CHAPTER 9

TRANSPLANT CENTER LABORATORY PROCEDURES
9.1 IMMUNE RECONSTITUTION ASSAYS

9.1.1 Introduction and Objectives

Two of the principal problems that occur following allogeneic transplantation are graft versus host disease (GVHD) and post-transplant immunodeficiency. After marrow transplantation, the severity of acute GVHD increases as the difference in HLA antigens between donors and recipients increases. Recipients of unrelated-donor marrow have an immunodeficiency of greater severity compared to recipients of related marrow than can be explained by the increased severity and frequency of their acute GVHD. Thus, other factors beside GVHD may contribute to the prolonged immunodeficiency observed in recipients of unrelated marrow. Among the reasons hypothesized for the increased immunodeficiency are the effects of GVHD on the thymus (4), defects in differentiation of donor T cells in the recipient, and histoincompatibility between donor-derived T cells and patient antigen presenting cells. It is of interest to determine the extent to which this prolonged immunodeficiency occurs following cord blood transplantation.

There is no permanent carry over of antigen specific T lymphocyte or B lymphocyte functions. Antigen specific immune function after transplantation depends on the development of new antigen specific T lymphocytes through the recipient thymus and on subsequent development and maturation of new functional B cells. Most of the patients in the post transplant period are receiving IVIG. Therefore, development of B lymphocyte function can only be assessed by immunization of the patients with neo-antigens. For this reason, the primary objectives of this study are to characterize the regulation of the production of new T cells in the recipient, evaluate the antigen specific T cell response to infectious antigens (herpes simplex, HSV; varicella, VZV; cytomegalovirus, CMV) and following immunization (tetanus toxoid, TT), determine the role of cytokines (IL-2 and IL-7) in antigen specific function, and to investigate the capacity of recipient T cells to interact with B cells to induce specific antibody production. Data will be analyzed considering patient age at transplant, primary disease, and degree of HLA mismatch. Each assay will be performed in a single laboratory to eliminate inter-site variation.

9.1.2 Background

Previous studies demonstrated that there was no significant carry over of antigen specific T cells following transplantation. Thus, antigen specific T cell function in recipients following marrow transplant is provided by T cells that differentiated in the recipient’s thymus. Immunophenotypic analyses were undertaken to determine if the T cells present in the peripheral blood of recipients early following transplant had immunophenotypic characteristics similar to those of lymphocytes present in the cord blood of newborn infants and the peripheral blood of fetuses. Early following transplantation, CD3+ T cells co-expressed CD4 and CD8, and CD1-expressing T cells were detected in some patients (1), much like neonates.
The phenotypic evaluation of transplant recipients was confounded by the fact that the marrow used for allogeneic transplantation was contaminated by a significant number of T cells. The evaluation of patients receiving T cell-depleted marrow represented a more accurate assessment of post-transplant T cell ontogeny. When patients with severe combined immune deficiency (SCID) were evaluated following T-cell depleted transplant, no T cells were identified by flow cytometry for the first 8-12 weeks following transplantation. The appearance of phenotypic T cells at 12 weeks parallels normal fetal lymphoid development in which phenotypic T cells first exit from the thymus at 12 weeks of gestation (2). Longitudinal analyses revealed that the first detectable CD3+ cells in the peripheral blood of transplant recipients were CD3dim and did not express either CD4 or CD8 (i.e. double negative cells). Within a week to ten days of the initial appearance of CD3dim cells, CD3bright cells, some of which co-expressed CD4 and CD8, were detectable. These findings are consistent with the fact that lymphocytes that are normally only found in the thymus in post-natal life (CD3dim and CD3bright, double positive cells) are found in the peripheral blood of recipients early following transplantation.

Recent thymic immigrants expressing CD3 and CD4 also express the high molecular weight isoform of CD45, CD45RA. Normal cord blood CD4+ T cells express CD45RA but not the low molecular weight isoform, CD45RO which is found on “memory” T cells. A cross sectional analysis of histocompatible recipients without GVHD showed an age dependent decline in the number of CD45RA-expressing CD4 T cells/mm3 more than one year post-transplant. This is similar to the age dependent decline in the production of new CD4 T cells observed in normal individuals and patients receiving chemotherapy (3,4.)

Histocompatible recipients who had chronic GVHD or received unrelated marrow had reduced numbers of CD45RA-expressing CD4 T cells. This was not age dependent and suggested that recipients of unrelated marrow had a decreased capacity to generate new CD4 T cells regardless of age. The inability to generate new CD4 T cells may play a central role in the post-transplant immune deficiency seen in the recipients of unrelated transplants.

When histocompatible recipients with GVHD were evaluated for their antigen specific response to the antigen tetanus toxoid following immunization, the only recipients with chronic GVHD who were able to generate an antigen specific response were those who were also capable of producing new CD45RA-expressing CD4 T cells. Thus, the capacity of recipients with GVHD to generate new T cells was predictive of their antigen specific T cell function. Conversely, those recipients, regardless of age, who were unable to produce significant numbers of new CD4 T cells did not develop antigen specific responses following immunization with tetanus toxoid.

During development, the capacity of T cells to respond to the mitogen phytohemagglutin (PHA) can be detected first at 12 weeks of gestation, whereas antigen specific responses are not detected until 20 weeks. In histocompatible recipients without GVHD, the immunization of transplant recipients (like that of normal individuals) resulted in the detection of antigen specific function in 75% of patients after one immunization and 95% of patients after two immunizations. However, if patients were receiving significant immunosuppression and/or had ongoing GVHD, the rate of successful immunization following initial tetanus toxoid immunization was markedly lower (5).
**CORD BLOOD TRANSPLANTATION STUDY MOP**

*In vitro* antigen specific immune function was evaluated with and without exogenous interleukin-2 (IL-2). The longitudinal evaluation of antigen specific proliferation *in vitro* following tetanus toxoid immunization has shown that antigen specific, IL-2 dependent T cells appear in the peripheral blood approximately 2-4 weeks prior to the appearance of antigen specific IL-2 producing T cells in normal histocompatible transplant recipients. In histocompatible recipients with GVHD or recipients of unrelated marrow, the appearance of the antigen specific IL-2 producing subpopulation can be significantly delayed. Thus the basis for the lack of *in vivo* antigen specific immune function seen in some patients may be caused by a selective delay in the ontogeny of antigen specific IL-2 producing cells or defects in antigen presentation resulting in an absence of antigen specific T cells.

Similar studies have been performed in transplant recipients suffering from infections with herpes viruses (CMV and VZV.) Whereas patients without GVHD rapidly developed antigen specific proliferation in the absence of exogenous IL-2, many recipients of unrelated marrow or histocompatible recipients with chronic GVHD had the presence of antigen specific T cells detected only in the presence of added IL-2. This implied that there was a delay in the development of the antigen specific IL-2 producing cells but that antigen specific IL-2 dependent T cells had developed normally (6). Clinically, patients suffered from recurrent herpes virus infections until they had detectable *in vitro* proliferative responses without the addition of IL-2. Thus the ability to clinically control herpes virus infections correlated with the development of antigen specific IL-2 producing T cells.

IL-7 is a lymphokine with a central role in T cell development in the thymus. Preliminary analysis of IL-7 levels in sera of transplant recipients showed that SCID recipients (who are young) had higher levels of IL-7 following transplantation than older patients with leukemia or aplastic anemia (7). The relationship between IL-7 and the ability to produce new CD45RA T cells is an area of intense study.

Several investigators have studied changes in the immunophenotype of peripheral lymphocytes over time for histocompatible transplant patients with or without GVHD as well as recipients of unrelated marrow. Recipients without GVHD developed normal immunophenotypic T cells by 6-12 months following transplantation whereas recipients with GVHD or the recipients of unrelated marrow had significant delays in the development of normal numbers of CD4+ T cells and B cells (8).

Other investigators have reported that SCID recipients of haploidentical marrow depleted of T cells had no T cells identifiable by flow cytometry during the first three months following transplantation. However, once phenotypic T cells were present, the proliferative response to PHA also developed and was normal by one year (9).

The induction of the cell surface protein CD40 ligand on T cells is central to cooperation between T cells and B cells. Cord blood T cells have reduced CD40 ligand expression following polyclonal stimulation. This might contribute to the defects in antibody production against carbohydrate antigens seen in infants.
9.1.3 Specific Aim 1 - Production of New Lymphocytes

The hypothesis for this aim is that following cord blood transplant, the patients without GVHD will develop antigen specific T lymphocytes within 9 months, and that the development of antigen specific T lymphocytes is delayed in patients with GVHD.

Peripheral blood samples will be obtained to evaluate immunological responses according to the schedule in Table 9.1.3.1.
## SCHEDULE OF IMMUNE EVALUATION

<table>
<thead>
<tr>
<th>Forms</th>
<th>Months Post-Transplant</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Immunophenotyping 1</td>
<td>X</td>
</tr>
<tr>
<td>PHA Response 2</td>
<td>X</td>
</tr>
<tr>
<td>Antigen Specific Blastogenesis 3</td>
<td>X</td>
</tr>
<tr>
<td>Tetanus Toxoid Immunization</td>
<td>X</td>
</tr>
<tr>
<td>ΦX174 Stimulation</td>
<td>X</td>
</tr>
<tr>
<td>CD40 Ligand Expression 4</td>
<td>X</td>
</tr>
</tbody>
</table>

1 CD3, 4, 8, 56, 19/20, 4/8, RA/RO/4
2 3 doses of PHA ± IL-2
3 Tetanus toxoid, varicella zoster, CMV, herpes simplex, ± IL-2, IL-7
4 After PHA/PMA stimulation
CORD BLOOD TRANSPLANTATION STUDY MOP

Specimens will be prepared as below and analyzed by two color immunofluorescence using the monoclonal antibody combinations displayed in Table 9.1.3.2. Three color immunofluorescence will be used for CD4/CD45RA/CD45RO.

Table 9.1.3.2
MONOCLONAL ANTIBODY PANEL FOR IMMUNOPHENOTYPING-
IMMUNE RECONSTITUTION

<table>
<thead>
<tr>
<th>FITC Antibody</th>
<th>PE Antibody</th>
<th>Detects</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>IgG2a</td>
<td>Non-specific Binding</td>
</tr>
<tr>
<td>IgG2a</td>
<td>IgG1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Non-specific Binding</td>
</tr>
<tr>
<td>Anti-CD3&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Anti-CD4</td>
<td>Helper T Cell Subset</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>Anti-CD8</td>
<td>Cytotoxic T Cell Subset</td>
</tr>
<tr>
<td>Anti-CD4</td>
<td>Anti-CD8</td>
<td>Double Positive T Cells</td>
</tr>
<tr>
<td>Anti-CD45RA&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Anti-CD45RO</td>
<td>Naive vs Memory T Cells</td>
</tr>
<tr>
<td>Anti-CD16</td>
<td>Anti-CD3</td>
<td>NK Cells</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>Anti-CD19</td>
<td>B Cells</td>
</tr>
</tbody>
</table>

<sup>1</sup> Plus additional isotype controls as required  
<sup>2</sup> CD3+ cells will be characterized as CD3<sub>dim</sub> or CD3<sub>bright</sub> 
<sup>3</sup> Three-color immunofluorescence using CD4

9.1.4 Specific Aim 2 - Antigen Specific T Cell Function

The hypothesis for this aim is that cord blood transplant patients will develop a proliferative response to the mitogen PHA by three months post transplant, a response to tetanus toxoid immunization by six months, and a response to herpes viral antigens by 6 months. The secondary hypotheses that exogenous IL-2 and IL-7 will hasten the antigen specific responses and occurrence of GVHD will delay the antigen specific responses will be evaluated. The antigen specific response will be correlated with the appearance of T cells as determined in specific aim 1.

Peripheral blood mononuclear cells from patients will be tested according to the schedule in Table 9.1.3.1. Cells will be stimulated with both PHA and specific antigens (tetanus toxoid, varicella zoster virus, cytomegalovirus, and herpes simplex virus) in a microtiter culture system as described below. When adequate numbers of cells are available, the mitogen and antigen specific studies will be done in the presence of exogenous IL-2 and IL-7.
9.1.5 Specific Aim 3 - T and B Cell Cooperation

The hypothesis for Aim 3 is that cord blood transplant recipients produce antibodies to ΦΧ 174 antigen synchronously with development of expression of CD40 ligand.

Immunizations will be performed and peripheral blood mononuclear cells isolated from patients will be tested according to the schedule in Table 9.1.3.1. Procedures for immunization, antibody analysis and CD40 ligand induction are specified below. Use of the phage antigen ΦΧ174 allows assessment of a specific antibody response in patients receiving immune globulin. Antibody isotype, avidity, and class switching will be studied.

9.1.6 Collecting and Shipping of Peripheral Blood Samples

1. 10 cc heparinized blood (green top) at room temperature delivered within 24 hours.

2. Samples should be delivered Monday through Thursday from 7:30 AM to 6:30 PM (Pacific Time), and on Friday from 7:30 AM to 2:00 PM.

3. Samples should be delivered to:

   Robertson Parkman, M.D.
   Children’s Hospital of Los Angeles
   4650 Sunset Boulevard, Mail Stop #62
   Los Angeles, CA 90027
   Telephone: 323-669-2196    Fax: 323-660-1904

9.1.7 References


9.2 THAWING COBLT CRYOPRESERVED CORD BLOOD UNITS FOR TRANSPLANTATION

The COBLT Study requires certification of Transplant Centers in receiving and thawing CBUs prior to shipment of a COBLT CBU for transplant.

9.2.1 CBU Thawing Procedures

COBLT CBUs are cryopreserved in 10% DMSO and 1% Dextran. 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 side 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 reinfusion to the patient.

The COBLT CBU thawing procedure is designed to enable the technologist to steriley 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 40/albumin solution, allowing for adjustment to a suitable volume for reinfusion into patients of varying sizes. The final product is stable for at least six hours. COBLT CBU thawing procedures are included in the Investigators Brochure shipped with each COBLT CBU for transplant. A copy of the Investigators Brochure can be found in Appendix B of the Transplant Center Manual of Procedures (MOP) and Appendix F of the CBB SOP.
9.2.2 CBU Thawing Certification Requirements for Transplant Centers

Certification will be granted to a center if at least one staff member completes the following steps:

1. Attends a COBLT thawing wet workshop training session or visit a COBLT CBB for on-site training.
2. Receives three CBUs from a COBLT CBB, thaw all three CBUs using the COBLT thawing procedure, and report data to the CBB and Medical Coordinating Center (MCC).
3. Meets the COBLT Study requirements of:
   - ≥ 75% cell viability
   - ≥ 60% viable nucleated cell recovery.

Certification will be granted to a non-COBLT Transplant Center if at least one staff member:

1. Visits a COBLT CBB or Transplant Center to watch and perform the COBLT CBU thawing procedure.
2. Receives three CBUs from a COBLT CBB, thaw all three CBUs using the COBLT thawing procedure, and report data to the CBB and the MCC.
3. Meets the COBLT Study requirements of:
   - ≥ 75% cell viability
   - ≥ 60% viable nucleated cell recovery.

9.2.3 Requesting CBUs for Thawing Certification

Transplant centers may request CBUs for thawing certification from COBLT CBBs at Duke University and University of California – Los Angeles. Each center is assigned to a specific CBB by the MCC. Centers should contact the Data Coordinator at the MCC to determine their assignment. To request COBLT CBUs for thawing certification, the Transplant Center Thawing Coordinator should contact the COBLT CBB Coordinator at their assigned CBB using the CBU Request for Thawing Certification. A maximum of four CBUs may be requested at one time. The request should be faxed to the CBB Coordinator at the appropriate CBB as designated below.

The CBB Coordinator will confirm shipment of the certification CBUs by completing and faxing the CBU Request for Thawing Certification to the Thawing Coordinator requesting the CBUs at the time of shipment. A copy of the fax will be sent to the Data Coordinator at the MCC.

The CBB Coordinator will complete and include the Thawing Certification CBU Packing Information for all CBUs included in the shipment. The CBUs will be shipped to the Transplant Center in a ‘dry shipper’ or on dry ice.
9.2.4 Obtaining Certification to Thaw COBLT CBUs

Upon receipt, the Transplant Center staff should store and thaw CBUs according to current COBLT Thawing Procedures as described in the Investigators Brochure (Appendix B of the Transplant Center MOP, Appendix F of the CBB SOP). For each COBLT CBU thawed, a CBU Thawing Form should be submitted to the MCC to confirm that COBLT Study requirements on cell viability and recovery are met. Upon completion of all study requirements, the MCC will issue certification numbers to Transplant Center staff. Instructions for completing the CBU Thawing Form can be found in Chapter 10 of the Transplant Center MOP.

9.2.5 Maintaining Certification

To maintain certification, staff members from both COBLT and non-COBLT Transplant Centers will be required to thaw and report data to the MCC on a minimum of one COBLT CBU every six months. This requirement may be met by performing a minimum of one transplant every six months. Centers can request practice CBUs from their designated COBLT CBB to maintain certification.

9.2.6 Requesting Cell Wash/Infusion Bag Sets

Thawing Coordinators should order Cell Wash/Infusion Bag Sets from the MCC using the COBLT Bag Order Form located in Chapter 10 of the Transplant Center Manual of Procedures (MOP). A log documenting the use of each bag set must be kept at each Transplant Center.
CBU REQUEST FOR THAWING CERTIFICATION
(Fax request to CBB Coordinator.)

<table>
<thead>
<tr>
<th>TO:</th>
<th>FROM:</th>
</tr>
</thead>
<tbody>
<tr>
<td>COBLT CBB</td>
<td>Center</td>
</tr>
<tr>
<td>Phone #</td>
<td>Phone #</td>
</tr>
<tr>
<td>Fax #</td>
<td>Fax #</td>
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<tr>
<td>Date:</td>
<td># of Pages:</td>
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</table>

Number of CBU(s) Requested: ______ For Receipt the Week of: _____________
(A maximum of 4 CBU(s) can be requested at one time.)

Name of Person Receiving CBU(s): _______________________________________
Shipping Address: _______________________________________________________
Contact Phone Number: _____________________________________________

CONFIRMATION
(Fax confirmation to the Transplant Center staff requesting the CBUs and to the Medical Coordinating Center (MCC), 301-299-3991 FAX, at the time of shipment.)

<table>
<thead>
<tr>
<th>TO:</th>
<th>FROM:</th>
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<tbody>
<tr>
<td>Center</td>
<td>COBLT CBB</td>
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<td>Phone #</td>
<td>Phone #</td>
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<tr>
<td>Fax #</td>
<td>Fax #</td>
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<tr>
<td>Date:</td>
<td># of Pages:</td>
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</table>

Date CBU(s) Shipped: ___/___/___

Shipped CBU(s) ISBT Number(s):
- W __________ __ __ ___________ 00 __
- W __________ __ __ ___________ 00 __
- W __________ __ __ ___________ 00 __
- W __________ __ __ ___________ 00 __

CBB Contact Name and Phone Number: ________________________________

Cord Blood Study MOP - 12/99  9-11
Complete information below for all CBUs included in the shipment to the Transplant Center.

<table>
<thead>
<tr>
<th>CBU ID#: W __ __ __ __    __ __    __ __ __ __ __ __   00  _</th>
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**Cryopreserved CBU Information**

<table>
<thead>
<tr>
<th>% Viability</th>
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</tr>
</thead>
<tbody>
<tr>
<td>% Mononuclear cells</td>
<td></td>
</tr>
<tr>
<td>Total viable nucleated cell count x 10^8</td>
<td></td>
</tr>
<tr>
<td>Total viable mononuclear cell count x 10^8</td>
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**Maternal Infectious Disease Results**

<table>
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<tbody>
<tr>
<td>Anti-HBc</td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td></td>
</tr>
<tr>
<td>Anti-HCV</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td></td>
</tr>
<tr>
<td>HIV-1/2</td>
<td></td>
</tr>
<tr>
<td>HIV p-24 Antigen</td>
<td></td>
</tr>
<tr>
<td>HTLV I/II</td>
<td></td>
</tr>
</tbody>
</table>

Comments: _____________________________________________________________________________

Shipped By: ______________________________

Shipped To: ______________________________ Date Sent: ______________________________
9.3 METHODS FOR BUSULFAN DOSE ADJUSTMENT

Nursing Instructions

1. Complete the enclosed pink charge slip form. Use an addressograph, if available, for patient’s name and ID code in upper left corner. Also include age, actual weight and weight used for busulfan dose, if different, and the actual amount of busulfan administered (mg q 6 hr). Write the actual draw times on this slip.

2. In the comment space, include disease and the number of busulfan doses planned per protocol.

3. In the result section, indicate the target CSS (ng/mL) given by the protocol.

4. Draw 1-3 mL of blood into green top tubes. Label with patient name, ID code, sample number, date, and actual time of draw.

5. Place labeled samples immediately on wet ice and refrigerate as soon as possible.

6. **Draw schedule for Test Dose and Dose 1:** (all items post-dose): (suspension only, 15 minutes) 30 minutes, 1, 1.5, 2, 3, 4, 5, and 6 hours.

7. **Draw schedule for Dose 5 and 9:** pre-dose, 1, 2, 4, and 6 hour post.

8. Send the pink charge slip to the send-out laboratory so it may accompany the samples to Seattle.

Sample Processing

1. Use a capped 3 mL plastic tube for the plasma.

2. Use appropriate labels for identification.

3. Spin samples as soon as possible at 4°C. Separate plasma from RBCs and freeze plasma immediately at -20°C.

4. Plasma samples are to be shipped on dry ice using an overnight carrier.

5. Send to:
   Linda Risler
   Fred Hutchinson Cancer Research Center
   1100 Fairview Avenue North, D2-245
   Seattle, WA 98109

6. Enclose a pink charge slip and any other pertinent information with the samples.
9.4 PRE- AND POST-TRANSPLANT SAMPLES

9.4.1 Blood Draws for Chimerism Studies

Principle

A peripheral blood sample is required for chimerism studies: pre-transplant and post-transplant between Days 28 and 42, Day 100, and 1 year.

Specimen

Draw 3 mL peripheral blood in a EDTA (purple top) at the specified time periods.

Procedure

Ficoll cells to isolate mononuclear cell fraction, wash and pellet mononuclear cell layer, transfer to 1 labeled nunc vial and store at -20 °C at the home institution.

Label the vial with the following identifying information: COBLT ID, COBLT name code, date drawn, and type of sample.

File laboratory worksheet in laboratory manual and patient file.

9.4.2 CBU Sample for Retrospective HLA Typing

Principle

All non-COBLT cord blood units must have samples made available for retrospective allele level DNA based typing by a COBLT reference HLA laboratory.

Specimen

Samples form all non-COBLT units must be obtained from the wash during the thawing process.

Procedure

Remove 50 mL of the (usually 2nd) supernatant and place in a 50 mL (Falcon) conical tube.
Spin at 3000 rpm for 5 minutes to pellet cells
Remove supernatant and discard
Resuspend cells in approximately 600 µL dextran albumin
Add approximately 200 µL into 3 microfuge tubes
Spin at 14,000 rpm for 4 minutes to pellet cells
Remove supernatant, using a pipette
Freeze in -20 C freezer
Mail on dry ice
**CORD BLOOD TRANSPLANTATION STUDY MOP**

Label the sample with the following identifying information: CBU ID, date obtained, and type of sample.

Complete Non-COBLT CBU Sample Shipping Notification form. Fax form to the designated COBLT HLA laboratory and the Medical Coordinating Center. The form must accompany the sample at the time of shipment.

### 9.4.3 Blood Draws for Retrospective HLA Typing

**Principle**

A pre-transplant peripheral blood sample is required for retrospective HLA typing for all recipients.

**Specimen**  
For patients with normal WBC, 7 mL peripheral blood (yellow top-ACD or purple top-EDTA). Note that in smaller patients, 2mL of peripheral blood is usually sufficient if acquisition of 7 mL is problematic.

For patients with low WBC, 20 mL peripheral blood (yellow top-ACD or purple top-EDTA) or a minimum of 5 mL peripheral blood PLUS 2 buccal swabs.

For heterozygous HLA, 4 buccal swabs should be obtained. Additional sample may be required if homozygosity must be confirmed.

Study-approved buccal swab kits must be used to collect samples. Kits can be obtained from the COBLT Medical Coordinating Center. All buccal swab samples should be sent to Dr. Baxter-Lowe’s lab.

**Procedure**

Label the sample with the following identifying information: COBLT ID, COBLT name code, date drawn, and type of sample.

Complete Recipient Sample Shipping Notification form. Instructions for shipping samples are provided on the form.

Fax form to the designated COBLT HLA laboratory and the Medical Coordinating Center. The form must accompany the sample at the time of shipment.