Newborn Genetic Screening

C. Cord Blood Processing, Storage and Shipment

I. Cord Blood Processing

A) Cord bloods need to be processed in a timely fashion. Samples can only be left for a maximum of two days before processing, with the exception of unresolved pendings which can be left for a maximum of two weeks. If the blood remains unprocessed for a longer amount of time, the sample becomes hemolyzed and does not retain its integrity.

B) If, due to illness or emergency, samples could not be processed by the responsible recruiter in a timely fashion, the recruiter should alert the other recruiters, the recruiter supervisor, and lab personnel so that the blood can be processed on time.

II. Lab Preparation

Before processing any sample, it is important to do the following to protect yourself and the samples:

A) Prepare a clean field to work on by wiping the counter with a clean Sani-cloth (germicidal disposable cloth).

B) Wash your hands and put on a fresh pair of gloves.

C) Take out clean racks for vacutainers and cryovials. Clean all dirty racks in 10% bleach/water solution.

NOTE: Everyone is responsible for cleaning up after themselves. We have to work together to maintain a clean working environment.

III. Precautions

There are a number of precautions to take when processing blood samples.

A) **DO NOT** uncap more than one person's sample at a time!!!!

B) Place a Label for each tube with the DAISY ID as each hospital ID is removed.

C) When placing a cryovial cap onto the counter, make sure it is open-side up!!!

D) Wipe your gloves clean with a Sani-cloth after working with each individual cord blood. Do not allow blood to collect on your gloves.

E) Clean the outside of bloody vacutainers before processing.

F) Uncap vacutainers away from yourself.

G) Maintain a consistent method of filling cryovials. Collect as much of the sample as possible; fill the vials between 0.5ml and 1.25ml marks. Try not to overfill the vials.

IV. Processing Blood Samples

A lavender-top vacutainer should be kept at room temperature until it can be processed.

A) Collect a whole blood sample for HLA typing at Roch Molecular Systems (these samples are shipped every two weeks).
   1) Place the label on a 2mL Sarstedt cryovial with the child's:
      DAISY ID#
      Initals
   2) Gently agitate the lavender-top vacutainer back and forth, about four times.
   3) Aliquot 0.5mL of whole blood from the lavender top tube into the 2mL Sarstedt vial.
   4) Store the sample in the cryobox labeled **ROCHE**, which is located in the laboratory refrigerator. Enter the DAISY ID and the child's initials into the computer in the electronic copy of the Roche Shipping List Excel file.
   5) High Priority samples should be sent in the next shipment batch-See Procedure for High Priority Roche Testing
   6) New Roche Box:
      a. a. To start a new shipping list for Roche in Excel, first open the L: drive in Excel→click on the Roche folder→open the generic Roche Shipping List Form document.
      b. b. Enter your information (DAISY ID's and initials) into this generic Roche Shipping List Form document as usual.
      c. c. Save the document under a new batch by going to File→Save As→click on the Roche Sets 200_ folder→type in the title of the new document accordingly (Batch??_??_??Box?)→click on the Save button.
      d. High Priority samples should be sent in the next shipment batch-See Procedure for High Priority Roche Testing
   7) Shipments to Roche are sent every two weeks in two-box batches.

B) Collect a whole blood sample for the DAISY Study.
   1) Aliquot 1.25mL of whole blood from lavender top tube into a 2mL Sarstedt cryovial labeled with the child's:

DAISY ID#	For example: 30000
Date	110695
61	61

   2) Store the sample in the appropriately labeled cryobox for whole blood located in the laboratory refrigerator.
   3) **If the mom does not agree to the storage of the cord blood or to DNA storage**, still aliquot this whole blood sample, but place it in the cryobox labeled "St. Joe's Whole

Blood-No Storage" in the lab refrigerator. This box is "temporary" storage (only for QC purposes) and its contents eventually get destroyed.

C) Balance the lavender top tubes in the centrifuge and spin them for 10 min. Start at a low speed, between 3-4, and gradually increase the speed until it ends up between 7 and 8. The centifuging process will separate the whole blood into three distinct layers. The top (clear yellow) layer is the plasma layer. The thin middle (white) layer is called the buffy-coat layer. The buffy coat layer is composed of white blood cells (WBCs). The bottom red layer is composed of red blood cells (RBCs).

D) Collect a plasma sample for antibody testing at the Barbara Davis Center.
1) Aliquot between 10 to 100uL (3-4 drops) into a 0.5mL Sarstedt conical vial labeled with the child's:
DAISY ID #
Date
CB
2) Store the sample in the appropriately labeled cryobox located in the –70C lab freezer.
3) **If mom consented to NO storage of blood DO not collect sample**

E) Collect a plasma sample for the DAISY Study.
1) Aliquot 1.0mL of plasma into a 2mL Sarstedt vial labeled wirh the child's:
DAISY ID# For example: 30000
Date 110695
21 21
2) **Without disturbing the buffy coat layer (!!!!!!),** discard the rest of the plasma.
3) Store the sample in the appropriately labeled cryobox in the -70C lab freezer.
4) **If mom consented to NO storage of blood DO not collect plasma or this sample.**

F) Collect the buffy coat sample for the DAISY Study.
1) It is easier to collect the buffy coat layer immediately after aliquoting the plasma layer. Using the same pipette from plasma aliquoting, pull up the buffy coat layer with some of red blood cells (this gives some volume to the buffy coat so it doesn't dry out in the freezer).
2) Aspirate the buffy coat sample into a 2mL Sarstedt vial labeled with the child's:
DAISY ID# For example: 30000
Date 110795
31 31
3) The vial should contain at least 0.5mL of sample, but it can be filled up to the 1.25mL mark.
4) Store the sample in the appropriately labeled cryobox located in the –70C lab freezer.
5) If the mom does not consent to storage of the cord blood or no to DNA storage, do not collect the buffy coat!!!!!! This is the DNA portion of the blood that would be used for future screening.

G) Collect the RBC sample for the DAISY Study.
1) Using the same pipette from plasma and buffy coat aliquoting, pull up the bottom red blood cell layer.

2) Aspirate this sample into a 2 mL Sarstedt vial labeled with the child's:
   DAISY ID# For example: 30000
   Date 110795
   RBC RBC
3) The vial should contain at least 0.25mL of sample.
4) Next, snap freeze the red blood cell samples for about 10 seconds by gently lowering them into the liquid nitrogen.
5) Remove the samples from the liquid nitrogen and store them in the appropriately labeled cryobox in the –70C lab freezer.
6) **If mom consented to no storage of blood Do not collect this sample**

V. Recording in the NEC Log Book

A) Once all the samples are collected, they need to be entered into NEC Log Book. Record the child's:
DAISY ID#
Mother's name (last name, first initial)
Initials
Date (consent date)
Number of aliquots of each sample
Cryobox numbers for those aliquots

For example:

	30007	**(BA)**
	Bond, J.	**CB-20**
	2 1	**2-11**
110795	**3 1**	**3-8**
	6 1 2	**6-5**
	R 1	**R-2-16**

This tells me that Bond, J. delivered at St. Joseph Hospital and agreed to participate in our study. Her newborn, with the initials (BA), was assigned DAISY number 30007. We have a cord blood (CB) sample for antibody testing in box CB-20, 1 vial of plasma stored in box 2-11, 1 vial of buffy coat stored in box 3-8, 2 vials of whole blood (one will go to Roche, the second vial will be stored) and is stored in box 6-5, and 1 vial of red blood cells stored in box 2 space 16.

B) If a sample was shipped to another laboratory, underline the sample in the log book with the appropriate color pen: Roche Molecular Systems = red.

C) For moms who do not agree to the **DNA storage** of their child's cord blood, the NEC Log Book would look like this:

	30007	**(BA)**
	Bond, J.	**CB-20**
No DNA storage!	**2 1**	**2-11**

110795	**3**	**0**	**3-X**
	6	**1**	**6-X**
	R	**1**	**R-2-16**