percent of cells that are CD19+/CD16+CD56+. The data system will calculate the total CD19+ and CD16+56+ in the population.

Quality Control

1. At regular intervals as defined by institutional procedures, the Laboratory Supervisor will select previously assayed data files for blinded counting by each qualified lab staff member to determine accuracy. Any staff person whose counts are outside 10% of the average will undergo re-training.

2. At regular intervals as defined by institutional procedures, the Laboratory Supervisor will select a cord blood sample to be tested in at least 3 parallel assays by each qualified lab staff member to determine reproducibility. Any staff person whose assays are outside 10% of the average will undergo re-training.

3. At regular intervals as defined by COBLT participating cord blood banks, a cord blood sample will be selected for parallel assay by COBLT cord blood banks to determine reproducibility between facilities. Any staff person whose assays are outside 10% of the average will undergo re-training.

4. All training is administered according to institutional training procedures.

Procedure Notes

1. Prepare FACS buffer by adding 5 g of bovine serum albumin to 500 ml of phosphate buffered saline pH7.4. This buffer is stored at 4°C in a bottle labeled with contents, data of preparation, the identity of the responsible technologist, and the expiration date (one month following preparation).

2. Prepare the FACS lysis buffer by adding 45 ml of purified water to 5 ml of 10x Becton Dickinson FACS Lysing Solution. Store at 4°C in an aluminum foil covered 50 ml conical tube labeled with contents, date of preparation, the identity of the responsible technologist, and the expiration date (one month following preparation).

References

Becton Dickinson. ProCount Progenitor Cell Enumeration Kit Instruction Manual. Becton Dickinson. San Jose, CA; 1996.

Rose N, Friedman H, Fahey J, ed. Manual of Clinical Laboratory Immunology. Preparation, Staining, and Analysis by Flow Cytometry of Peripheral Blood Leukocytes. 3rd ed. Washington DC American Society for Microbiology; 1986: 226-235.

Shapiro HM. Practical Flow Cytometry. Goals and Methods in Data Analysis. 3rd ed. New York, New York. Wiley-Liss, Inc.; 1995: 179-182.

CHAPTER 7

QUALITY ASSESSMENT

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QUALITY ASSESSMENT

7.1 OVERVIEW OF CBB-SPECIFIC QUALITY ASSURANCE/QUALITY CONTROL PROGRAM

An extensive, site-specific Quality Assurance/Quality Control (QA/QC) program must be established and maintained by each collection facility under the direction and supervision of designated personnel. This program must verify the quality and integrity of cord blood units (CBUs) collected under this NHLBI project, as well as equipment used in the collection, processing, storage, and shipping of the units. In addition, there must be a review and approval process for policies and procedures, and documentation of compliance with regulatory requirements and standards.

Quality audits must be performed on a routine basis. It is the immediate obligation of the investigators, and by extension of the NHLBI, to assure conformity to Standard Operating Procedure (SOP) requirements relative to the eligibility or ineligibility of harvested CBUs for permanent storage.

As identified in the SOPs, the Steering Committee has established well-defined criteria for determining the integrity of potential CBUs for permanent CBB storage. Compliance with standards itemized in the SOPs is crucial for the welfare of cord blood donors and recipients, as well as for investigators and the NHLBI. To facilitate compliance and promote congruity between sites, the SOPs identify the functions and responsibilities of specific personnel, and provide detailed logs and flow sheets.

Adherence to the SOPs is more likely to be assured by instituting internal and external inspections, and uniform data forms completion standards. Inspection of completed data forms, including their audit against source records, is a recognized method of assuring the quality of research data. Internal and external audits provide quantitative and qualitative information relative to site compliance within established parameters. Internal audits of randomly selected patient records every 3 months by cross-trained personnel will provide direction for internal correction of any noted deficiencies. External verification of data forms against source records by an MCC monitoring team strengthens the integrity of data and the project, and provides sites with specific performance evaluations. Site monitoring is of particular value immediately following the initiation of a new research project. In the start-up phase, a monitoring team can be a critical communication link regarding application of the SOPs to the daily operations of the program. It encourages accountability and conformity between personnel as well as between sites.

External oversight by the MCC for the study will be instituted to inspect data collection forms and verify them against source documents. Within 6 months of a collection site's start-up, the monitoring team will review all data forms for randomly selected units. If the data audited satisfactorily demonstrates adherence to the SOPs, monitoring visits will occur every year. In the event of major breaches of protocol, the NHLBI may impose additional conditions on an investigator or, if necessary, potentially terminate the site's participation in the project.

The MCC and/or NHLBI will conduct external laboratory audits to inspect and certify the performance

of all CBU processing and storage laboratories. External audits will be completed, at a minimum, at 1 year intervals. Again, in the event of major breaches of protocol, the NHLBI may impose additional conditions on an investigator or, if necessary, potentially terminate the site's participation in the project.

In order to remain in compliance with federal regulatory requirements, principal investigators at each NHLBI banking site are held accountable for implementing and managing all regulatory responsibilities of the research project at both their immediate site and all satellite collection facilities. Those responsibilities are recognized to include the following:

- Promote the rights of research subjects
- Adhere to the NHLBI Cord Blood Bank SOPs
- Maintain all IRB administrative obligations
- Support monitoring audits of the sponsoring agency
- Source document the initiation, maintenance, and closure of the study
- Comply with quality assurance criteria

In turn, by virtue of holding an IND for the project, the NHLBI is charged with accountability for each of its sites. If the sites are not compliant to NHLBI directives, then NHLBI is liable and placed at risk of breaching federal regulations. In order to most effectively protect patients, investigators, sponsoring agencies, and the general research community, it is essential that layers of accountability, with documented adherence and oversight, be structured into the generic research process and its application in this project.

These issues are of particular clinical import for the COBLT Study in that the banked product is intended for use in human transplants. The increased inherent risks involved in representing the rights of two research subjects, the donor and recipient, and the clinical imperative of ensuring product integrity for transplant, intensify the need for regulatory scrutiny. Implementing universal, regulatory quality control standards for all participating collection sites will serve to protect patients and ultimately the project. Some of those standards are suggested below.

At the local level, the SOPs and customized informed consents will be submitted for initial review and approval to an IRB which satisfies federal and state requirements for IRB committee composition and function. No site will initiate collections without providing the MCC, as the NHLBI representative, with notification of such IRB approval. Sites will inform their IRBs of any major SOP amendments and any consent revisions, and will receive IRB approval prior to implementation. Annual progress reports will be supplied to the NHLBI, initial and updated Form FDA 1572s, laboratory accreditation and normal values, investigator and sub-investigator CVs will be sent to the MCC, and records of all correspondence will be retained.

Strict adherence to the elements of informed consent will be maintained at all points in the consenting process, in compliance with the approved consent, and COBLT and institutional SOPs. The rights of the recipient patient can best be met by pursuing an intensive, documented informed consent process, and by insuring the clinical integrity of the product. Confidentiality and linkage will be issues at all sites which require internal audits as well as external oversight by the MCC and/or NHLBI.

Follow up for infectious and genetic disease tracking will be maintained by each collection facility per SOP criteria, as well as records of neonatal follow up contact. Quarterly reports tracking screening, quarantine, permanent storage, and shipping of cord blood units (including demographic profiles) are integral quality assurance components to be maintained by each site. Similarly, data must be managed in common, universal formats that allow for confidentiality, linkage, verifiable results and accurate transmission. Laboratory quality control issues are of particular significance and are addressed specifically in the policies and procedures identified in each site's COBLT QA/QC Manual.

Though these particular guidelines may not be collectively acceptable, at this point, the records for any finalized, acceptable standards will be audited at regular intervals (every year) by the MCC and/or NHLBI for accuracy, compliance, and follow up. The financial implications of such requirements can be significant.

The design of the COBLT Study has been engineered by a dedicated, multidisciplinary team. The successful implementation of the project demands strict adherence to the SOPs, federal regulations, and accepted industry standards. Any collection site that gains authorization to contribute cord blood units to the project (i.e. contract and non-contract banks), must be required to meet ALL itemized standards in the SOPs without exception, and within quality control guidelines.

7.2 ASSESSING QUALITY

7.2.1 Purpose

To define the policies, tasks, and responsibilities related to quality assessment and help ensure the provision of high quality products and service.

7.2.2 Scope

Assessment tasks are designed to monitor whether personnel, procedures, reagents, equipment, supplies, cord blood product, and record keeping meet their expected functions in a reliable, reproducible manner.

7.2.3 Policies

7.2.3.1 a. This program shall support the goals and mission statements of the medical centers and hospitals associated with each Umbilical Cord Blood Bank.

b. The primary goal of the Cord Blood Banks is to provide a safe, reliable and efficient source of umbilical cord blood stem and progenitor cells for transplant in an environment that supports and fosters research and continuing development and quality improvement.

7.2.3.2 This program shall incorporate the principles of continuous quality improvement when assessing umbilical cord blood processes.

a. Assign responsibility.

b. Delineate scope of responsibility.

c. Identify important aspects of responsibility.

d. Identify key indicators.

e. Establish thresholds for evaluation.

f. Collect and organize data.

g. Evaluate care.

h. Take action.

I. Assess the effectiveness of the action.

j. Communicate findings.

7.2.3.3 Quality assessment requirements shall be based on appropriate State regulatory bodies and on the following agencies and their current documents:

a. FDA: Code of Federal Regulations, Title 21.

b. AABB: Standards for Blood Banks and Transfusion Services; Accreditation Requirements Manual.

c. CAP: Transfusion Medicine Inspection Booklet.

d. JCAHO: Accreditation Manual for Hospitals.

e. CLIA: Clinical Laboratory Improvement Act, 1988, Title 42, Part 493.

f. FAHCT: Standards for Hematopoietic Progenitor Cell Collection Processing and Transplantation.

7.2.3.4 Each bank should have a designated quality team consisting of directorial and supervisory staff. This team shall audit, on an ongoing basis, key systems and critical control points related to the quality and safety of cord blood collection, processing, handling, testing, and distribution.

7.2.3.5 The quality control program shall be under the surveillance of the quality team. Items evaluated on a daily or weekly basis shall be reviewed by a trained individual at least weekly. Secondary review should occur at least monthly by another trained, designated individual. Items evaluated monthly shall have secondary review completed quarterly. Items evaluated quarterly or at a less often interval shall have a secondary review annually.

7.2.4 **Elements to be Assessed**

Personnel training and competency, as well as the quality assessment of procedures, equipment, supplies, and records, are integral parts of assuring component quality.

7.2.4.1 Personnel

a. New employees are oriented to the Umbilical Cord Blood Bank policies pertinent to their sections and are trained for their assigned job functions.

b. Job competency is assessed annually in accordance with institutional competency testing.

c. Personnel are expected to confirm data and identification as they perform procedures and computer entries.

d. Personnel are expected to record data/test results promptly at the time the results are determined or read.

e. Employee performance and test results are reviewed for completeness and accuracy as part of regular supervisory reviews.

f. Performance evaluation is discussed with employees at least annually.

7.2.4.2 Procedures

a. Procedures are written, reviewed, and implemented as required. Changes and revisions are documented.

b. Before new or revised procedures are implemented, they must be validated and staff must be trained.

c. Procedures will be reviewed annually by the Director and Medical Director.

d. Procedures retired from use must be archived and retained.

7.2.4.3 Reagents and Specimens

a. Reagents used for routine donor testing must meet appropriate FDA criteria (see CFR Title 21, Part 660). Licensed reagents (when available) must be used and in-date.

b. When a reagent/solution is not covered by FDA criteria or when the licensed reagent is not available or is rare, unlicensed or expired reagents may be used with proper documentation, quality control, and/or supervisor approval.

c. (1) New bottles of reagents shall be dated upon opening and tested for reactivity before being placed into use.

(2) Control specimens shall be tested in the same manner as patient samples.

(3) Control results must be verified as acceptable before reporting test results.

(4) Test results from procedures for which controls and calibration are not established or available shall be reviewed by the Medical Director before reporting.

d. Reagents that appear to be contaminated (cloudy or turbid) shall not be used for testing or processing.

e. Reagents shall be used as prescribed by the manufacturer.

f. Any component of a reagent "kit" shall be used only within that kit lot unless otherwise specified by the manufacturer.

Coordinators shall establish intended use and performance specifications for all equipment items. These specifications and ranges of performance will be defined in the equipment procedure and/or checklist.

g. Vendors should be selected based on their ability to provide equipment that meets these performance standards. Selection considerations might include equipment design, validation of intended use, training and service support, licenser, and the company's commitment to quality.

h. Before a piece of equipment is placed into service, it will be entered into inventory and validated in-house for its intended use.

i. Equipment should be used as procedures and manufacturer's directions dictate.

j. Quality control and preventative maintenance shall be performed on all pieces of equipment as specified in procedures and/or checklists. These steps shall meet regulatory requirements and manufacturer's recommendations.

k. Thermometers or other temperature sensing devices shall be placed in each refrigerator, freezer, incubator, heat block, water bath, or other equipment used for testing or storage of cord blood, reagents, or samples.

7.2.4.4 Supplies

a. Are units collected, processed, and stored according to procedure?

b. Does the equipment used in the processing and storage of umbilical cord blood function properly?

c. Do cord blood units meet quality control specifications?

7.2.4.5 Records

a. All donor records are considered confidential and are subject to COBLT confidentiality policies. Computer access to data is limited by staff verification codes. Donor test results may be given only to the donor (or an individual designated by the donor with written authorization). Notifications shall be done only by the Medical Director or designated personnel.

b. All records must carry facility identification and comply with regulatory standards. Records should have a title that designates intended use, observed test results and interpretations, test date, and personnel identities. They must be legible and corrections must be clearly identified.

c. Test results and donor records must be reviewed for completeness and accuracy in a timely manner.

(1) Procedures and record systems are set up so that, whenever possible, current results can be compared to previous results. This allows staff to monitor accuracy of donor identification and detect significant changes during task performance.

(2) Computer procedures incorporate entry verification steps prior to data acceptance to help assure entry accuracy.

(3) Test results and critical documents are reviewed as specified in the section regarding supervisory review procedures and checklists.

(4) Significant abnormal results are flagged for medical review and action.

d. Records shall be stored and retained as specified in each of the Cord Blood Bank's Quality Assurance Manuals.

e. Records should be retrievable within a reasonable period of time.

7.2.4.6 Proficiency Testing

a. Proficiency testing is performed on an ongoing basis to assess general division performance of policies and procedures, personnel, equipment, reagents, and supplies.

7.2.4.7 Facilities and Safety

a. The environmental conditions in the laboratory shall provide a safe and adequate place to work while fulfilling all pertinent regulatory requirements.

b. Each employee will participate in safety training programs as defined by the duties performed by that employee.

7.2.5 **Assessment Schedule**

Quality assessment tasks and the frequency with which they are performed will be specified in the section regarding procedures and on appropriate checklists.

7.2.6 **Variances and Corrective Action**

7.2.6.1 Variances and deviations noted in any of the elements listed in the above section should be immediately called to a supervisor's attention and documented on a "Continuous Improvement worksheet" and "Corrective Action Report" or Cord Blood Bank equivalent. This Variance report is forwarded to the Responsible Coordinator and Director.

7.2.6.2 Corrective action will be determined by the Laboratory Director.

7.2.6.3 Reagents, equipment, and supply items that do not meet performance specifications must be taken out of service for repair or removal.

7.2.6.4 Variance reports are compiled in a master log and reviewed quarterly by the QA unit for trend analysis.

7.2.7 **References**

1. FDA: Code of Federal Regulations, Title 21.

2. AABB: Standards for Blood Banks and Transfusion Services; Accreditation Requirements Manual.

3. CAP: Transfusion Medicine Inspection Booklet.

4. JCAHO: Accreditation Manual for Hospitals.

5. CLIA: Clinical Laboratory Improvement Act, 1988, Title 42, Part 493.

6. FAHCT: Standards for Hematopoietic Progenitor Cell Collection Processing and Transplantation.

7.2.8 **Quality Assessment Task and Frequency Summary**

Tables 7.2.8.1 and 7.2.8.2 provide examples for summarizing the framework and overview for each Cord Blood Bank.

1. Personnel

• Training	Prior to working independently
• Competency Testing	Annual
• Continuing Education	On-going

2. Procedures

• Validation	Prior to implementation and changes
• Quality Assessment Review	Annual

3. Records

• Result Verification	At computer entry
• Supervisor Review	Daily per procedures
• File Rotation/Storage	Annual or as specified

4. Reagents and Supplies

5. Equipment and Supplies

• Initial Validation	Prior to use
• Preventative Maintenance	See checklists
• Recertification	After repairs or changes in use
• Cord Blood Collection Processing	At the time of assembly of collection kits and again prior to use
• Centrifuges	
Function	Monthly
Speed and Timer	Quarterly
Temperature	Monthly
Function and Safety Check	Annual
• Computer	
Clean Screens	As Needed
Vacuum Printers	As Needed
Keyboard Covers	Quarterly
Validation of Specific Activity	Semi-annual
• Heating Blocks/Water Baths	
Temperature	Each day of use
Quadrant Checks	Annual
Periodic Maintenance	Annual
• Heat Sealers	
Function Check	Each use
Service/Cleaning	Annual
• Microscopes	
Cleaning	As Needed
Function and Safety	Annual
Service Check	Every 5 years
• Pipetter Recalibration	Annual
• Scales/Balances	
Weight Check	Each day of use
NIST Reference Weight	Monthly

- Sterile Connecting Device

Weld Check	Each use
Clean	Daily
Function and Safety Check	Annual

- Thermometers

Safe T Vue	Upon receipt/annual

- Timers/Wall Clocks — Upon receipt/annual

- Transport Coolers

Function	Upon receipt
Shipping Temperatures	Winter/Summer

- Water Baths/Heat Blocks

Temperature	Each day of use
Quadrant Check	Annual
Periodic Maintenance	Annual

- Miscellaneous

Air Vents Clean/Inspect	Quarterly
Electrical Safety Checks	Annual
Eye Wash	Weekly
Fire Drill Check	Quarterly
Fire Extinguisher Service Check	Monthly

- Internal Self-Assessment — On-going

Table 7.2.8.1
Quality Plan - Framework

	Systems							
	Donor Suitability	Collection	Transport	Processing	Testing	Review and Labeling	Storage and Distribution	Investigation of Adverse Events
Critical Control Points								
Organization Issues								
Personnel Selection, Training, and Education								
Validation, Calibration, Preventative Maintenance, and Proficiency Testing								
Supplier Qualification								
Process Control								
Documentation/Record Keeping/Record Review								
Label Control								
Incident/Error Review								
Internal Assessment								
Process Improvement								

Table 7.2.8.2
Quality Plan - Overview

Organization Issues	Personnel Selection, Training, and Education	Validation, Calibration, Preventative Maintenance, and Proficiency Testing	Supplier Qualification	Process Control
• Description of Umbilical Cord Blood Bank • Organization Chart • Mission Statements • Management Responsibility • Quality Unit	• Position Description • Performance Standards • Department Orientation • Training Program • Competence Assessment • Performance Appraisal • Continuing Education	• Equipment Quality Standards • Equipment Specifications/Validation • Equipment Calibration • Equipment Preventative Maintenance	• Reference Laboratories • Vendors • Reagent Receipt	• Definition of Critical Control Points/Key Elements • SOPs for Critical Control Points/Key Elements • System Checks for Critical Control Points/Key Elements • Flow Charts • Change Control SOPs
Documentation/Record Keeping/Record Review	**Label Control**	**Incident/Error Review**	**Internal Assessment**	**Process Improvement**
• Revision of SOPs • SOP Index • SOP Archive • Record Review, Retention, and Storage	• Label Control SOP • Label Storage/Destruction	• Incident/Error SOP • Incident/Error Data Collection • Incident/Error Review • Corrective Action • Report to Quality Unit • Lookback	• Audit SOP • Audit Schedule • Report to Quality Unit	• Identification of Processes in Need of Improvement • Assessing Quality • Continuous Quality Improvement Policies and Processes

7.2.9 **CBU Quality Assessment Program**

The COBLT Study will use a Quality Assessment Program to monitor CBU processing activities at each CBB. The program will be initiated at each CBB following the start of CBU collections, and will continue until all collection and processing activities have been completed.

The quality of the processed CBU will be determined by comparing pre-freeze and post-thaw measurements on the following: viability, nucleated cell count, mononuclear cell count, CD34+ count, colony-forming units, and burst-forming units. This data will be submitted to the MCC using the CBU Processing QA Report which can be found in Chapter 8. Data on 10 CBUs will be required at the start of the collection and processing activities at each CBB. Following completion of this requirement, data on one CBU will be submitted to the MCC every three months.

7.3 **PROCESS IMPROVEMENT POLICY**

7.3.1 **Purpose**

To define a process by which opportunities for improvement detected by the Quality Plan are linked to selection and implementation of solutions in a continuous fashion.

7.3.2 **Scope**

Tasks are designed to move the program from identification of areas for improvement to realization of solutions.

7.3.3 **Policies**

7.3.3.1 On detection of error in a Corrective Action Report or through the activities of the Compliance Officer and the Quality Unit, the Responsible Coordinator shall collate all relevant data on patterns and influencing factors for presentation to the Quality Unit.

7.3.3.2 Based upon this report, the Quality Unit shall define potential solutions, obtain input from the Advisory Panel, select the most appropriate solution, and design a plan for its implementation.

7.3.3.3 The implementation shall be documented by changes in the relevant SOPs.

7.3.3.4 The Responsible Coordinator shall monitor the performance of the solution by personal observation and examination of data patterns and influencing factors as in Step 1.

7.3.3.5 The conclusions drawn from this monitoring shall be presented to the Quality Unit for consideration of the effectiveness of the solution.

a. Where necessary, this process shall be repeated from Step 2 until satisfactory resolution is achieved.

CHAPTER 8

DATA COLLECTION

Chapter 8

DATA COLLECTION

8.1 OVERVIEW

The initial subsection of this chapter describes some of the steps taken to maximize donor confidentiality as well as maintain a linkage between donor and the unit. Note that no information on the identity of the donor is submitted to the MCC. The linkage is maintained entirely at the cord blood banking centers, which are responsible for physical security of the process.

Subsequent sections include all of the data forms used to capture information related to maternal history, eligibility, delivery, and cord blood unit collection. In addition, all forms for centrally stored data describing the receipt, processing, infection screening, characterization of the unit and release of the unit from quarantine and from permanent storage into the storage bank are provided. Forms submission requirements are summarized in Table 8.1.

For each CBU, the collection, processing, testing and storage activities will be completed during a quarantine period. CBUs will be released from quarantine storage to long term storage following submission of a CBU Exclusion and Quarantine Release Form indicating the long term storage freezer locations of the CBU and associated samples. After the CBU is released to long term storage and becomes available as a potential stem cell source, data related to that CBU cannot be modified or deleted, i.e. all data records pertaining to that CBU will be locked. The locking sequence is as follows:

<u>Category 1</u>

All records including the CBU Disposition Form will be locked when the following condition is met:

1.A Immediately after a CBU is shipped for transplant.

<u>Category 2</u>

All records excluding the CBU Disposition Form will be locked when the following condition is met:

2.A Immediately after the CBU is placed in the COBLT Search Registry.

For locked data records, only three types of data can be subsequently updated: 1) additional HLA testing may further characterize the unit, 2) a CBU disposition form may be filed to indicate use of or discarding of the CBU, and 3) freezer locations of samples and CBUs may be modified.

Requests to unlock a bar code may be made to the Data Manager at the MCC. Requests must be made by the CBB Principal Investigator (PI), or his/her designate, and must contain the full ISBT ID bar code to be unlocked, the reason of the request, and the requestor's COBLT certification number. In addition, the request should include the current status of the CBU (e.g., shipped for transplant, eligible for search, currently reserved by a transplant center). Requests may be faxed or e-mailed to the MCC data management e-mail address (cobltdm@emmes.com).

The CBB PI and CBB Coordinator will be notified immediately by the MCC when the bar code is unlocked. Records will remain unlocked for 4 days. During this time, data may be added, modified, or deleted.

Note that the sources for these data are varied; some describe clinical characteristics of the mother and delivery, some derive from the processing and distribution tasks, and some come from the special laboratory testing. Because of the data volume, the HLA reference labs will submit electronic records of their testing results. The remainder of the data will be incorporated into the MCC developed data system at the banks following the data specifications which follow in the form subsections.

TABLE 8.1
FORMS SUBMISSION REQUIREMENTS

Mother consented but no collection obtained	
If a label has not been assigned	No forms are expected
If a label has been assigned	CBU Disposition Form indicating that the label set was discarded
Collected CBU but processing not initiated	Medical History Form CBU Collection and Receipt Form CBU Disposition Form
Collected CBU and processing initiated but not completed	Medical History Form CBU Collection and Receipt Form CBU Processing Form CBU Disposition Form
Collected, processed, cryopreserved, and quarantined CBU, AND either an exclusion criteria is met or CBU is transferred to long term storage	Medical History Form CBU Collection and Receipt Form CBU Processing Form CBU Cryopreservation Form Donor and Delivery Information Form Maternal Sample Form Graft Characterization Form CBU Exclusion and Quarantine Release CBU Disposition Form (due at removal from quarantine or long term storage)
CBU label set released but not assigned, and discarded	CBU Disposition Form indicating that the label set was discarded

8.2 DATA COLLECTION FORMS

8.2.1 Volunteer Cord Blood Donor Identification Form

8.2.2 **Medical History Form**

MEDICAL HISTORY FORM
EXCLUSION CRITERIA FOR COLLECTED CORD BLOOD UNITS

Part I: Background Information

1. Date Informed Consent Signed	Exclude unit if mother/donor has not signed or reaffirmed an informed consent.
2. Ethnic Background	See question 3.
3. Father's Age	If question 2, Ethnic Background, is coded 88-Unknown for the father, and the father's approximate age is unknown, then exclude the unit.
4. Serious Illness	If mother answers 1-Yes then: Refer the history form to the Medical Director at the CBB.
5. Childhood Deaths	If mother answers 1-Yes then: Refer the history form to the Medical Director at the CBB.
6. Related Marriages	Exclude unit if mother answers 1-Yes.
7. Genetic Mother	Exclude unit if mother answers 2-No.
8a. Chronic Blood Transfusion	If mother answers 1-Yes then: Refer the history form to the Medical Director at the CBB.
8b. Inheritable Deficiencies	If mother answers 1-Yes then: Refer the history form to the Medical Director at the CBB.
8c. Cancer/Leukemia	If mother answers 1-Yes then: If the cancer is documented as basal or squamous cell skin cancers do not exclude the unit. Exclude the unit if any other type of cancer or leukemia in a first degree relative of the infant (mother, father, siblings) is reported.
9a. Hemolytic Anemia	Exclude unit if mother answers 1-Yes.
9b. Spleen Removed to Treat a Blood Disorder	Exclude unit if mother answers 1-Yes.
9c. Gallbladder Removed for a Non-Traumatic Reason	If mother answers 1-Yes then: Refer the history form to the Medical Director at the CBB.

10a. Red Blood Cell Disease	Exclude unit if mother answers 1-Yes.
10b. White Blood Cell Deficiency	Exclude unit if mother answers 1-Yes.
10c. Platelet Disease	Exclude unit if mother answers 1-Yes.
10d. Metabolic/Storage Disease	Exclude unit if mother answers 1-Yes.
10e. Other Diseases	If mother answers 1-Yes then: Refer the history form to the Medical Director at the CBB.

Part II: Blood Donor Information

Questions With No Time Restrictions

11a. Refused as Blood Donor	If mother answers 1-Yes then: If refusal was for anemia, or because of mechanical problems with donating blood (e.g., unsuccessful donation, hematoma, etc.), then the unit is acceptable. If refusal was due to a history of risk behavior for transfusion-transmittable disease, then refer the form to the Medical Director if no other exclusion criteria is recorded. For answers other than the above, refer the history form to the Medical Director at the CBB.
11b. Cancer, Blood Diseases, etc.	Exclude unit if mother answers 1-Yes, unless the cancer is documented as basal or squamous cell skin cancers.
*11c. Yellow Jaundice, etc.	Exclude unit if mother answers 1-Yes.
11d. Chagas Disease or Babesiosis	Exclude unit if mother answers 1-Yes.
11e. Creutzfeld-Jacob Disease (CJD)	Exclude unit if mother answers 1-Yes.
11f. Risk CJD	Exclude unit if mother answers 1-Yes.
11g. Had Dura Mater Transplant	Exclude unit if mother answers 1-Yes.
11h. Growth Hormone or Tegison	Exclude unit if mother answers 1-Yes.
*11i. Needle Use	Exclude unit if mother answers 1-Yes.
*11j. Money for Drugs/Sex	Exclude unit if mother answers 1-Yes.

*11k. Clotting Factor	Exclude unit if mother answers 1-Yes.
*11l. AIDS/Sex	Exclude unit if mother answers 1-Yes.
11m. Organ Transplant	Exclude unit if mother answers 1-Yes.
11n. Allogeneic Tissue Transplant	If mother answers 1-Yes then: Refer the history form to the Medical Director at the CBB.
<u>Questions Restricted to 1977 and the African Countries of Cameroon, Central African Republic, Chad, Congo, Equatorial, Guinea, Gabon, Niger, or Nigeria</u>	
12a. Lived in One of Listed Countries in the Last 3 Years	Exclude unit if mother answers 1-Yes.
12b. Travel to One of Listed Countries	Exclude unit if mother answers 1-Yes to traveling to one of the listed countries since 1977 AND receiving a blood product while there.
*12c. Sexual Contact with Resident	Exclude unit if mother answers 1-Yes.
<u>Questions Restricted to the Last 3 Years</u>	
*13a. Traveled Outside USA/Canada	If mother answers 1-Yes then: Exclude units collected from mothers who have resided in an area endemic for malaria within the past 3 years. Endemic areas are listed in Health Information for International Travelers published by the Centers for Disease Control (CDC). The most current publication must be used when determining whether a mother has traveled to an endemic area. Updated information on regions may be obtained 24 hours a day by calling the CDC Malaria Hotline at (404) 332-4555. If the mother resides in the USA/Canada or is from an area <u>not</u> endemic for malaria, and has traveled outside the USA or Canada in the PAST YEAR, determine whether or not the mother has traveled to an area endemic for malaria. Units collected from mothers who have traveled to a malarial area in the PAST YEAR where travel was limited to daylight hours (e.g. flight connection or cruise ship port visits) are acceptable. Otherwise, if yes, exclude unit.
13b. Malaria in Last 3 years	Exclude unit if mother answers 1-Yes.

Questions Restricted to the Last 12 Months

*14a. Allogeneic Blood Transfusion	Exclude unit if mother answers 1-Yes.
*14b. Tattoo, Ear/Skin Piercing, etc.	If mother answers 1-Yes then: It is important to determine if the mother has been exposed to transfusion-transmittable disease through non-sterile needles or contact with someone else's blood. Exclude units if the mother reports contact with blood through nonsterile needle sticks, a human bite that results in a wound which breaks the skin (including bites from children), electrolysis, or ear/body part piercing or acupuncture procedures if needles were previously used or not sterile or if the mother does not know if the needles may have been reused. Exclude the unit if the mother reports having a tattoo in the last 12 months. Units are not excluded if the mother reports ear or body piercing using an ear piercing gun that has sterile disposable supplies, or receiving autologous blood products/tissue.
*14c. Contact with Yellow Jaundice, etc.	Exclude unit if mother answers 1-Yes. Close contact is defined as having either sexual contact, routine sharing of the same household, kitchen, and/or toilet facilities within the last year with a person who has had yellow jaundice at any time after age 11, or membership in a group where multiple cases of hepatitis have occurred.
*14d. Treated/Tested Syphilis, Gonorrhea	Exclude unit if mother answers 1-Yes.
*14e. Shots/Vaccinations	If mother answers 1-Yes then: Exclude the unit if the mother reports having received HBIG, or a live viral vaccine, or an unlicensed vaccine, or rabies vaccine following a bite by a rabid animal or one suspected to be rabid.
*14f. Sex With Man Who Had Sex With a Man	Exclude unit if mother answers 1-Yes.
14g. Jail 72 Hours	Exclude unit if mother answers 1-Yes.

Additional Questions

*15a. Pills or Medications	If mother answers 1-Yes then: If pills or medication were reported as prenatal vitamins or iron supplement, then unit is acceptable. Otherwise, refer the history form to the Medical Director at the CBB.
*15b. TB Infection/Exposure	Exclude unit if mother answers 1-Yes.
15c. AIDS Question	No exclusion.
Part III: Interview Information	
16. Consent Withdrawn at Any Stage	Exclude unit if mother answers 1-Yes.
17. Present for Genetic History	No exclusion.
18. Mother Questioned in Private	Exclude unit if mother answers 2-No.

* Questions to be re-asked following consent reaffirmation for interviews conducted pre-delivery.

8.2.3 **Donor and Delivery Information Form**

EXCLUSION CRITERIA FOR
DONOR AND DELIVERY INFORMATION FORM

1. Mother's Age	Exclude unit if mother's age is < 18 years.
2. Evidence of Placental Infection	Exclude unit if 1-Yes.
3. Membrane Rupture	*Data collection - no exclusion*
4. Prenatal Antibiotics	Exclude unit if 1-Yes.
5. Mother Afebrile	Exclude unit if 2-No.
6. Date/Time of Delivery	*Data collection - no exclusion*
7. Type of Delivery	*Data collection - no exclusion*
8. Gestational Age at Birth	*Data collection - no exclusion*
9a. Infant Gender	*Data collection - no exclusion*
9b. Infant Weight	*Data collection - no exclusion*
10. Single Birth	Exclude unit if 2-No.
11. Infant Afebrile	Exclude unit if 2-No.
12. Evidence of Infant Sepsis	Exclude unit if 1-Yes.
13. Free of Congenital Infection, etc.	Exclude unit if 2-No.
14. Free of Congenital Abnormalities	Exclude unit if 2-No.
15. Pregnancy or Birth Complications	If answer 1-Yes then: Refer the form to the Medical Director at the CBB.

8.2.4 **Maternal Sample Form**

8.2.5 **CBU Collection and Receipt Form**

8.2.6 **CBU Processing Form**

8.2.7 **CBU Cryopreservation Form**

8.2.8 **Graft Characterization Form**

8.2.9 **CBU Exclusion and Quarantine Release Form**

8.2.10 **CBU Disposition Form**

8.3 **LOGS**

8.3.1 **Label Release Log**

8.3.2 **Shipping Log**

8.3.3 **HLA Request Log**

8.4 **REPORTS**

8.4.1 **CBB Monthly Recruitment Report**

8.4.2 **CBU Processing QA Report**

8.5 **HLA TYPING DATA COLLECTION**

HLA data for newly typed CBUs from the typing laboratories will be sent weekly to the MCC. This data will consist of:

- Specimen Identification
- Typing Date
- Assigned types at each of the HLA A,B and DRB1 loci
- Potentially present nucleotide sequences for the above
- Typing Method

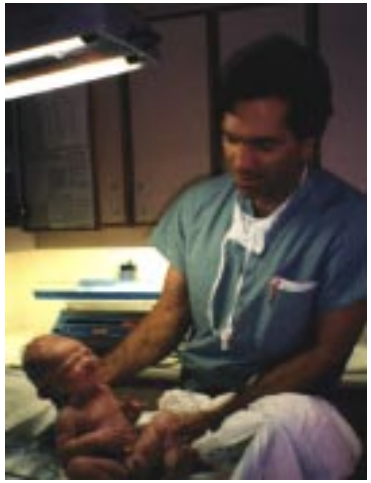
Submission of electronic records will be via the Internet.

Additional confirmatory typing of specimens will be reported with notation as to the date and results. Additional loci may also be typed at the time of confirmatory typing.

APPENDIX A

EDUCATIONAL BROCHURE

Your donation can help others



You could help save the life of a child or an adult by donating umbilical cord blood to the National Cord Blood Project following the birth of your baby.

Q. *What is umbilical cord blood?*

A. Umbilical cord blood is the blood that remains in the placenta following a birth. It is normally thrown away.

Q. *What is the National Cord Blood Project?*

A. This project will establish three cord blood banks nationwide to collect and store umbilical cord blood. The project is funded by the National Heart, Lung, and Blood Institute.

Q. *What is stored umbilical cord blood used for?*

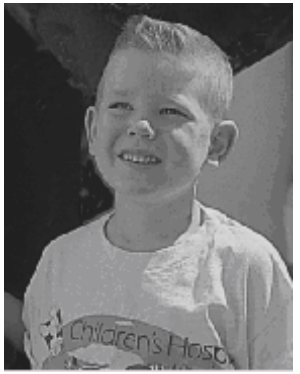
A. The umbilical cord blood contains stem cells. These cells can be used to replace blood-forming cells in a person being treated for cancer or other life-threatening diseases. Trans-planting these stem cells offers hope to many patients.

Making this donation--priceless to the life of another--costs you or your insurer nothing.

Q. *When is it collected?*

A. Following delivery of your baby the placenta and umbilical cord are given to a trained staff member. The collection process will not change your labor experience in any way. Blood will not be taken from your baby. The birthing process, your privacy and that of your baby are of the greatest importance.

John Mash, age 6, two years after his cord blood transplant for leukemia



Q. *What happens after the umbilical cord blood is collected?*

A. A nurse from the Cord Blood Bank will visit you. The nurse will talk to you about the Cord Blood Project and ask a few questions about your medical history. You will be asked to give a small blood sample, to be taken from your arm.

Q. *Is there any cost to me?*

A. There is no cost to you or your insurance company.

By donating, you can help save a life.

If you have any questions, please talk with your delivering physician, or call

A national project sponsored by the National Heart, Lung, & Blood Institute.

Participating Cord Blood Banks:
Carolinas' Cord Blood Bank/
Duke University Medical Center
Georgetown University Medical Center
University of California, Los Angeles

Participating Cord Blood Transplant Centers:
Dana Farber Cancer Institute
Duke University Medical Center
Fred Hutchinson Cancer Research Center
Indiana University
University of California, Los Angeles/
Childrens Hospital Los Angeles
University of Minnesota

Medical Coordinating Center:
The EMMES Corporation

National Cord Blood Project

The birth of your child could save a life

APPENDIX B

SAMPLE INFORMED CONSENT FORM

B.1 INFORMED CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Umbilical Cord Blood Banking for Transplantation

You are being asked to take part in a research study that is trying to find better ways to treat patients with leukemia, bone marrow failure, and certain rare inherited diseases (for example, immune deficiency, inborn errors of metabolism, and storage diseases). Before agreeing to join the study, you need to understand the purpose of the study and what you will be asked to do. This process is called informed consent.

This is an informed consent form and it gives details about the study. Once you have had a chance to read this form and discuss the study with your doctor, you will be asked to sign the form if you wish to take part. You will also be given a copy of the form.

Purpose of the Study

Blood cells produced by a baby before birth circulate through the baby's body, umbilical cord, and placenta. These blood cells bring oxygen and nutrition from the mother's blood to the baby. When the baby is born, the umbilical cord is cut and the baby is separated from the placenta and the mother. The placenta, or "afterbirth," is delivered several minutes later and is usually thrown away. The placenta contains one-third to one-half of a cup of blood which is rich with blood cells. These blood cells could be used to replace the blood cells in a person who has leukemia, bone marrow failure, or a certain rare inherited disease. The replacement process is called transplantation.

Insert Banking Program Name Here has a program to collect the usually discarded blood cells from the placenta and umbilical cord and use them as blood cells for transplantation or for research.

Your Involvement in the Study

By volunteering to take part in this study, you are agreeing to do the following things:

1. Allow the blood from the umbilical cord and placenta taken after your baby's delivery to be tested and frozen for research. If our tests show that the cord blood is suitable for transplantation, it will be stored until it is given to a person who needs a bone marrow transplant or until it is no longer suitable for storage.

   If the cord blood is not suitable for transplantation, it may be thrown away using standard hospital practices or it may be used in laboratory research that has been approved by *Insert IRB Name Here,* an institutional review board. The cord blood will not be cloned or used for any commercial purpose. Cord blood used in laboratory research will not be used for transplantation. If it is used for research, your identity and your baby's identity will not be known.

2. Allow a sample of the cord blood to be tested for some genetic diseases that can be passed through blood cells, like Gaucher's Disease and Adrenoleukodystrophy (ALD). The tests are

done to protect the person who will eventually receive the cord blood.

Allow an additional sample of cord blood to be frozen and stored for future testing for such infectious diseases as hepatitis, cytomegalovirus (CMV), HTLV I and II, and HIV (the virus that causes AIDS). These tests will only be done if the cord blood is selected to be used in a transplant.

All test results are confidential. Any test performed will be done for the safety of patients who may receive the cord blood. We will try to inform you of any confirmed positive test results which may affect your health or your baby's health. We will offer a counseling referral if needed. If you do not want to be told of these test results, you should not sign this consent form or take part in the study.

3. Allow a sample of the cord blood to be tissue typed. Tissue typing gives a "fingerprint" of the blood cell by analyzing the cell DNA. This DNA fingerprint will be needed to match the cord blood cells to a patient's blood cells.

4. Give up to 30 mL (about two to three tablespoons) of your blood. The blood will be taken from your arm by a qualified person and tested for viruses such as hepatitis, cytomegalovirus (CMV), HTLV I and II, HIV (the virus that causes AIDS), and syphilis. The tests will be used to find out if the cord blood can be stored in the cord blood bank. The testing may include tissue typing (DNA fingerprinting) of the cells. Some of the blood will be frozen and stored for future testing if tests become available for currently unknown diseases.

   All test results are confidential. Any test performed will be done for the safety of patients who may receive the cord blood. We will try to inform you of any confirmed positive test results which may affect your health or your baby's health. We will offer a counseling referral if needed. If you do not want to be told of these test results, you should not sign this consent form or take part in the study.

   Insert as Appropriate:
   The law in *Insert State Name Here* requires that we give the names of persons who test positive for certain diseases to public or state health agencies of confirmed positive test results for certain diseases, including HIV and syphilis. These agencies may contact you if you have confirmed positive test results.

5. Answer questions about your and your family's medical history, including questions about your pregnancy, drugs you are taking, and past medical problems in your family and in the father's family. There are also questions about your current and past lifestyle, including sexual history and drug use. These questions are similar to questions asked when a person donates blood. The information is confidential and will be used to find out if the cord blood can be stored in the cord blood bank.

6. Allow your and your baby's medical charts to be reviewed, specifically for pregnancy and delivery information and hospital tests up to the time of your discharge from the hospital.

7. Allow your baby's blood screening test results to be checked. The tests look for diseases such as Sickle Cell Anemia and Thalassemia, and are required by the state of California/North-South Carolina.

Insert as Appropriate:

8. Allow us to contact you (about six months and one year after your baby's birth) to ask about any changes in your baby's health which might affect the cord blood's suitability for transplantation. Our contact will coincide with the regular "Well Baby" check-ups that should be a part of routine care for all babies at two months, six months, and one year of age. We may ask for your permission to contact your pediatrician or family doctor at these times.

Possible Risks and Discomforts

Taking blood from your arm for tests may cause bruising, infection, fainting, pain, or discomfort. All normal precautions will be taken to keep these side effects from happening.

We will make every effort to protect your and your baby's confidentiality. In the rare event your identity becomes known, a transplant patient could try to contact you.

In the rare event your name and positive test results (for example, HIV tests) became known, you could be treated unfairly by others.

Anticipated Benefits to You and Your Baby

There will probably be little or no direct benefit to you or your baby from taking part in this study. However, it is possible that our tests will detect an infection or genetic disease which would affect you or your baby and which might not have been otherwise detected. This early detection could result in earlier treatment and improved health care.

If, in the future your child needs a bone marrow transplant and the cord blood was banked and still available, it could be used for your child. It is also possible that your baby's blood will match a brother's or sister's blood if a transplant is ever needed. Keep in mind, though, if the cord blood has already been given to another person for transplant, it will not be available for your child or children.

Another benefit to you and your family is the satisfaction of potentially helping others.

Anticipated Benefits to Society

Currently, many patients who need a transplant cannot find a donor. The cord blood bank will provide another source of donor blood cells, allowing more people the chance to receive a potentially life-saving transplant.

Alternatives to Participation in This Study

There are companies who collect, process, and store cord blood for family use only. They charge a fee for this service. If you choose to use one of these companies to store your baby's cord blood, you

should contact them immediately to make the arrangements. If you decide to use one of these companies, you will not be allowed to take part in this study.

You may also choose not to take part in this study. If you decide not to take part, the cord blood will be thrown away using standard hospital practices.

Financial Obligation

You will not be charged and there will be no cost to your insurance company for anything connected to this study. You will not be paid for taking part in this study.

Privacy and Confidentiality

No information identifying you or your baby will be given to anyone unless required by law or unless you request that the information be given. A Certificate of Confidentiality has been obtained for this study. The purpose of this certificate is to prevent any and all persons not connected with the study or courts from gaining access to your identity.

Information collected in this study will be reported to the National Heart, Lung and Blood Institute, the Food and Drug Administration, the International Cord Blood Transplant Registry, and the scientific community. Your name and your baby's name, or any other identifying information, will not be included in any study reports or papers.

Information collected in this study will be reviewed by authorized officials from the National Heart, Lung and Blood Institute, the Medical Coordinating Center (The EMMES Corporation), the Food and Drug Administration, or other agencies. Access to study information by these individuals will be provided under a guarantee of confidentiality and only for the purpose of making sure that this study is following proper procedures.

Participating In and Withdrawing From the Study

If you choose to take part in this study, your participation is voluntary. You are also free at any time to stop participating in the study, without any effect on your future care at *Insert Banking Program/Parent Medical Center Name Here*. If you choose not to take part in the study, this decision will not affect your or your baby's right or access to health care or other services which you are entitled to receive at *Insert Banking Program/Parent Medical Center Name Here.*

Your baby's cord blood cannot be withdrawn from the study after it has been released to a patient for transplant.

Withdrawal by the Investigator

There is no guarantee that your cord blood will be collected or stored. There are many reasons why this could happen.

Blood may not be collected because the collection team is not available or because the baby's birth is

complicated with fever in the mother, early rupture of membranes, mixing of the mother's and baby's blood, or a birth defect or genetic disease in the baby.

Blood may not be stored because the amount of cord blood collected is too small, the cord blood is infected, or there are problems in the processing or freezing of the cord blood.

In addition, there is always the possibility that an unexpected problem may arise and result in the cord blood not being collected or stored.

You will not be told whether or not the cord blood is stored unless you request to be told. You can contact us at _____ (*Address*) or _____ (*Phone Number*).

Identification of Investigators

If you have any questions about this study, feel free to contact _____ (*Name of Bank Director and/or Medical Director*) at _____ (*Address*) or _____ (*Phone Number*) .

Rights of Research Subjects

At any time, you may withdraw your consent and end your participation in this study without a penalty. If you have any questions about your rights as a research subject, you may contact the Office for Protection of Research Subjects at _____ (*Address*) or _____ (*Phone Number*).

VOLUNTEER DONOR INFORMED CONSENT FOR CLINICAL RESEARCH

Title:

Umbilical Cord Blood Banking for Transplantation

Purpose:

The purpose of this study is to collect and store umbilical cord blood and use it to replace the blood-forming cells in a person with a serious blood disease.

Signature of Research Subject:

I have read and understand the information provided above. I have been given an opportunity to ask questions and all of my questions have been answered to my satisfaction. I have been given a copy of this form as well as a copy of the Subject's Bill of Rights.

By signing this form, I willingly agree to participate in the research it describes.

_____
Name of Subject

_____ _____
Signature of Subject Date

Signature of Investigator or Designate:

I have explained the research to the subject and answered all of her questions. I believe that she understands the information described in this document and freely consents to participate.

_____
Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date
(must be same as subject's)

Signature of Witness:

My signature certifies that the subject has signed this consent form in my presence as her voluntary act and deed.

_____
Name of Witness

_____ _____
Signature of Witness Date
(must be same as subject's)

B.2 PRELIMINARY INFORMED CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Umbilical Cord Blood Banking for Transplantation

You have asked if you could donate your baby's cord blood to the *Insert Cord Blood Bank Name Here*. We welcome your donation, but because you will deliver your baby soon, we do not have time to tell you everything you need to know to make an informed decision about participation in this research project before the birth of your baby.

Since cord blood must be removed from your placenta (afterbirth) within 10-15 minutes of your baby's delivery, we will streamline the process of obtaining your consent for collection by having you read and sign this short form before the birth of your baby. We will attempt to collect your baby's cord blood and if the collection is successful, we will provide you with detailed information about the program and obtain complete consent within 12-48 hours of your baby's birth.

By signing this informed consent form you are agreeing to the following:

1. You wish to donate your healthy baby's umbilical cord blood to the *Insert Cord Blood Bank Name Here* at *Insert Institutional Name Here*, and you give your permission for a Collection Specialist to collect the cord blood from the placenta and cord shortly after delivery.

2. You understand that if a sufficient volume of cord blood is collected for processing and banking, a Collection Specialist will come and talk to you about the study in detail before you are discharged from the hospital. At that time the Collection Specialist will explain the program to you, have you sign a long consent form, and take a complete medical history from you. If you do not sign the long consent form, the cord blood will not be saved.

3. You agree to allow a nurse to take a little extra blood (20-30 ml, about one to two tablespoons) at the same time she draws blood as part of your medical care for admission to Labor and Delivery.

4. You understand that if the volume of cord blood collected is insufficient for processing and banking, it may be used for research projects at *Insert Institutional Name Here*. If this occurs, no further consent will be done and you give permission for the use of your baby's cord blood for research by signing this form. The cord blood will not be cloned or used for any commercial purpose.

You will not be charged and there will be no cost to you or your insurance company for anything connected to this study. You will not be paid for taking part in this study.

You are also free to stop participating in the study at any time. If you choose not to take part in the study, or to stop participating, this decision will not affect your or your baby's right or access to health care or other services which you are entitled to receive at *Insert Institutional Name Here*.

By signing this form, you willingly **agree to participate**. If you have any questions about this study,

please contact _____ *(Principal Investigator)*, Director of the _____ *(Name of Cord Blood Bank)*, at _____ *(Address)* or _____ *(Phone Number)*.

_____	_____	_____
Name of Mother	Signature	Date
_____	_____	_____
Name of Person Obtaining Consent	Signature	Date

APPENDIX C

EXAMPLES OF NOTIFICATION LETTERS FOR POSITIVE INFECTIOUS AND GENETIC DISEASE TESTS

NOTIFICATION LETTER FOR CONFIRMED ANTI-HTLV-I POSITIVE TEST RESULT

Date

Dear Donor:

Thank you for your recent placental blood donation. Let me assure you, THIS IS NOT A LETTER ABOUT AIDS.

We test all blood for Human T Cell Lymphotropic Virus Type I/II antibodies (anti-HTLV-I). This recently discovered virus may be associated with some diseases in a small number of people exposed to it. This virus is **not** associated with HIV-1/2, the AIDS viruses. When we tested your blood, your first test was positive and a second, different test for HTLV-I was also positive.

Having HTLV-I antibodies in your blood does not mean that you will definitely develop any associated illness. However, these positive test results may be important information for your future health. For these reasons, we have enclosed an HTLV-I fact sheet, and we recommend you share these test results with your doctor.

Blood with positive test results cannot be given to another person. Therefore, we were unable to use the placental unit you donated. In addition, we are informing you that you must never be a blood donor.

If you want more information about this letter or would like to schedule an appointment to discuss your test results, call *insert phone number here* and ask for *insert name here*.

Thank you for your support.

Sincerely,

Insert name here

Notes: Enclose anti-HTLV-I Supplemental Positive Fact Sheet.

FACT SHEET FOR POSITIVE TEST RESULT FOR THE
HUMAN T LYMPHOTROPIC VIRUS, TYPE I ANTIBODY (Anti-HTLV-I)

What does a positive Anti-HTLV-I test result mean?

FIRST, HTLV-I IS NOT THE AIDS VIRUS (HIV-1). HTLV-I DOES NOT CAUSE AIDS.

A positive anti-HTLV-I test result means that the person is infected by HTLV-I; this virus was recently found in the United States. Most people infected with HTLV-I come from (or have parents who come from) Japan, the Caribbean basin, South America, or Africa.

This test result means that a sample of the donor's blood tested positive, more than once, on a screening test for anti-HTLV-I. A different, more specific test for anti-HTLV-I confirmed the results of the screening test.

How does HTLV-I infection affect a person's health?

HTLV-I infection does not appear to affect the health of most infected persons. Less than 1% of infected persons will develop cancer of the blood (leukemia) or a neurologic disease sometime during their lives.

How does a person become infected by HTLV-I?

- From sexual intercourse with an infected partner.
- From sharing needles **for illegal drugs or steroids**.
- From an infected woman who breastfeeds.
- Rarely, from an infected blood transfusion.

What should a person with a positive anti-HTLV-I test result do?

- A person with a positive anti-HTLV-I test result should have a medical check-up. The purpose of a check-up is to find out if there are any signs of blood or neurologic disease. A doctor may recommend anti-HTLV-I tests for sexual partners and, depending on the results, may advise that condoms be used to prevent further spread of HTLV-I infection by sexual intercourse.
- **Do not** donate blood, semen, body organs, or other tissue.
- **Do not** share needles or syringes.
- **Do not** breastfeed.

Should a person with a positive anti-HTLV-I test result donate blood?

No. Another person (patient) could be infected by blood donated by people with positive anti-HTLV-I test results.

Please carefully read the letter that accompanies this Fact Sheet.

NOTIFICATION LETTER 1 FOR HIV - 1/2 TEST RESULT

Date

Dear Donor:

Thank you for your recent placental blood donation.

In the information that we gave you when you donated, we explained that your blood would be tested for certain viruses. When we tested your blood, one of these tests was abnormal. It is important that we discuss the results of this test with you.

Please call *insert name, phone number, between the hours of here* to schedule an appointment to discuss your test results. To protect your privacy, it is our policy not to discuss specific test results over the phone.

Sincerely,

Insert name here

Notes: Do not enclose any Fact Sheet. Do not enclose test result form. Certified mail/return receipt requested.

NOTIFICATION LETTER 2 FOR HIV TEST RESULT

Date

Dear Donor:

Thank you for your recent blood donation or blood sample. We are writing to let you know the results of some of the tests we did on your blood and how these test results will affect your future as a blood donor.

We test all blood for evidence of human immunodeficiency viruses (HIV). Some of your HIV test results are abnormal. The enclosed test result report form identifies the names of the tests done and the test results.

These abnormal test results indicate HIV infection and are important information about your health. For these reasons, we have enclosed a copy of an informational pamphlet which explains the meaning of your test results and includes recommendations you should follow to protect your health and the health of others. **We strongly recommend that you share these test results with your doctor.**

Blood with positive test results cannot be given to another person. Therefore, we were unable to use the placental blood that you donated. In addition, we are informing you that you must never be a blood donor.

If you want more information about this letter or would like to schedule an appointment to discuss your test results, please call *insert phone number here* and ask for *insert name here*.

Sincerely,

Insert name here

Notes: Enclose HIV Ag Fact Sheet. Enclose test result form. Certified mail/return receipt requested.

FACT SHEET FOR HUMAN IMMUNODEFICIENCY VIRUS

What does a positive human immunodeficiency virus screening test result mean?

The blood test screens for the presence or absence of viral particles, such as antigens, or protein material, such as antibodies, to see if your body may have been exposed to certain infectious agents, such as viruses. The cord blood bank uses screening tests on placental blood donations for the same reason. However, other things (vaccinations, for example) may cause screening tests to be abnormal when there is no viral infection.

A positive screening test for HIV antigen may indicate that you have been infected by a human immunodeficiency virus (HIV), the virus that causes AIDS. Positive screening test results, however, require the use of a more specific test, a **confirmatory** test, to make sure you really have been infected with HIV.

What does a confirmatory test result mean?

Confirmatory tests are different than screening tests and confirmatory test methods are approved by the FDA. They are used to determine if the screening test is truly abnormal (positive). **It is important that you see your personal physician or contact *insert name of CBB Medical Director here* for follow-up testing and information if your confirmatory test is positive.**

Insert appropriate test here
*Since your confirmatory test for HIV antigen (neutralization) is **positive**, you may have been exposed recently to human immunodeficiency virus. You can be exposed to HIV by sharing needles, being exposed to the blood of infected persons, or by sexual contact with an infected person. If you are infected with HIV, you can spread the virus to others by sharing needles, through blood transfusions or through other exposure to your blood, and by having sexual contact. You may not have any symptoms associated with this infection, but you can still have the virus and could infect others.*

*Since your confirmatory anti-HIV test result (Western blot) is **indeterminate**, we cannot be certain, based on the tests that we performed on your donation, that you have not been exposed to a human immunodeficiency virus. A very small number of persons having an indeterminate anti-HIV test result may have been infected with HIV. Most indeterminate results, however, do not indicate that you have been exposed to HIV. These results are caused by other factors in a person's blood and are not related to exposure to the HIV virus or AIDS. If you have an indeterminate confirmatory test, you are probably not infected with HIV virus. However, your personal physician is best able to review your results to determine if it is likely that they represent a true positive. Your physician may also want to perform additional tests. You are not eligible to continue to donate blood, even if your doctor determines that you have not been exposed to HIV.*

*Since your confirmatory anti-HIV test result (Western blot) is **positive**, you have been infected with human immunodeficiency virus. You can be infected with HIV by sharing needles, being exposed to the blood of infected persons, or by sexual contact with an infected person. Persons infected with HIV can spread the virus to others by sharing needles, by blood transfusions or exposure to your blood, and by*

sexual contact. You may not have any symptoms associated with this infection, but you still have the virus and could infect others. There are several types of human immunodeficiency viruses and the cord blood bank tested your blood for anti-HIV-1 and anti-HIV-2. Both of these viruses can cause problems with your immune system (AIDS). You are no longer eligible to donate blood. ***It is important that you see your personal physician for follow-up testing and information if your confirmatory test is positive.***

There are several types of human immunodeficiency viruses. Your blood was tested for HIV antigen as well as antibodies to HIV-1 and HIV-2 (anti-HIV-1/2). Both of these viruses can cause problems with your immune system (AIDS). You may test confirmed positive for HIV antigen, but negative for antibodies. If this happens, this probably indicates a very recent exposure to HIV (within the past few weeks).

You are no longer eligible to donate blood. **Note**: The law in certain states requires that we notify public or state health departments of confirmed positive test results for HIV. These agencies may contact donors who have had confirmed positive HIV test results.

Your personal physician can evaluate your test results in terms of your overall health and help you decide how best to take care of yourself. If you don't have a personal physician with whom you feel comfortable, call the local medical society or HIV/AIDS service organization for a referral. You'll need to build a comfortable, ongoing relationship, so it is important to find a doctor or clinic with staff knowledgeable about AIDS. Recent medical research indicates that early treatment for people infected with HIV helps them live longer and delays the onset of symptoms and HIV-related illnesses. People are learning to live with HIV as a chronic illness.

What do's and don'ts should you follow regarding your HIV antigen test?

- Do eat a balanced diet, get enough sleep, exercise, and take care of yourself.
- Do try to avoid infections by not eating raw eggs or raw or undercooked meat. Maintain good kitchen hygiene. Don't clean up bird droppings or dog or cat feces. Avoid being in a closed room with others who have respiratory infections.
- Do try to avoid stress. Listen to your body. It will tell you when you're under stress (through tension in your neck or shoulders, an upset stomach, or some other way). You must discover how you best deal with stress.
- Do not share things that can be contaminated with blood or body fluids, such as needles, toothbrushes, razor blades, or tweezers.
- Do not donate blood, plasma, semen, body organs, or other tissues. Do not breastfeed.
- Do not use drugs, alcohol, or nicotine. Drugs may hamper your immune system. If you use them, get help. Talk to your HIV/AIDS counselor, or call the National Institute of Drug Abuse Hotline, toll free at 1-800-662-HELP.

How can you protect others?

- Abstinence, that is, not having sexual intercourse of any kind, is the best way to prevent the spread of HIV from one person to another through sex. If you are sexually involved with someone, it is important to talk with your partner. You and your partner need to take

precautions to prevent new or further infections. If you and your partner choose to be sexually active, find out how to use condoms and spermicides. Have oral, vaginal, or anal sex only when using a condom and spermicide.

Condoms are effective but not foolproof. They can break, tear, or slip off. By properly using latex condoms every time you have sex, the risk of infection is reduced. Never use the same condom more than once.

Condoms are important whether you are heterosexual or homosexual, and even if your partner is also infected. Continue to protect yourself and your partner during sex from sexually transmitted diseases, including repeated infections with HIV.

- Inform your current sexual partner(s) and anyone else you may have had sex with since you became infected. This isn't easy to do, but it is very important. Your counselor may help you to make it easier with less blame and guilt. Urge current and ex-partners to seek HIV/AIDS counseling and testing. This can help stop the spread of the virus to others.

- Tell your doctor, dentist, and other health care workers about your test results. These people may need to know this to plan the best care for you. Discuss with them whether or not to put the test results in your medical or dental records. If you are concerned that your doctor or dentist might refuse to treat you, or if you don't have one, contact the county medical or dental society or an HIV/AIDS service organization in your area. They are listed in telephone directories.

What about emotional support?

Learning that you are infected with HIV can be frightening. Most people have found their family and friends supportive and caring. Choose carefully, however, with whom you share your medical information--the more people you tell, the less control you have over this information. Many people do not know as much about HIV infection and AIDS as you do. They may need to be reminded that HIV is not spread by casual, nonsexual contact such as a handshake or hug.

There are many others who are facing the same problem you are. Many of these people have found help by joining support groups. If you'd like to learn more about support groups in your area, or if you prefer individual, professional help, ask your HIV/AIDS counselor for referrals, or call a local HIV/AIDS service organization.

What if I do get sick?

It is important for you to know when to call your doctor. You should be aware of the following warning signs. If any of these persist for more than a few days, contact your doctor.

- Tender or enlarged lymph glands in your neck, jaw, armpit, or groin. They'll be large enough to feel easily.
- Unexplained shortness of breath, which can be serious and should be evaluated medically if it persists longer than a few minutes.

- Constant fatigue, which is not the feeling you have when you don't want to go to work, but a real exhaustion that won't go away.
- Unexplained diarrhea.
- Persistent or recurring fever, either a persistent high-grade fever of over 100°F or one that keeps going up and down for at least two weeks. Chills or night sweats, i.e., waking up with your bed and bedclothes wet.
- Unplanned weight loss of ten pounds or more.
- Unusual bruising or bleeding.
- Persistent white spots or unusual blemishes in the mouth.
- Severe motor, behavioral, or cognitive changes. An example of a motor problem might be suddenly finding yourself unable to hold something in your hand. An example of a behavioral problem might be suddenly becoming uncontrollably talkative, if you are normally a rather quiet person. An example of a cognitive change might be losing the ability to add correctly or having trouble remembering.

For more information, contact:

- The National AIDS Information Hotline (toll free): 1-800-342-AIDS.
  For Spanish-speaking persons, Linea Nacional de SIDA: 1-800-344-SIDA.
  For deaf or hearing-impaired persons, TTY/TDD Hotline: 1-800-AIDS-TTY.
- Your doctor or health care worker.
- Your local or state public health department.
- The HIV/AIDS service organization of your choice.

HUMAN IMMUNODEFICIENCY VIRUSES (HIV) RELATED TEST RESULTS

Name of Donor: _____

Hospital ID: _____

Date of This Report: _____

Test Name	Result		
	Repeat Reactive	Non-Reactive	
Antibodies to HIV-1/HIV-2 combination screening test (ELISA)			
Antibodies to HIV-1 screening test (ELISA) (This test is not done routinely.)			
Antibodies to HIV-2 screening test (ELISA) (This test is not done routinely.)			
	Positive	Negative	Indeterminate
Anti-HIV-1 confirmatory test - Western blot			
Anti-HIV-2 supplemental test - Western blot (unlicensed)			
	Repeat Reactive	Non-Reactive	
HIV-1 p24 antigen screening test (ELISA)			
	Positive	Indeterminate	
HIV-1 p24 antigen confirmatory test			

Notes: Send with all HIV-related notification letters.

NOTIFICATION LETTER FOR HbsAg CONFIRMED POSITIVE TEST RESULT

Date

Dear Donor:

Thank you for your recent placental blood donation. We are writing to let you know the results of some of the tests we did on your blood and how these results will affect your future as a blood donor. Let me assure you, THIS IS NOT A LETTER ABOUT AIDS.

We test all blood for infection with the hepatitis B virus. Hepatitis is an inflammation of the liver and is usually caused by a virus. When we tested your blood, the first test was positive and a second test confirmed this result. These positive test results may be very important for your health and for the health of others. For these reasons, we have enclosed a hepatitis B fact sheet and a summary of your hepatitis-related test results which we recommend you review with your doctor.

Blood with positive test results cannot be given to another person. Therefore, we were unable to use the placental blood you donated. In addition, we are informing you that you must never be a blood donor.

If you want more information about this letter or your test results, please call *insert phone number here* and ask for *insert name here*.

Sincerely,

Insert name here

Notes: Enclose HbsAg Fact Sheet. Enclose test result form. Mail First Class.

FACT SHEET FOR POSITIVE TEST RESULT FOR
HEPATITIS B SURFACE ANTIGEN (HbsAg)

What does a positive hepatitis B surface antigen (HbsAg) test result mean?

A positive test for HbsAg means that a person has a hepatitis B virus infection in his or her blood. An infected person can spread the infection to others by sharing needles **for illegal drugs or steroids**, by sexual intercourse, or by a blood transfusion. A mother can spread the infection to her newborn baby.

All people with a positive test result for HbsAg have some form of hepatitis B, but most will not have any symptoms of the disease. Some people, even when their infection seems mild, may go on to have serious liver disease over time.

How does hepatitis B infection affect a person's health?

Many people are "carriers" of the infection for many years without feeling ill. Others may have a recent infection that will lead to acute viral hepatitis with fever, jaundice (yellowing of the skin and whites of the eyes), and dark urine. Very rarely, a person with a positive HbsAg test result will develop cirrhosis (liver failure) or cancer of the liver.

What are the other tests for viral hepatitis performed and what do they mean?

Three hepatitis-related tests, besides HbsAg, are performed for each donor. When one test result is abnormal, we report all hepatitis-related test results to the donor. The donor may wish to review these test results with a doctor. These are the other tests:

Hepatitis B Core Antibody (Anti-HBc). This test detects the body's response to a hepatitis B virus infection. A positive test result usually means the person has recovered from a past infection. For some people, this test result may be a sign of current hepatitis B infection.

Antibody to Hepatitis C Virus (Anti-HCV). This test detects the body's response to a hepatitis C virus infection. A positive test result usually means the person has a hepatitis C infection.

Alanine Aminotransferase (ALT). The ALT test measures an enzyme in liver cells that leaks into the blood when the liver is infected or irritated. The liver can be infected by a virus or irritated by exercise, alcohol, toxins, or certain medications. Rarely, being overweight can irritate the liver. A person with an abnormal ALT test result may have a viral infection or inflammation of the liver.

What should a person with a positive HbsAg test result do?

- Donors should see their doctor for a medical check-up and take their hepatitis-related test results with them. That is the only way to find out what the test result means for them.
- Tell your doctor or dentist about this test result before receiving treatment.
- Some doctors may recommend hepatitis testing for sexual partners and other people in the household. The doctor may advise them to have a hepatitis B vaccination, if they do not have

positive hepatitis test results. This may be important for a woman who is considering pregnancy, because hepatitis B can be a serious illness for a pregnant woman and her baby.

- Stop donating blood, semen, body organs, and other tissue.
- Do not share things that can be contaminated with blood or body fluids, like toothbrushes, razor blades, or needles.

Should a person with a positive HbsAg test result donate blood?

No. Another person (patient) could be infected by blood donated by people with positive HbsAg test results.

Please carefully read the letter that accompanies this fact sheet.

NOTIFICATION LETTER FOR ANTI-HBc EIA REPEAT REACTIVE TEST RESULT

Date

Dear Donor:

Thank you for your recent placental blood donation. We are writing to let you know the results of one of the tests we did on your blood and how these results will affect your future as a blood donor. Let me assure you, THIS IS NOT A LETTER ABOUT AIDS.

We tested your blood for hepatitis B core antibodies (anti-HBc). When we tested your blood, the test was positive for hepatitis B core antibodies.

Hepatitis is an inflammation of the liver and is usually caused by a virus. Hepatitis B core antibodies merely indicate that a person might have been infected in the past and is now fully recovered. **Often people with these antibodies never even felt sick or knew that they had the virus.**

Please read on carefully; the rest of this letter should answer all of your questions.

Some important things to know about this test result are as follows:

- This test result does not mean that you have an illness or an infectious disease. If you are feeling well, there is no need for a medical check-up or additional laboratory testing.
- Sometimes this test is positive even if there was no infection.
- There is no evidence that people with this test result are considered capable of spreading hepatitis by casual or sexual contact.
- Although there is no evidence that you could spread an infection by casual or sexual contact, there is a slight chance that a unit of your blood could transmit hepatitis to a person (patient) receiving it.

Just in case you do want to discuss these results with your doctor, we have attached a summary of your hepatitis-related test results, along with a fact sheet on hepatitis B core antibodies.

If you want more information about your test results, please call *insert phone number here* and ask for *insert name here*.

Sincerely,

Insert name here

Notes: Enclose Anti-HBc Fact Sheet. Enclose test result form. Mail First Class.

FACT SHEET FOR POSITIVE TEST RESULT FOR
HEPATITIS B CORE ANTIBODY (ANTI-HBc)

What does a positive hepatitis B core antibody (anti-HBc) test result mean?

A positive anti-HBc test result usually means the person has recovered from a past hepatitis B infection. Hepatitis is an inflammation of the liver and is usually caused by a virus. It is possible that people with this test result might never have felt sick or known of their infection. These test results **do not** necessarily mean that you could infect other people through casual or sexual contact. In some cases, this test result may be positive even if there is no disease.

- This result does not mean that you have an illness or an infectious disease. If you are feeling well, there is no need for a medical check-up or additional laboratory testing.
- There is no evidence that people who have this test result are capable of spreading hepatitis by casual or sexual contact.
- Although there is no evidence that you could spread an infection by casual or sexual contact, there is a slight chance that a unit of your blood could transmit hepatitis to a person (patient) receiving it.

What does a positive anti-HBc test result mean about a person's health?

Usually, a positive anti-HBc test result means the person has recovered from a past infection and cannot be infected by the hepatitis B virus again. These people may never have felt sick or known of their infection. More than 5% of people in the United States have this test result and it does not affect their health. If the results of other hepatitis-related tests are normal, the person is unlikely to have viral hepatitis or another liver-related illness.

What are the other tests for viral hepatitis performed and what do they mean?

Other hepatitis-related tests, besides anti-HBc, are performed for each placental blood donor. When one test result is abnormal, we report all hepatitis-related test results to the donor. The donor may wish to review these test results with a doctor. These are the other hepatitis-related tests performed on all donated umbilical blood:

Hepatitis B Surface Antigen (HbsAg). This test detects the presence of the hepatitis B virus in a person's blood. A positive test result means the person is infected by the hepatitis B virus.

Antibody to Hepatitis C Virus (anti-HCV). This test detects the body's response to hepatitis C virus infection. A positive test result usually means the person has a hepatitis C infection.

What should a person with a positive anti-HBc test result do?

- If the anti-HBc test result is the only abnormal hepatitis-related test result after a routine donation, and the person is feeling well, there is no need to make a special appointment to see a doctor.

- A person should tell a health care provider about the positive anti-HBc test result during the next routine visit.

Should a person with a positive anti-HBc test result donate blood or pheresis products?

People with one positive anti-HBc test result remain eligible to donate blood and pheresis products. However, if their anti-HBc test is positive on a future donation, only the plasma part of their blood will be used and they will no longer be eligible to donate blood or pheresis products.

NOTIFICATION LETTER FOR ANTI-HCV EIA REPEAT REACTIVE
SUPPLEMENTAL TEST POSITIVE

Date

Dear Donor:

Thank you for your recent placental blood donation. We are writing to let you know the results of one of the tests we did on your blood and how these results will affect your future as a blood donor. Let me assure you, THIS IS NOT A LETTER ABOUT AIDS.

We tested your blood for antibodies to the hepatitis C virus (anti-HCV). Hepatitis is an **inflammation** of the liver and is usually caused by a virus. When we tested your blood, your first test was positive for antibodies to the hepatitis C virus. A supplemental test for anti-HCV was also positive.

Because positive test results usually mean either a past or present infection with the hepatitis C virus, these positive test results may be important for your health. For these reasons, we have enclosed a hepatitis C fact sheet and a summary of your hepatitis-related test results, which we recommend you review with your doctor.

Blood with positive test results cannot be given to another person. Therefore, we were unable to use the placental blood you donated. In addition, we are informing you that you must never be a blood donor.

If you want more information about this letter or your test results, please call *insert phone number here* and ask for *insert name here*.

Sincerely,

Insert name here

Notes: Enclose Positive Supplemental Anti-HCV Fact Sheet. Enclose test result form. Mail First Class.

FACT SHEET FOR POSITIVE SUPPLEMENTAL TEST RESULT FOR
HEPATITIS C VIRUS ANTIBODY (ANTI-HCV)

What does a positive supplemental test result for antibodies to hepatitis C virus (anti-HCV) mean?

This test result means that a person's blood sample tested positive, more than once, on a screening test for hepatitis C virus infection. To provide more information, an additional supplemental test for anti-HCV was performed. The results from this test were also positive.

A positive test result for anti-HCV usually means the hepatitis C virus is still present in the body. However, in some cases, it means the person used to be infected with the hepatitis C virus, but has recovered. The person might never have known of his or her hepatitis C infection.

How can hepatitis C infection affect a person's health?

Most people with these positive anti-HCV test results have no obvious illness. Some are in the early stages of liver disease. The hepatitis C virus can cause hepatitis (yellow jaundice), cirrhosis (liver failure), or potentially, liver cancer. The only way to tell which people have liver disease is by a medical check-up. People taking prescription medicine should notify their doctors about positive anti-HCV test results.

What are the other tests for viral hepatitis and what do they mean?

Three hepatitis-related tests, besides anti-HCV, are performed for each donor. When one test result is abnormal, we report all hepatitis-related test results to the donor. Donors may wish to review these test results with their doctor. These are the other tests:

Hepatitis B Surface Antigen (HBsAg). This test detects the presence of the hepatitis B virus in a person's blood. A positive test result means the person is infected by the hepatitis B virus.

Hepatitis B Cord Antibody (Anti-HBc). This test detects the body's response to a hepatitis B virus infection. A positive test result usually means the person has recovered from a past infection. For some people, this test result may be a sign of current hepatitis B infection.

Alanine Aminotransferase (ALT). The ALT test measures an enzyme in liver cells that leaks into the blood when the liver is infected or irritated. The liver can be infected by a virus or irritated by exercise, alcohol, toxins, or certain medications. Rarely, being overweight can irritate the liver. People with an abnormal ALT test result may have a viral infection or irritation of the liver.

What should a person with a positive supplemental test result for anti-HCV do?

- A person with a positive supplemental anti-HCV test result should see a doctor for a medical check-up. Usually the check-up will indicate normal health. If a medical check-up detects liver disease, the doctor may recommend changes in personal behavior or medication.

- Tell your doctor or dentist about this result before receiving treatment.
- Do not donate blood, semen, body organs, or other tissue.
- The U.S. Public Health Service recommends that people with positive anti-HCV test results check with their doctors for specific guidance about sexual behavior. Hepatitis C infection may be spread by sexual intercourse, although it is very uncommon and probably requires multiple exposures.

Should a person with a positive supplemental test result for anti-HCV donate blood?

No. Another person (patient) could be infected by blood donated by people with positive anti-HCV test results.

Please carefully read the letter that accompanies this fact sheet.

HEPATITIS-RELATED TEST RESULTS

Name of Donor: _____

Hospital ID: _____

Date of This Report: _____

Please read the enclosed fact sheet for further explanation of your test results.*

Test Name	Result		
	Repeat Reactive (Positive)	Non-Reactive (Negative)	
Hepatitis B Surface Antigen (HbsAg) ELISA (Screening Test)			
HBsAg Confirmatory Neutralization			
Antibody to Hepatitis B Core (Anti-HBc) ELISA (Screening Test) Total Antibody Test **			
Antibody to Hepatitis C Virus (Anti-HCV) ELISA (Screening Test)			
	Positive	Negative	Indeterminate
Antibody to Hepatitis C Virus (Anti-HCV) Supplemental Test, Performed When Anti-HCV EIA is Repeat Reactive (RIBA) Unlicensed			
***Donor ALT Level _____ IU/L. (Alanine Aminotransferase or SGPT) Cutoff: 120 IU/L. Cutoff: If your ALT level is equal to or above this value (considered a high elevation), your blood cannot be given to another person.			

Comments: _____

* Insert this statement when fact sheets are included with notification letter.

** Anti-HBc total antibody test: IgM and IgG.

*** If the ALT level is below the cutoff, insert the actual ALT level or the statement "Within Normal Limits." If the ALT level is equal to or greater than the cutoff, the actual ALT level must be inserted.

Notes: Send with all hepatitis-related notification letters.

NOTIFICATION LETTER FOR POSITIVE SYPHILIS SCREENING TEST

Date

Dear Donor:

Thank you for your recent placental donation. We are writing to let you know the results of one of the tests we did on your blood.

We test all blood for infection with syphilis. Syphilis is a sexually transmitted disease that can be treated with medication. When we tested your blood, your first test was positive and a second, different test for syphilis confirmed this result.

These positive test results may be important for your health and for the health of others. For more information, we have enclosed a copy of your test results and a syphilis fact sheet. We **strongly advise** that you inform your doctor about this test result and follow his or her recommendations for medical treatment.

If you have any questions after reading this letter and the enclosed fact sheet, please call *insert phone number here* and ask for *insert name here*.

Note: The law in *insert State Name here* requires that we notify the public or state health department of confirmed positive test results. We have notified the health department of your syphilis test results, and it is possible that they will contact you about these test results.

Sincerely,

Insert name here

Notes: Enclose Confirmed Positive Syphilis Fact Sheet. Enclose Syphilis Test Result Form. Mail First Class.

FACT SHEET FOR CONFIRMED POSITIVE TEST RESULT FOR SYPHILIS

What does a positive syphilis test result mean?

Syphilis is a sexually transmitted infectious disease that has several stages and can have severe medical consequences if left untreated. A positive test result almost always means that a person has a syphilis infection now or has had infection in the past.

How does a syphilis infection affect a person's health?

Syphilis is a serious health problem. Treatment with antibiotics is effective and very important.

If syphilis infection is left untreated, complications that could occur include:

- Joint disease.
- Cardiac disease.
- Neurologic problems.
- Women who become pregnant and who have not been treated may have children with severe congenital abnormalities.

How does a person become infected by syphilis?

- Having sexual intercourse with an infected partner.
- An infected mother can spread syphilis to a fetus or to a newborn during delivery.
- Very rarely, from an infected blood transfusion.

What should people with positive syphilis test results do?

If test results are due to a current or untreated infection:

- Contact a doctor.
- Tell sexual partners immediately so that they can be tested and receive medical treatment if necessary.
- Follow the doctor's advice about safe sex practices.

If test results are due to a previously treated infection:

- If a person is feeling well, there is no need to see a doctor or take any special action.
- A person should contact his/her doctor if he/she is not feeling well or if he/she has been treated for syphilis in the past but has reason to believe that these test results show a new infection.

Should people with a positive syphilis test result donate blood?

A person who has a positive syphilis test result and has been treated may donate blood twelve months after the diagnosis or positive test result for syphilis, whichever came first.

Please carefully read the letter that accompanies this fact sheet.

SYPHILIS TEST RESULTS

Name of Donor: _____

Hospital ID: _____

Date of This Report: _____

Test Name	Result		
	Reactive (Positive)	Non-Reactive (Negative)	Other
Check syphilis screening test used:			
☐ RPR			
☐ PK-TP			
☐ Other: _____			
Check syphilis confirmatory test used:			
☐ FTA-ABS			
☐ RPR* (when performed as part of the PK-TP confirmatory testing)			
☐ Quantitative RP1**			
☐ Other: _____			

* Reactive RPR test results may indicate current infection.

** This result is provided for you to share with your physician. This information can be used as a baseline for monitoring treatment if your syphilis confirmatory test (FTA-ABS) is positive. If your syphilis confirmatory test (FTA-ABS) is negative or inconclusive, this information is not relevant.

Insert the following note when confirmatory testing is performed by Public Health Department:
Note: Confirmatory testing was performed by the state/public health department.

Notes: Enclose this form with syphilis-related notification letters.

DRAFT NOTIFICATION LETTER FOR POSITIVE GENETIC SCREENING TEST: ADRENOLEUKODYSTROPHY

Date

Dear Donor:

Thank you for allowing us to collect your newborn's umbilical cord blood on *insert date here*. As discussed with you at that time, a sample of your baby's blood would potentially be screened for certain genetic diseases that might be passed to a patient needing these cells for transplantation. Because your baby's blood was recently identified as a close match for a patient, it was tested to see if your child had a disease called "Adrenoleukodystrophy." YOUR CHILD DOES NOT HAVE THIS DISEASE. It will not influence your child directly. You, the child's mother, may also be a carrier. Therefore, we recommend that you seek genetic counseling since you could have a boy with this disease.

If you have a male child, he should be evaluated by his pediatrician to see if he has signs and symptoms of this disease and have his blood tested. Moreover, it is possible that the test performed on the frozen umbilical cord blood was inaccurate. Enclosed is a Fact Sheet on Adrenoleukodystrophy; please take this sheet and this letter to your pediatrician. This is only necessary if you have a male child.

If you plan to have additional children, you have a 50:50 chance of having a boy and all boys will have the disease. It will be important for your daughter (from whom the cord blood was collected) to know about this risk as well.

A genetic counselor will be made available to you. Please call us to schedule an appointment at *insert phone number and name here*.

Sincerely,

Insert name here

Notes: Enclose Positive Test for Adrenoleukodystrophy Fact Sheet. Mail First Class.

DRAFT FACT SHEET FOR POSITIVE GENETIC SCREENING TEST FOR ADRENOLEUKODYSTROPHY

What does it mean to be a "carrier" of Adrenoleukodystrophy?

One in ??? individuals in the United States are "carriers" for Adrenoleukodystrophy. This is the progressive neurological disorder that results in blindness, deafness, and early death. This disease has been treated with "Lorenzo's Oil" and by bone marrow transplantation. This disease almost always occurs in males and very rarely in females. The most important aspect of being a "carrier" is having the knowledge that you are a carrier and that you have all male children tested for this disease. If you are considering the possibility of having additional children, it is important that you talk with a genetic counselor. You have a 50:50 chance of having a boy and boys will develop the disease.

Will being a "carrier" effect my personal health or the health of my baby?

Being a "carrier" has no effect on your personal health or the health of your female children.

Why should I seek the advice of a genetic counselor?

You need to know the risks of having a child affected with the disease as well as the tests available for prenatal diagnosis (e.g., amniocentesis).

How do I determine if I am really a carrier?

These tests were developed using fresh blood samples from potential patients and not frozen umbilical cord blood. Therefore, it is possible that the test result is not accurate. It is possible that you are not a carrier. We recommend that you and your child be rechecked to determine if you are carriers. A blood sample can be obtained by your doctor and sent to a genetics laboratory for retesting. You will be notified of the results within one week.

What do I do if I have a male child?

All male children born to you must be tested for this disease. Early diagnosis and medical treatment could be advantageous to their well being.

What are the signs and symptoms of Adrenoleukodystrophy?

Adrenoleukodystrophy presents with ... to be completed by Bill Krivit.

DRAFT NOTIFICATION LETTER FOR POSITIVE GENETIC SCREENING TEST:
GAUCHER'S DISEASE

Date

Dear Donor:

Thank you for allowing us to collect your newborn's umbilical cord blood on *insert date here*. As discussed with you at that time, a sample of your baby's blood would potentially be screened for certain genetic diseases that might be passed to a patient needing these cells for transplantation. Because your baby's blood was recently identified as a close match for a patient, it was tested to see if your child had a disease called "Gaucher's Disease." YOUR CHILD DOES NOT HAVE THIS DISEASE. It will not influence your child directly. You, the child's mother, may also be a carrier. Therefore, we recommend that you seek genetic counseling since you could have a boy with this disease.

It is possible that the test performed on the frozen umbilical cord blood was inaccurate.

A genetic counselor will be made available to you. Please call us to schedule an appointment at *insert phone number and name here*.

Sincerely,

Insert name here

Notes: Mail First Class.

APPENDIX D

EXAMPLES OF FORMS AND LOGS FOR NOTIFICATION OF INFECTIOUS DISEASE RESULTS

RECORD OF DONOR NOTIFICATION

CONFIDENTIAL

Donor Name _____ Hospital ID _____

Telephone Numbers (h) _____ (w) _____

*Social Security Number _____ *Date of Birth _____

Reason for Donor Notification _____

Notification Letters Sent

Date Letter 1 was Mailed _____ Date Letter 2 was Mailed _____

Restricted Delivery Receipt Returned to Region? Yes _____ No _____

Letter Returned Marked No Forwarding Address? Yes _____ No _____

Telephone Calls To or From Donor

Date/Time of Contact _____/_____ *ID confirmed? Yes _____ No _____

Date/Time of Contact _____/_____ *ID confirmed? Yes _____ No _____

Comments _____

_____

_____

Appointments for Personal Interview Donor Notification

Date/Time of Appointment _____/_____

If First Appointment Not Kept or Cancelled: Date/Time of Appointment _____/_____

Signed Information Release Form Received? Yes _____ No _____ N/A _____

Test Results Mailed to Donor's Physician? Yes _____ No _____ N/A _____

***Ask the donor at the beginning of each telephone contact to state this information for identify verification.**

INFORMATION RELEASE REQUEST

I authorize *insert Cord Blood Bank name here* to release the results of my blood test for *insert name of blood test here* to me and/or to the following doctor or clinic:

Your name (only if you are requesting that your test results be sent to you)

_____

Your address (only if you are requesting that your test results be sent to you)

_____

_____

Doctor's Name _____

Name of Facility _____

Street Address _____

City, State, Zip Code _____

Donor/Guardian Signature _____

Donor/Guardian Printed Name _____

Donor Social Security Number _____

Today's Date _____

Please return this form to: *insert Cord Blood Bank name and address here*

_____

CBB Staff Use Only

Hospital ID Number _____

AUTHORIZATION FOR RELEASE OF TEST RESULT INFORMATION

I authorize *insert Cord Blood Bank name here* to release the results of my blood test for ** *HIV, anti-HTLV-I/II/anti-HTLV-unable to distinguish viral type* to the doctor or blood center listed below and that I donated blood to that facility on or about *insert date here*.

Doctor's Name _____

Name of Facility _____

Street Address _____

City, State, Zip Code _____

Donor Signature _____

Donor Printed Name _____

Donor Social Security Number _____

Today's Date _____

CBB Staff Use Only

Hospital ID Number _____

Donation Date _____

**** Select appropriate test name.**

DONOR COUNSELING WORKSHEET

CONFIDENTIAL

Date of Counseling _____

Hospital ID Number _____

Place a check mark next to the items that were discussed with the donor:

1. Specific test results requiring notification _____

2. Information from the appropriate fact sheet _____

3. Placental donation that tested positive and was destroyed _____

4. Donor's name and other identifying information has been added to a confidential list of deferred donors _____

5. Donor is no longer eligible to donate blood _____

6. Donor was referred to his/her personal physician for further medical evaluation and followup _ _____

7. Written materials were provided _____

8. Local support resources were discussed (if applicable) _____

9. Donor has/has not donated blood since 1977 _____

   If yes, list locations and dates to the best of the donor's recollection _____ _____

Ask donor to sign an Authorization For Release of Test Result Information Form.

_____ _____

Counselor Signature Date

CORD BLOOD TRANSPLANTATION STUDY
NOTIFICATION LOG

Bar Code Label	Type of Letter Mailed	Date Mailed	Comments

APPENDIX E

HLA REFERENCE LABORATORY APPENDIX

HLA-A ALLELE DESIGNATIONS

0101
0102
0103
0105N
0201
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0204
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6809
6810
6901

7401
7402
7403
8001

HLA-B ALLELE DESIGNATIONS[1]

0702	1404	1526N	1804	3510
0703	1405	1527	1805	3511
0704	1501	1528	1806	3512
0705	1502	1529	1807	3513
0706	1503	1530	2701	3514
0707	1504	1531	2702	3515
0708	1505	1532	2703	3516
0709	1506	1533	2704	3517
0710	1507	1534	2705	3518
0711	1508	1535	2706	3519
0712	1509	1536	2707	3520
0713	1510	1537	2708	3521
0801	1511	1538	2709	3522
0802	1512	1539	2710	3523
0803	1513	1540	2711	3524
0804	1514	1542	2712	3525
0805	1515	1543	2713	3526
0806	1516	1544	2714	3527
0807	1517	1545	3501	3528
0808N	1518	1546	3502	3701
1301	1519	1547	3503	3702
1302	1520	1548	3504	3801
1303	1521	1549	3505	3802
1304	1522	1550	3506	3803
1401	1523	1801	3507	3901
1402	1524	1802	3508	3902
1403	1525	1803	3509	3903

[1] 62 63 71 72 75 76 77 Serology types will be pre-processed and mapped to 15 or 70

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HLA-DrB1 ALLELE DESIGNATIONS

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PACKING INFORMATION

Cord Blood Bank (CBB) Instructions: Complete all information below. Then fax this sheet to the requesting Transplant Center AND include a copy with the shipped cord blood unit (CBU).

Shipping to: _____ FAX: _____

This dry shipper contains **a frozen cryopreserved human placental umbilical cord blood unit (CBU)** for COBLT Recipient ID: _____

Contents of Dry Shipper:

COBLT CBU ID: _____

Accompanying Paperwork:
Transplant Center Feedback Sheet
Receipt Procedures
Procedure for Thawing Cryopreserved Cord Blood Unit (CBU) for Transplantation
Product Information
COBLT Bar Code Labels

Name of CBB Supplying the CBU: _____

CBB Shipper Number: _____

Federal Express Tracking Number: _____

For questions, contact:

CBB Contact Person: _____

Phone #: _____ Fax #: _____

Alternate CBB Contact:_____

Phone #: _____ Fax #: _____

Transplant Center Instructions: Upon receipt of this information, call the CBB contact person listed above for the combination of the dry shipper lock or if you have questions/concerns.

TRANSPLANT CENTER FEEDBACK SHEET

CBU ID #: _____ Recipient ID #: _____

CBU Information:

Volume (mL) - 25:
ABO Rh Type:
HLA A:
HLA B:
HLA DRB1:
CBU Collection Weight (gm):
Total Viable NCC x 10^8:
CFU-GM x 10^5:
CD 34+ x 10^6:
CD 3+ x 10^6:
Infant gender:

Maternal Sample Test Results:

CMV IgM Antibody:
Anti-HBc:
Syphilis:
Anti-HCV:
HbsAg:
Microbial Culture:
HIV-1/2:
HIV p-24 Ag:
HTLV-I/II:
Hemoglobinopathy Screen:

Complete the following information prior to packing the CBU in the dry shipper

Charged Weight of Unpacked Dry Shipper: _____ _____ _____ lb.

Complete the following information upon receipt of the CBU and fax to:
Name and FAX Number of CBB and MCC - Computer Generated

Transplant Center Code: ___ ___ ___ _____ Time Zone (ET, CT, MT, PT)

Date and Time of Receipt: ___/___/___ ___:___ (24 hour clock)

Confirm Label Checks:
CBU ID and Recipient ID on Packing Information / Transplant Feedback Sheet / CBU Registration _____
CBU ID on Unit / Transplant Feedback Sheet _____
Transplant Feedback Sheet / Accompanying Labels _____

Weight of Unpacked Dry Shipper: _____ _____ _____ lb.

Condition of CBU at Receipt: 1 G Satisfactory 2 G Unsatisfactory, *specify*:_____

Condition of Dry Shipper: 1 G Satisfactory 2 G Unsatisfactory, *specify*:_____

Did shipper temperature monitoring device indicate temperature > -120°C? 1 G Yes 2 G No

Please specify conditioning regimen intended for Recipient ID_____
_____

Information Completed By:_____ Study ID: __ __ __ __ Date: __/__/__

RECEIPT PROCEDURES

1. Verifying and Storing the Cord Blood Unit (CBU)

1.1 Open the top of the dry shipper using the combination lock number supplied by the CBB contact person. Locate the Transplant Center Feedback Sheet included in the packing information. Verify that the COBLT CBU ID and the COBLT Recipient ID recorded in the packing information, on the Transplant Center Feedback Sheet, and on the COBLT Confirmation of Registration/CBU Release Request match.

If there is a discrepancy, DO NOT PROCEED. Immediately call the designated CBB contact person listed in the packing information.

1.2 Open the main storage compartment of the dry shipper and locate the plastic bag containing the CBU canister. Transfer the CBU from the dry shipper into the vapor phase of liquid nitrogen in a liquid nitrogen freezer at ≤ -120°C. Verify that the identification number on the CBU matches the CBU ID number on the Transplant Center Feedback Sheet. **THESE STEPS MUST BE COMPLETED AS QUICKLY AS POSSIBLE TO MINIMIZE THE TIME THE CANISTER CONTAINING THE CBU IS EXPOSED TO THE AMBIENT TEMPERATURE.**

If there is a discrepancy, proceed. After safely storing the CBU canister, immediately call the designated CBB contact person listed in the packing materials.

1.3 After the CBU is safely stored, inspect the condition of the dry shipper and locate the temperature monitoring device packed with the CBU canister. Contact the CBB immediately if there is any indication that the CBU was damaged or exposed to temperatures > -120°C.

1.4 After all checks have been performed and any discrepancies resolved, notify the appropriate individuals at your institution that the CBU has arrived, and complete the receipt information on the Transplant Center Feedback Sheet. Fax the sheet to the CBB responsible person listed in the packing materials and to the MCC.

2. Returning the Dry Shipper (Contract Centers Only)

2.1 Make arrangements to return the dry shipper to the CBB immediately following storage of the CBU. Return the dry shipper via Federal Express using the enclosed return shipping label. Pack the Styrofoam packing, bubble paper, and the shipper lid. Lock the lid with the combination lock.

2.2 On the day the shipper is sent, inform the CBB designated contact person so that they can expect its arrival.

Immediate return of the shipper is essential because it is needed for another patient's product. If there are any questions, please call the designated CBB contact person.

THAWING CRYOPRESERVED CORD BLOOD UNIT (CBU) CELLS FOR TRANSPLANTATION

Principle

Cells cryopreserved in DMSO have limited viability upon thawing, resulting in significant losses of cells available for transplantation. DMSO, the cryopreservant used to maintain cell viability at ultra low temperatures, is toxic to cells when warmed to 37° C. Intracellular DMSO creates a hypertonic environment which leads to sudden fluid shifts and cell death upon warming. Lysis of red blood cells leads to accumulation of extracellular free hemoglobin which can be nephrotoxic if infused intravenously. In addition, DMSO causes adverse effects in vivo after reinfusion, including blood pressure instability, fever, chills, and nausea. These problems can be ameliorated by mixing the thawed cells with a hypertonic solution, Dextran 40 + 5% albumin, immediately upon thawing. Cells can then be washed and further manipulated to remove DMSO, free hemoglobin, and other cellular products, as well as to perform other procedures before infusion to the patient.

NB: This procedure is designed to enable the technologist to sterilely thaw cryopreserved cord blood within a closed system while maximizing viable cell recovery. The final product can be resuspended in a variable amount of dextran/albumin solution, allowing for adjustment to a suitable volume for reinfusion into patients of varying sizes. The final product is stable for at least 6 hours and time should be taken to work carefully and calmly.

Specimen

Frozen CBU within a metal canister maintained in the vapor phase of liquid nitrogen in a liquid nitrogen freezer at -120° C. The cryo bag containing the CBU may be overwrapped with plastic and may have 1-2 sealed tubing segments attached.

Equipment

Laminar Flow Hood	
Refrigerated Blood Bank Centrifuge	Sorvall
Plasma Expressor	Baxter 4R4414
Coupler	Baxter 4C2405
Transfer Packs with Spike (#2, 300 mL)	Baxter 4R2014
Sterile Docker	Haemonetics SCD 312
Balance	Sartorius or Mettler
Heat Sealer	Sebra
Hemocytometer with Coverslip	Hausser Scientific, Bright-Line 1492
Instrument to Count WBCs	Coulter
BacT/Alert 120	Organon Teknika
Glass Microscope Slides (2)	
Nunc Tube	
Sterile Gloves	
Protective Freezer Gloves	
COBLT Bar Code Labels	From CBB
CBU Thawing Form	

Reagents

Albumin 25% Human UPS (12.5 grams in 50 mL)	Baxter
Dextran 40 (10% Gentran 40 in 0.9% NaCl, USP)	Baxter
Trypan Blue Vital Stain, 1% Solution	Gibco

Supplies

12 x 75 mm Tubes, Non-sterile	Falcon 2052/Fisher 149-596
12 x 75 mm Sterile Culture Tubes with Snap Caps	Labcon 3336-335-000/Port City, Inc.
Syringes: Sterile 1 cc, 20 cc	Becton-Dickinson: (1 cc) 309623, (20 cc) 309661
Injection Needles: Sterile 16 g, 19 g	Becton-Dickinson: (16 g) 305198, (19 g) 305187
Cell Wash/Infusion Bag Set	Pall Medical (791–03)
Hemoset (optional)	Abbott Labs #8948

Or

Y-Type Blood/Solution Set with Large Standard Blood Filter (170-260 micron filter)	Baxter
Hemostats (optional)	
Insul - Ice Mat (optional)	POLYFOAM Packers #970362
Cup of Regular Ice	
Bucket of Dry Ice	
Small Plastic Zipper-locked Bags* *Gas sterilize in house	
Alcohol Prep Pads	Baxter Healthcare #40000-110
Iodine Swabsticks	Baxter Healthcare #40000-040

Procedure

1. Begin preparations.

    a. Verify that the water bath temperature is 37° C.

    b. Prepare and label QC materials: counting vials, glass slide(s) for Wright's stain, tubes for viability, nunc tube to refreeze cryo bag segment(s), and bacterial culture bottles. Nunc tubes should be labeled using one of the cryogenic ISBT-128 bar code labels supplied with the CBU. OPTIONAL: Tubes for immunophenotyping and sterile tubes for progenitor assays.

    c. Assemble and bar code the necessary paperwork, completing as much as possible.

d. Mark transfer and label packs at 150 cc, 50 cc, and 25 cc with a permanent marker using a template prepared in the laboratory.

e. Place dry ice in bucket.

f. Place regular ice in cup, then inside the hood for QC.

g. Label the transplant bag and waste bag of the Cell Wash/Infusion Bag Set (Figure 1) and put 100, 125, 150, and 175 mL marks to the outsides of the transplant bag, using a template prepared in the laboratory, to aid in adding the correct volume of Dextran/albumin solution (Illustration 1). Additional marks for 50, 200, and 250 mL may also be added. Tare the scale and use the same tared scale to weigh the transplant bag and residual cells in Step 6d.

NB: To obtain accurate weights, always position the transplant bag and attached tubing identically. To do this, obtain a plexiglass tray with pins which fit into the holes in the transplant bag. Also place a block next to the scale to rest the tubing attached to the transplant bag. Always rest the tubing on the block at the same distance from the bag. It is suggested that marking the tubing 15 cm from the top of the transplant bag will help in positioning the tubing.

2. Prepare Dextran 40 + 5% albumin solution.

a. With sterile technique, add 25 gm (100 mL from 2 bottles) of stock albumin (25% Human UPS, 12.5 gm/50 mL) to a UPS bag containing 500 mL of Dextran 40. Final volume is 600 mL Dextran/albumin solution with a final concentration of approximately 5% albumin (actually 4%).

Or

From a UPS bag containing 500 mL of Dextran 40, drain 250 mL Dextran 40 and sterilely add 12.5 gm (50 mL from one bottle) of stock albumin (25% Human UPS, 12.5 gm/50 mL) to the remaining 250 mL of Dextran 40. Final volume will be 300 mL of Dextran/albumin solution with a final concentration of approximately 5% albumin (actually 4%).

b. Using sterile technique (sterile docking or spike), transfer 300 mL of Dextran/albumin solution into the labeled 300 mL sterile transfer pack. Heat seal the tubing and remove the transfer bag.

c. If 600 mL of solution is prepared, using sterile technique (either sterile docking or spiking), transfer the remaining 300 mL of Dextran/albumin solution into a second marked 300 mL sterile transfer pack. Label bag with a 24 hour expiration date and time and save for another thaw procedure.

3. Set up a closed system.

   a. Sterile dock the 300 mL Dextran/albumin transfer bag to the Cell Wash/Infusion Bag Set (Illustration 2). Using the volume marks on the transplant bag, allow 125 mL of Dextran/albumin to flow into the transplant bag (see Step 1g).

   b. Clamp off the tubing. Surround the bag with an ice mat to allow the solution to cool and place in hood (Illustration 3).

4. Thaw the cord blood and transfer to transplant bag.

   a. Remove the cryopreserved cord blood from the freezer carefully. Two technicians will perform label checks as per institution SOP.

   b. Working in vapor phase of liquid nitrogen, open the cassette, remove cryo bag plastic overwrap if present, and quickly separate segment(s) from the cryo bag. Place segment(s) in labeled nunc tube and put nunc tube in vapor phase of liquid nitrogen immediately to prevent thawing of the cord blood in the segment(s). Nunc tubes should be placed in a permanent storage location at a later time.

   c. Remove the frozen CBU from the cassette. Wipe down outside of cryo bag quickly and carefully place into a sterile zipper-locked bag. Thaw the CBU in the zipper-locked bag in the water bath until the product reaches a slushy/liquid consistency. Remove the zipper-locked bag containing the CBU from water bath, dry outside of zipper-locked bag, and place under the hood.

   d. Under the hood, remove CBU cryo bag from zipper-locked bag and, if necessary, dry the outside of the cryo bag. Clean the cryo bag port covers with iodine solution, cut the covers over the ports, and clean the cut and inner surfaces again with alcohol.

      NB: The ports on the cryo bag are covered with a plastic seal. In addition, there is an internal seal that the spikes will break as they enter the bag.

      Use sterile gauze to dry the ports. Spike into the cleaned ports with the spike lines attached to the Cell Wash/Infusion Set. Envelope the cryo bag with an ice mat. **Open the connection to the Dextran/albumin bag, but do not begin mixing the cells with the dextran/albumin until you read through Steps e and f below.**

   e. Hold the cold pack-wrapped cryo bag in one hand and the cold pack-wrapped transplant bag in the other hand, lower the cryo bag, and raise the transplant bag to allow the Dextran/albumin solution to run into the cryo bag (Illustration 4). (Approximately 25 mL of Dextran/albumin solution should flow into the bag by gravity over 1-2 minutes.) Adjust the cold pack to allow the cryo bag to expand to accommodate this additional volume (Illustration 5). Massage the cryo bag by hand to thoroughly mix the 50 mL of cells plus Dextran/albumin solution.

f. Continue to **gently mix** the cells in the cryo bag with the Dextran/albumin solution by alternatively raising the cryo bag (with ice mat) relative to the transplant bag (with ice mat) and then the transplant bag relative to the cryo bag. Allow gravity to facilitate mixing of the cells and remaining Dextran/albumin solution. Gradually and completely mix cells with Dextran/albumin over a minimum of 4-5 additional minutes. After complete mixing, lower the ice pack-wrapped transplant bag and raise the ice packed-wrapped cryo bag to allow the cells to run into the transplant bag (Illustration 6). A small amount of residual fluid and cells will remain in the cryo bag and tubing. Clamp off the lines between the cryo bag and transplant bag in preparation for the rinsing procedure.

g. To rinse the cryo bag, unclamp the tubing between the Dextran/albumin bag and the cryo bag. Allow approximately 25 mL of Dextran/albumin solution to run into the cryo bag. Close the clamp on the tubing between the Dextran/albumin bag and the cryo bag. Swirl the solution around the cryo bag to mix any remaining cells with the Dextran/albumin solution. Open the tubing between the cryo bag and the transplant bag and allow the rinse solution with cells to run from the cryo bag into the transplant bag (Illustrations 7a-c). Repeat this process a second time. The total volume in the transplant bag should now be approximately 200 mL. [125 mL Dextran/albumin + 25 mL cells in DMSO + 25 mL (rinse 1) + 25 mL (rinse 2)]. CAUTION: Do not add more than 250 mL to the bag. Overfilled bags may break when centrifuged.

Note: To enhance flow during rinsing, add air from the transplant bag to the cryo bag. Sometimes the liquid in the 5 mL section of the cryo bag will not drain out of the bag. If this happens, close the clamp between the 20 mL section of the cryo bag and transplant bag. The fluid will then run out of the 5 mL compartment. CAUTION, do not add more than 250 mL to the bag. Overfilled bags may break when centrifuged. Allow a few mLs of well-mixed Dextran/Albumin + cells to enter back into the cryo bag. Drain back into Transplant bag.

5. Centrifuge the cord blood to pellet the cells.

a. Place the Cell Wash/Infusion Bag Set and Dextran/albumin transfer bag into a centrifuge bucket. It is suggested that the bags be placed in a sterile zipper-locked bag prior to placement in the centrifuge bucket. The assembly must be supported and cushioned and all bags must be standing upright with ports facing up (Illustration 8). Bags can be cushioned by placing two 250 mL bags filled with water or saline placed on either side of the transplant bag. Weigh and tare the balance. Balance with a second bucket.

b. Pellet the cells at 880 G for 20 minutes at 4° C via centrifugation. On their Sorvall model, personnel at Duke University spin at 1800 rpm to achieve 880 G . Each center should validate its centrifuge prior to thawing a COBLT CBU.

6. Express supernatant and prepare cells for transplantation.

   a. After centrifugation, gently remove the Cell Wash Infusion Bag Set and Dextran/albumin bag, taking care not to disrupt the cell pellet at the bottom of the transplant bag.

   b. Hang the transplant bag on the plasma expressor without disturbing the pellet at the bottom of the bag.

   c. Express the majority of the supernatant from the transplant bag into the waste bag. Continue to apply pressure until the cells in the pellet at the bottom of the transplant bag start to move or when the net remaining cell solution reaches a volume of approximately 25 mL.

      Note: Clear the tubing between the transplant bag and the waste bag by adding air from the waste bag.

   d. After completion of expression of the supernatant, move the transplant bag to the tared scale (see Step 1g). The transplant bag and tubing must be placed in the same position used to tare the scale.

   e. Carefully open the tubing between the Dextran/albumin bag and transplant bag. Resuspend cells in 50 mL (or desired volume between 30-150 mL) of fresh Dextran/albumin. Record the weight from the tared scale of the washed and resuspended CBU on the CBU Thawing Form. Heat seal and remove the waste and Dextran/albumin bags.

      Note: If patient weight is $\leq$ 20 kg, use approximately 30 mL. If patient weight is $\leq$ 40 kg, use approximately 40 mL.

   f. Using sterile technique, remove a 0.5 mL sample of the cells for QC. Obtain a cell count and viability on the final product. Record values on the CBU Thawing Form.

      Calculate viable cell recovery using the following formula:

$$\frac{\text{Total viable nucleated cells in washed and resuspended CBU}}{\text{Total viable nucleated cells of cryo-preserved CBU}}$$

      Note: To standardize viability reporting, viability should be performed by Trypan Blue dye exclusion within 5 minutes of taking the QC sample. See Procedure Notes: Viability counts using Trypan Blue.

      If < 75% of total viable nucleated cells are recovered (calculated above), re-spin supernatant in waste bag to recover additional cells, as detailed in Procedure Note 1.

      OPTIONAL: Calculate the amounts needed for progenitor cell assays and immunophenotyping.

g. If the final cord blood product contains clumps, filter using a burette filter (100 mL burette hemoset, Abbott Labs #8948) or Fenwal Y-type filter set (blood component filter set). Do not use any filter small enough to deplete leukocytes (e.g. < 60 microns). A pore size 60 - 270 microns is recommended. Do not irradiate product.

h. Label the transplant bag with information per institutional SOP.

i. Remove 15 mL from the waste bag and add to sterility testing tubes.

Procedure Note

1. To attempt to recover additional cells from the waste bag, follow the procedure below.

   a. Heat seal the tubing between the transplant bag and the waste bag proximal to the transplant bag. The transplant bag can be transported to the transplant unit for infusion into the patient.

   b. Using sterile technique, attach the tubing on the waste bag to a new transfer bag. Pellet the residual cells in the waste bag. Centrifuge the bag at 880 G at 4 degrees centigrade x 15 minutes. Remove the bag from the centrifuge carefully without disrupting the cell pellet.

   c. Express the supernatant into the transfer bag, leaving a residual volume of approximately 10-20 mL.

   d. Resuspend the cell pellet in the remaining supernatant and remove an aliquot of 0.1 mL. Perform cell count and viability.

   e. If $\geq 1 \times 10^6$ cells/kg of patient body weight are recovered from the waste bag and if patient received a cell dose of 1-5 x 10^7 cells/kg in the initial transplant bag, infuse these additional cells. These cells may be added to the original transplant bag or may be given as a boost later on Day 0. Transport residual cells to the patient's room for reinfusion. If $< 1 \times 10^6$ but $> 1 \times 10^5$ cells/kg are recovered, cells should be cryopreserved in 10% DMSO for future testing or other uses. These cells can be stored in a nunc vial or small cryo bag at the discretion of the transplant center lab.

Quality Control

Cell count

Viability

Smear for Wright's stain

Progenitor assay (Optional) * Do not remove more than 0.5% of total cells.

CD34+ Procount (Optional) * Do not remove more than 0.5% of total cells.

Procedure Notes

Viability Counts using Trypan Blue

1. Add 10λ of post-thaw cells and 10λ of trypan blue, 1% solution, to a sterile tube.

2. Mix thoroughly and incubate for 5 minutes.

3. Remove 10λ and place under a coverslip on a hemacytometer.

4. Allow to settle.

5. Count 200 cells, scoring live versus dead cells.

NOTE: The live cells are NOT blue, the dead cells are blue. Results are expresses as percent of cells that exclude the dye (i.e., are alive).

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Pablo Rubinstein, M.D., Director of Placental Blood Bank, New York Blood Center, New York (personal training and communications).

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Cell Wash/Infusion Bag Set

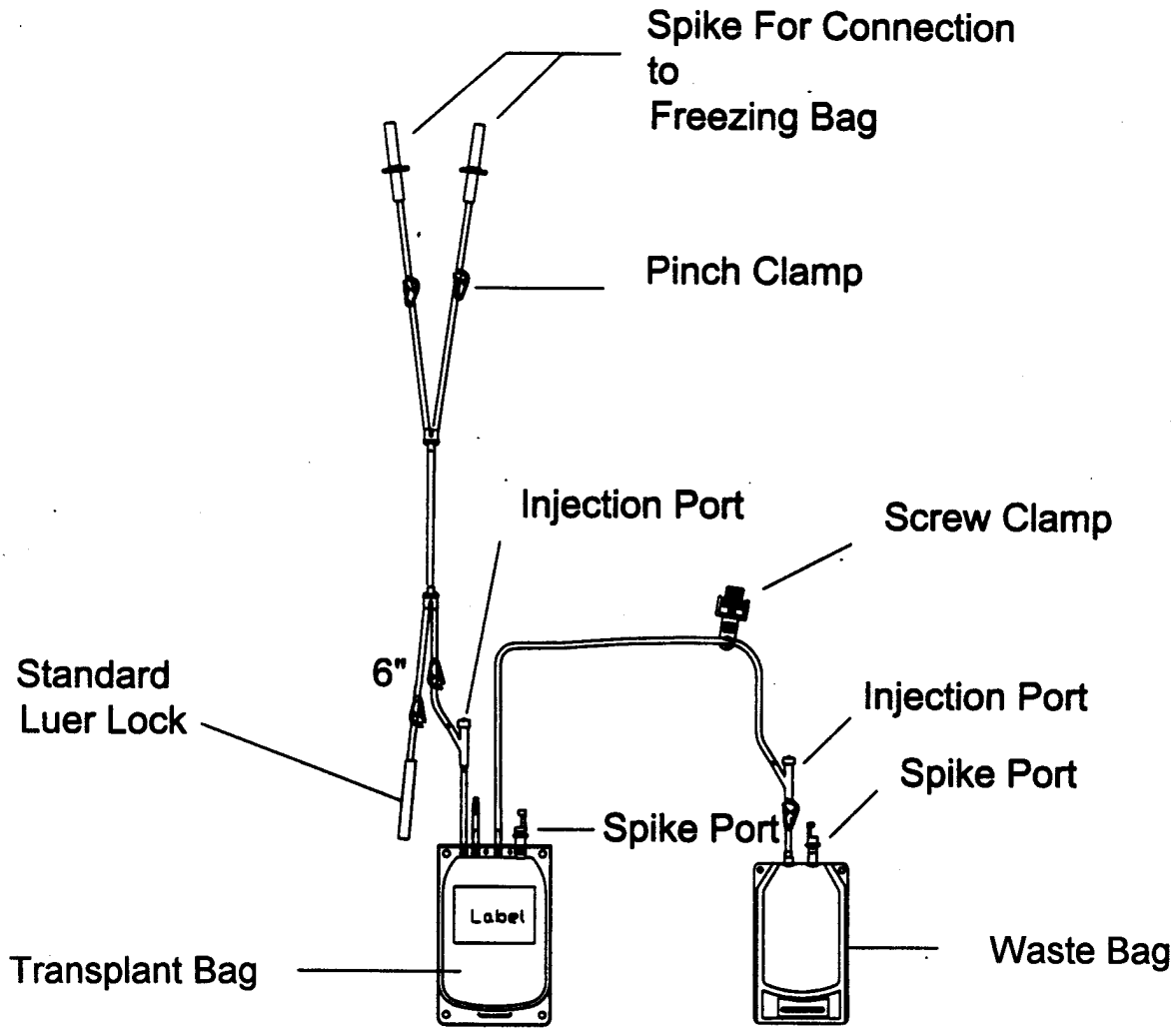


Figure 1 Transplant bag

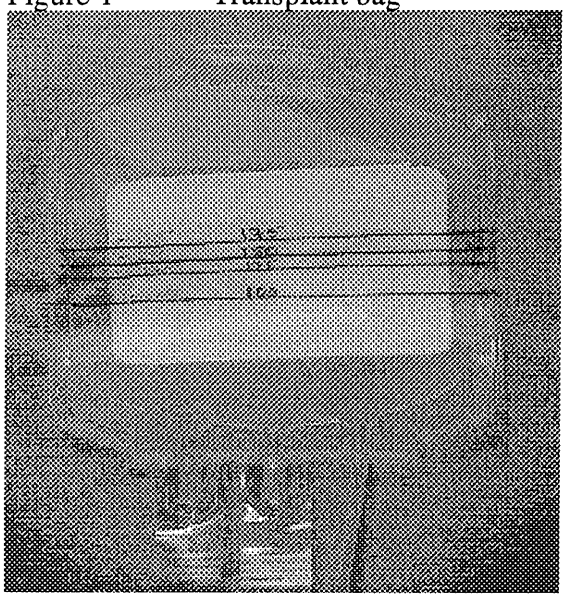


Fig 1. Transplant bag of the Cell Wash/ Infusion Bag Set with 100, 125, 150, and 175 mL marks on the outside to aid in adding the correct volume of Dextran/albumin solution.

Figure 2 Docked transplant bag

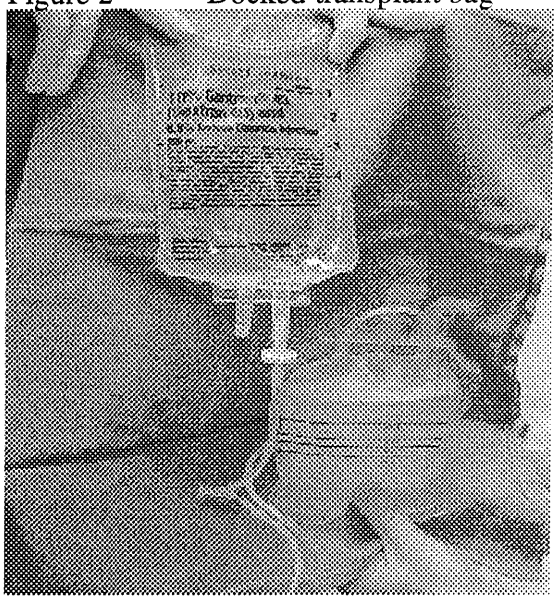


Fig 2. Transplant bag docked to Dextran/albumin of the Cell Wash/Infusion Bag Set with 100, 125, 150, and 175 mL marks on the outside to aid in adding the correct volume of Dextran/albumin solution.

Figure 3 Cold pack-wrapped cryobag

Fig 3. Cold pack-wrapped cryobag with velcro seal.

Figure 4 Lowered cryobag

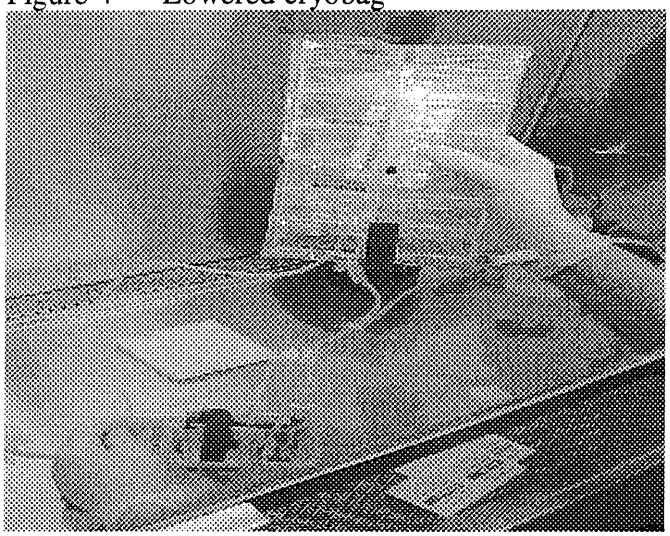


Fig 4. Illustration of lowered cryobag and raised transplant bag to allow the Dextran/albumin solution to run into the cryobag.

Figure 5 Bulging cryobag

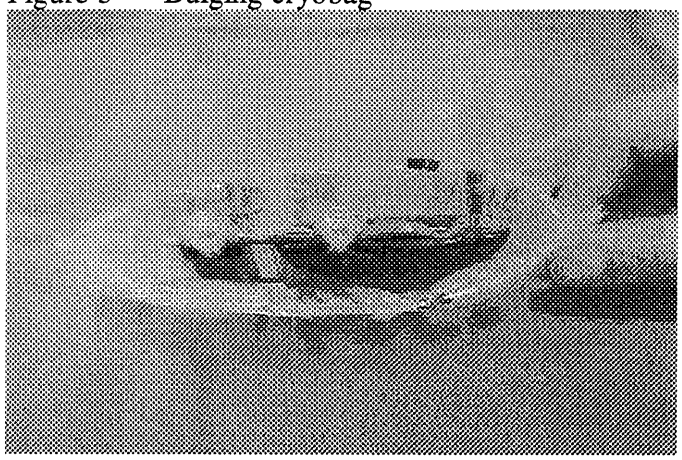


Fig 5. Bulging cryobag after initial addition of Dextran/albumin solution.

Figure 6 Lowered ice pack-wrapped transplant bag

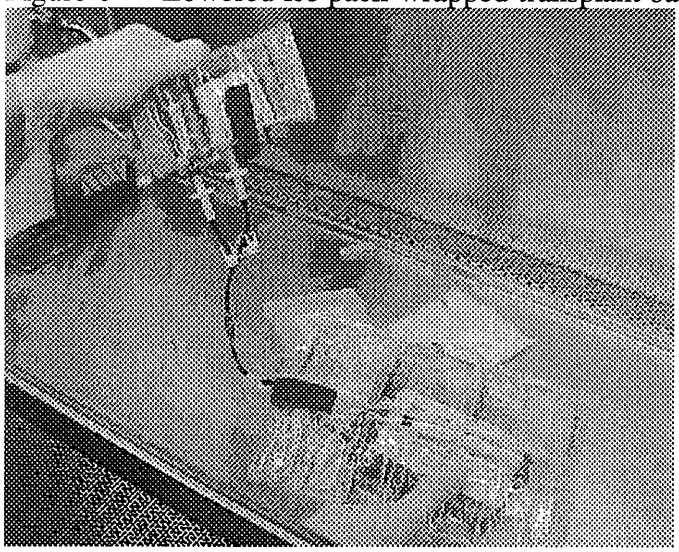
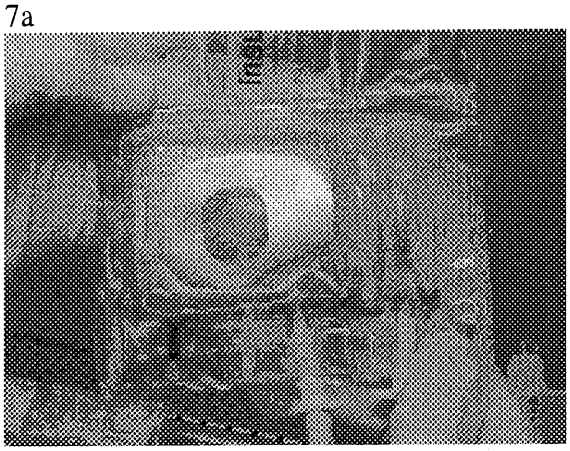


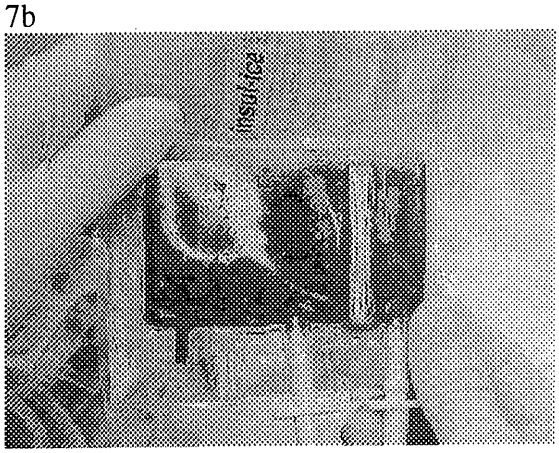
Fig 6. Illustration of lowered ice pack-wrapped transplant bag and raised ice pack-wrapped cryobag to allow the cells to run into the transplant bag.

Figures 7a-c Sequentially rinsed cryobags

7a



7b



7c

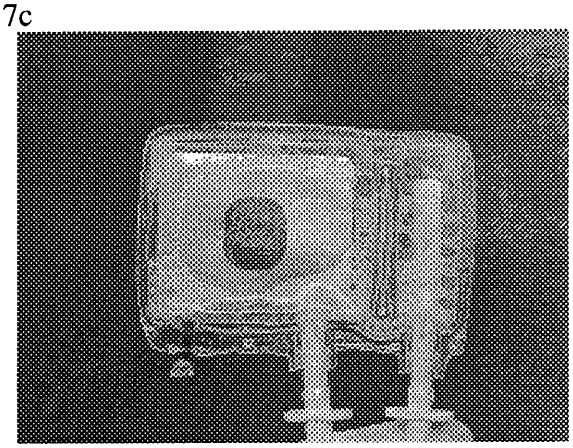


Fig 7a-c. Illustrations of sequentially rinsed cryobags with the spike lines attached to the Cell Wash/Infusion Bag Set.

Figure 8 Centrifuge bucket

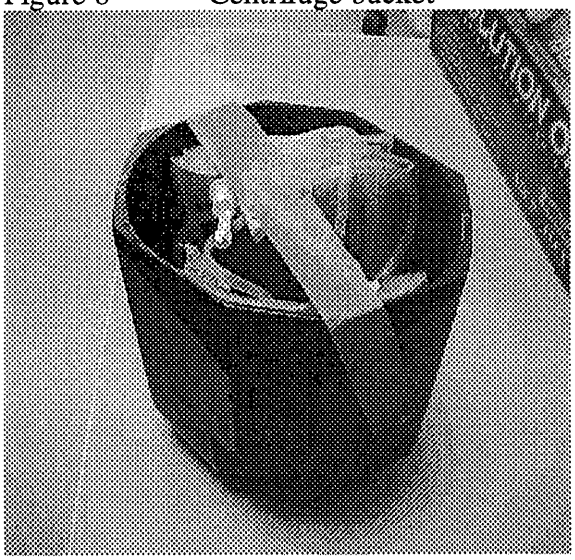


Fig 8. Cell Wash/Infusion Bag Set and Dextran/albumin transfer bag in a centrifuge bucket with tape securing the tubing and spike ports.

PROCEDURE FOR INFUSING THAWED CBU

Principle

Unrelated cord blood, banked for public use, can substitute for bone marrow as the source of reconstituting stem cells after marrow ablative therapy used to treat patients with cancer, bone marrow and immunodeficiency diseases, and selective genetic diseases. After selection for transplantation, the designated unit is shipped from the bank to the transplant center in a dry shipper before the patient begins cytoreductive therapy. At the transplant center, the unit is stored in the vapor phase of liquid nitrogen until the day of transplant, when it is thawed and washed in dextran/albumin, a process which increases cell viability and removes approximately 90% of the DMSO cryoprotectant. The washed cells are resuspended in dextran/albumin and transported to the patient's bedside in a labeled transplant bag for infusion.

The unit is infused into the patient's blood via the central venous catheter over 2-30 minutes. Every effort should be made to be sure that all the cells in the transplant bag and IV tubing are delivered to the patient, maintaining a closed system during the infusion procedure.

Materials

Labeled transplant bag
Y-infusion set
IV extension tubing
500 mL bag of Normal Saline
Blood filter, 170-260 microns

Procedure

1. Verify that the patient is stable and has received scheduled premeds for transplant.

2. Verify that the transplant bag label matches patient identifiers via institutional procedures.

3. Examine the transplant bag to be sure that cells are in solution and that large clumps are not present in the bag. If clumps are present, prepare to use blood filter in infusion set-up.

4. Close all clamps on the tubing on the Y-infusion set and blood filter.

5. Spike one arm of the Y-infusion set into the bag of normal saline.

6. Connect extension tubing to the distal arm of the Y-infusion set.

7. Prime all tubing with normal saline.

8. Spike the other arm of the Y-infusion set into the transplant bag.

9. Connect the distal end of the extension tubing to the patient's central venous catheter.

10. Open the clamps between the transplant bag, the extension tubing, and the patient's central line and allow cells to infuse into the patient's blood.

11. Rinse residual cells in the transplant bag and tubing. After all cells have dripped out of the transplant bag, close the clamps on the extension tubing and Y-set connected to the transplant bag. Open the clamps between the normal saline bag and transplant bag and allow approximately 25 cc of saline to run into the transplant bag. Close the clamps between the saline bag and transplant bag and open the clamps between the transplant bag and patient and infuse this saline to the patient. Repeat rinse x 1.

12. Monitor the patient's vital signs before, during, and after infusion per institutional practices.

PRODUCT INFORMATION

General Information

Bone marrow transplantation (BMT) from human leukocyte antigen (HLA)-identical sibling donors has been successfully utilized in the treatment of high-risk or recurrent hematological malignancies, bone marrow failure syndromes and selected hereditary immunodeficiency states and metabolic disorders. In an attempt to increase the availability of suitable donors and reduce the morbidity and mortality associated with allogeneic bone marrow transplantation, clinical investigators worldwide have evaluated placental and umbilical cord blood as an alternate source of hematopoietic stem and progenitor cells for transplantation (1 - 21).

As of June 2000, umbilical cord blood from sibling and unrelated donors has been used to reconstitute hematopoiesis in approximately 1200 patients with malignant and non-malignant disorders treated with myeloablative therapy. Reports from individual institutions and the International Cord Blood Transplant Registry (ICBTR) suggest that umbilical cord blood contains sufficient numbers of hematopoietic stem and progenitor cells for both early and late engraftment at least in recipients weighing less than 40 kilograms. The purpose of the Cord Blood Transplantation (COBLT) Study is to accurately describe 180-Day survival and other events after cord blood transplantation.

Drug Description

Umbilical cord blood is collected from the delivered placenta by insertion of a sterile transfusion set needle into the umbilical vein. Gravity causes the blood to drain into the collection bag, which is part of a sterile closed system. The anticoagulant citrate-phosphate-dextrose (CPD) is included in the bag. Collection volumes range from 40-300 mL. Informed consent is obtained from every mother and samples of the mother's blood are screened for CMV, Hepatitis B, Hepatitis C, HIV-1/2, HTLV-I/II, and syphilis. A sample of the cord blood unit is tested for microbial contamination and HLA type. Results from newborn sickle cell disease screening are obtained. A maternal medical history is obtained, and a six month follow-up of the baby is requested. A nucleated cell count, a CD34+ cell count, and a colony forming unit assay are performed on cells from the unit. Units are cryopreserved using a solution of 10% dimethyl sulfoxide (DMSO) and 1% dextran. Cryopreserved CBUs are permanently stored in liquid phase of liquid nitrogen. Small aliquots for additional testing and unit identification are also frozen. The collection bag, cryobag, test samples, and data forms are labeled with a study bar code. When selected for transplant, the unit is shipped to the transplant center using an express carrier. It is thawed in the laboratory at 37°C, washed with 10% dextran 40 and 5% human albumin to remove cryoprotectant, resuspended in fresh 10% dextran 40 and 5% human albumin, and infused into a patient who has received an appropriate conditioning regimen.

Pharmacological/Toxicological Effects

In early studies, there were reports of reactions to the cryoprotectant used for freezing the cord blood unit. A change in the thawing procedure (22) has reduced or eliminated that problem and improved viability of the infused cells. Reported graft versus host disease, even in patients who received two and three HLA antigen mismatched cord blood units, appears to have been less than would have been expected with marrow from an unrelated donor (16).

Pharmacokinetics

The median time to engraftment (ANC >500 on the first of three days) for patients who receive cord blood stem cell transplants has been reported to vary from 17 to 26 days (17). Hematopoietic recovery may have been related to the use of growth factors in this study. Others have suggested it may also be related to cell dose per kg of patient weight, and perhaps to other unidentified factors. Platelet engraftment is significantly delayed in recipients of cord blood compared to other types of stem cell transplants (median time to platelets >50,000 = 67 days in the Wagner study and 82 days in the Kurtzberg study).

Safety and Effectiveness

Nearly 375 unrelated donor UCB transplants have been performed at Duke University and University of Minnesota. In July 2000 (21), a detailed analysis of their combined data sets was performed to determine the potential influence of various factors (e.g., graft cell dose and donor/recipient HLA disparity) on rate of hematopoietic recovery and probabilities of engraftment, acute GVHD, chronic GVHD, non relapse mortality, relapse and overall survival. In comparison to prior reports on unrelated donor UCB transplantation, the present study benefits from standardized HLA typing with high resolution typing of HLA-DR, greater homogeneity in supportive care treatments between two centers, and the ability to internally verify data accuracy.

The results from the analysis demonstrated that cryopreserved UCB from HLA 0-3 antigen mismatched unrelated donors contains sufficient numbers of transplantable hematopoietic stem and progenitor cells for most small patients. The data presented indicated that the probabilities of grade III-IV acute GVHD and extensive chronic GVHD are low. Please see the reference list for complete citation and additional studies.

Risks and Toxicities

Recipients of cord blood transplants, like recipients of allogeneic marrow transplants, incur risks from pre-transplant conditioning and graft versus host disease (GVHD) prophylaxis which must be weighed against the risk of malignancy or other disease for which they are receiving a transplant. Compared to other forms of transplantation, the following risks may be increased in recipients of cord blood.

1. Failure to engraft or secondary graft failure can occur. It appears that both white cell and platelet engraftment are slower compared to other sources of stem cells. Graft failure is of special concern in larger patients. Additional stem cells will not be available from the same donor to treat graft failure.

2. Relapse of the underlying disease may occur, especially in patients with advanced disease at the time of transplant. Because of the naive nature of the cord blood cells, relapse may be an increased problem in this kind of transplant. Additional stem cells will not be available from the same donor to treat graft failure.

3. GVHD may be increased compared to marrow transplants, especially in patients with two or three HLA antigen disparity.

4. Infections can be life threatening in the transplant patient population, especially patients receiving immunosuppressive therapy for GVHD.

5. Unknown toxicity from residual cryoprotectant or other agents in the cord blood infusion is a theoretical possibility.

Despite these potential risks and toxicities, the published data to date suggest that cord blood transplants are an acceptable alternative to other forms of stem cell transplantation and prompted this study.

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