 <b>CLSA ÉLCV</b> Canadian Longitudinal Study on Aging Étude longitudinale canadienne sur le vieillissement	<b>Title:</b>	<b>Processing of the CPT Tube</b>		
	<b>Version Date:</b>	2015-APR-13	<b>Document Number:</b>	SOP_BCP_0004
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<b>Data Collection Site (DCS)</b>	<b>Version:</b>	4.1	<b>Number of Pages:</b>	10

### 1.0 Purpose:

The purpose of this document is to describe the procedure for processing the CPT tube as well as the isolation of mononuclear cells from human peripheral blood and cryopreservation for long term storage.

### 2.0 Scope:

This document is to be used by all DCS laboratory staff when processing blood samples collected in the CPT tube.

### 3.0 Responsibilities:

It is the responsibility of the CLSA laboratory staff to perform procedures as described in the current and approved version of the standard operating procedure.

### 4.0 Related Documents:

- **CHA\_BCP\_0003\_2** - Matrix Storage Box Chart – Mapping of Biospecimen Aliquot;
- **SOP\_BCP\_0040** - Operation and Maintenance – Biocision CoolCell;
- **SOP\_BCP\_0037** - Operation and Maintenance – Eppendorf 5702 Centrifuge;
- **SOP\_BCP\_0003** - Biospecimen Post Collection Processing;
- **SOP\_BCP\_0033** - Pipette, Eppendorf Research plus adjustable 100-1000 µL;
- **SOP\_BCP\_0030** - 2D barcode box scanner, Thermo Fischer Scientific Visionmate;
- **SOP\_BCP\_0035** - Individual tube scanner, Thermo Fischer Scientific Visionmate One;
- **SOP\_BCP\_0031** - 8 Channel Decapper, Thermo Fischer Scientific;
- **SOP\_BCP\_0044** - Brady Code Reader 3500 – Barcode Scanner;
- **SOP\_BCP\_0406** - Site Specific Waste Disposal SOP; and,
- **MAN\_BCP\_0217** - LabWare Guide

### 5.0 Definitions:

- **Peripheral Blood Mononuclear Cells (PBMC):** Any blood cell having a round nucleus. For example, a lymphocyte, monocyte or macrophage.
- **Vacutainer Cell Preparation Tube (CPT):** an evacuated tube with sodium citrate intended for the collection of whole blood and the separation of mononuclear cells.

### 6.0 Equipment:

- Brady Code Reader 3500 – Barcode Scanner;

- Centrifuge, Eppendorf 5702R with rotor A-8-17;
- Freezer, Isotemp Ultra Low Temperature Undercounter;
- Pipette, Eppendorf Model Research plus adjustable 100-1000  $\mu$ L;
- Pipetting Station, Argos Flexirack;
- Refrigerator, AGA Marvel Model 6CADM;
- 2D barcode box scanner, Thermo Fischer Scientific Visionmate;
- Individual tube scanner, Thermo Fischer Scientific Visionmate One; and,
- 8 Channel Decapper, Thermo Fischer Scientific.

## 7.0 Supplies:

- 15 mL Conical tubes, sterile;
- Non-latex gloves, disposable;
- Liquid waste container;
- 2D coded Matrix Screw Top storage tubes (0.5 mL, V-bottom);
- Transfer Pipettes;
- 1x Phosphate Buffered Saline (PBS);
- Sharps disposal container;
- Small absorbent gauze pad;
- CPT Vacutainer, filled; and,
- 16 mm plastic SAFETY-T-FLEX CAPS.

## 8.0 Procedure Steps:

### Overview

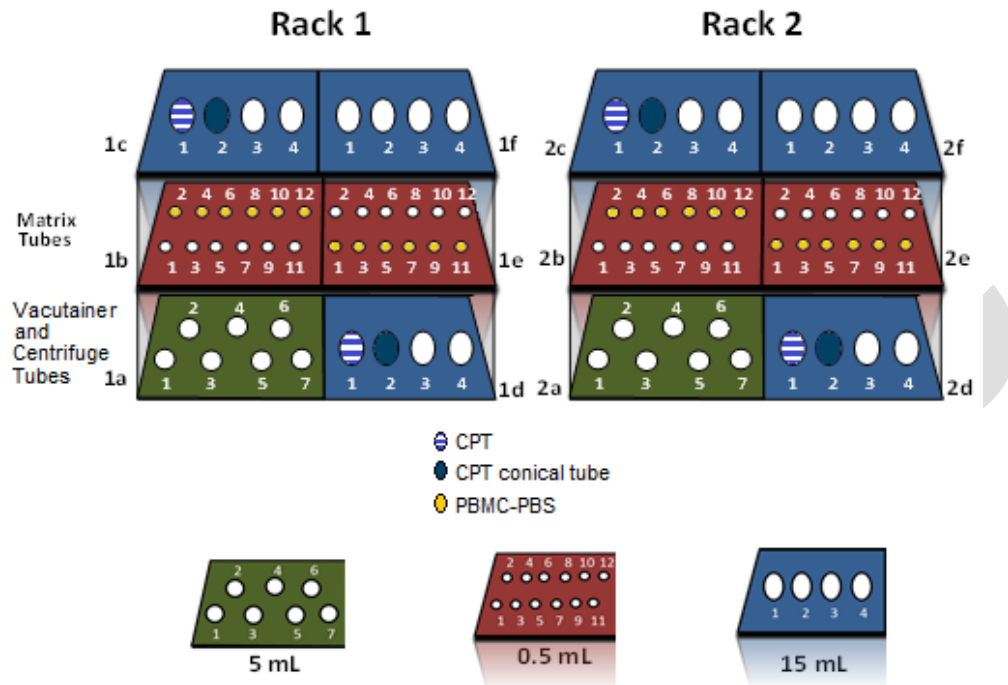
This protocol involves separating blood into different layers and extracting the PBMC layer to be washed and stored. Be cognizant when separating layers, where one ends and the other begins so as not to disturb the cell pellets or layers when aspirating supernatant. CPT tubes must be processed and frozen within 6 hours.

### 8.1 Preparation

- 8.1.1** Keep the CPT tube at room temperature at all times throughout the procedure. The media within the CPT tube (Ficoll) only works to separate PBMC at room temperature.
- 8.1.2** Place the A-8-17 rotor in the centrifuge when ready to process the CPT tube(s).

- 8.1.3 Set-up FlexiRack Pipetting Station. Use the appropriate inserts and empty matrix tubes. Refer to [Figure 1](#).

Figure 1. Set-up of the FlexiRack Pipetting Station for Processing of the CPT Tube.



## 8.2 First Centrifugation

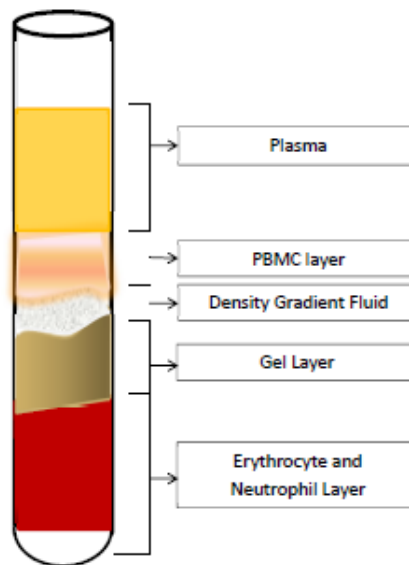
- 8.2.1 Refer to *SOP\_BCP\_0037 - Operation and Maintenance – Eppendorf 5702R Centrifuge* for instructions on using the centrifuge.
- 8.2.2 Remove the stopper from a CPT tube using a small absorbent gauze pad and replace it with 16 mm plastic SAFETY-T-FLEX CAPS.
- 8.2.3 Bring the CPT tubes from the FlexiRack Pipetting Station and gently invert each tube 8-10 times by hand.  
**NOTE:** Prior to centrifugation, the whole blood sample rests above the polyester gel layer.
- 8.2.4 In LabWare, in **Sample Processing**, under **Centrifuge Single Sample**, press the **Start** button.
- 8.2.5 Scan the linear barcode label on the CPT tube. Ensure the correct date and time are entered in the pop-up window.
- 8.2.6 Balance each CPT tube in the A-8-17 rotor. Do not load the CPT tubes directly opposite each other. Be sure that the tubes do not touch one another or the sides of the bucket and break when the rotor is swinging.
- 8.2.7 Close the centrifuge lid.
- 8.2.8 Centrifuge at 1.5 rcf (1500 g) for 20 minutes at 22°C with the SOFT brake turned **ON**.

- 8.2.9 Open the centrifuge lid five minutes after the centrifuge has stopped, in case there has been a breakage.

**NOTE:** If you have heard a breakage during centrifugation, wait half an hour before opening the centrifuge.

- 8.2.10 Remove the CPT tubes without disturbing the layers above the gel barrier.
- 8.2.11 Confirm that the layers in the CPT tubes look as they should. Refer to [Figure 2](#).
- 8.2.12 In LabWare, in **Sample Processing**, under **Centrifuge Single Sample**, press the **Stop** button and scan the linear barcode label on the CPT tube. Ensure the correct date and time are entered in the pop-up window.

Figure 2. CPT Vacutainer Post First Centrifugation



- 8.2.13 Place in the FlexiRack Pipetting Station. Refer to [Figure 3](#).

### 8.3 First Cell Wash REMINDER

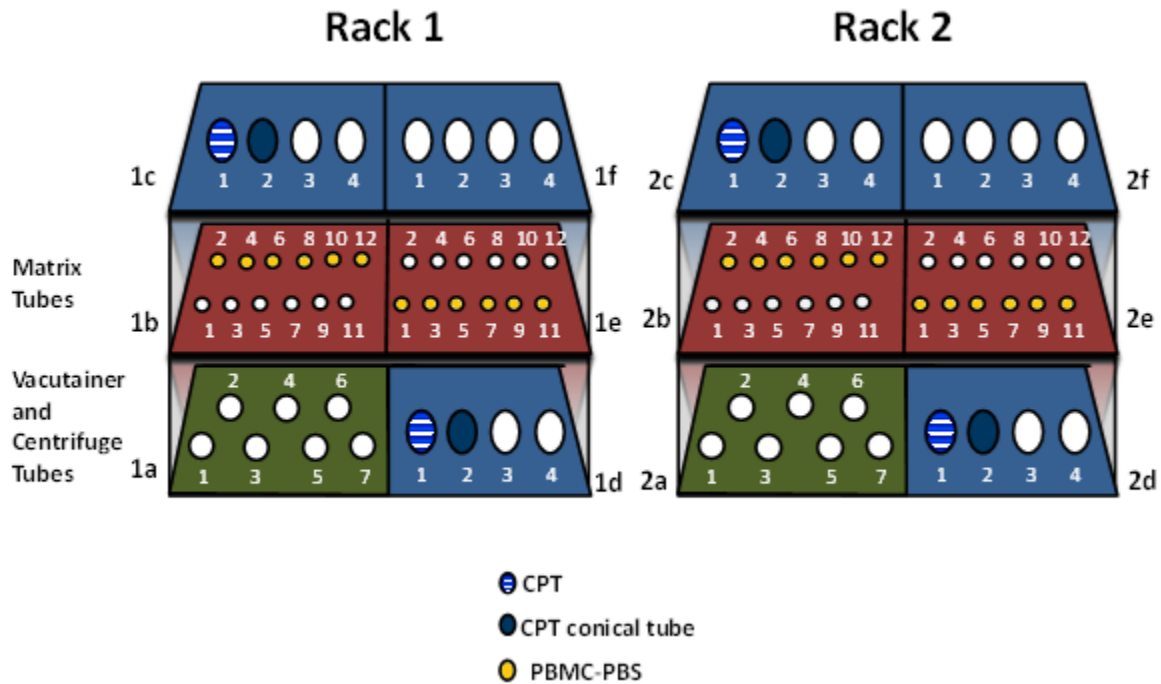
Label one sterile 15 mL conical tube for each CPT tube.

**NOTE:** It is important to label the CPT tube and the 15 mL conical tube with sequential numbers from one column of labels for a single participant (eg. 70029 and 70030). Refer to *SOP\_BCP\_0003 – Biospecimen Collecting & Processing*.

- 8.3.1 Remove the 16 mm plastic SAFETY-T-FLEX CAP from the CPT tube.
- 8.3.2 Use a new transfer pipette and slowly aspirate the plasma layer to within 1-2 mm of the PBMC layer. Starting at the top of the plasma, aspirate this layer at a constant rate, slowly following the liquid down the side of the tube. This will minimize movement and help to avoid disturbing the PBMC cell layer which may at this point resemble a 'cloud' rather than a distinct layer.
- 8.3.3 Discard the plasma in the liquid waste container.

Baseline

Figure 3. Layout of the FlexiRack Pipetting Station for Processing of the CPT Tube



**8.3.4** Aspirate the remaining phases above the gel barrier with care, which includes the residual plasma layer, lymphocyte and monocyte layer and density gradient fluid into the corresponding sterile 15 mL conical tube. Do not aspirate any pieces of the gel layer; this is more likely to occur if the tube has been agitated.

**8.3.5** Repeat procedure steps 8.3.2 to 8.3.5 for each CPT tube.

**8.3.6** If particles from the gel barrier were dislodged during the aspiration procedure in step 8.3.5 remove them now using a new transfer pipette.

**8.3.7** Pour PBS into each labeled sterile 15 mL conical tube up to the 14 mL mark.

**NOTE:** Don't pour PBS directly from the stock bottle. Instead, pour PBS into a sterile urine container, correctly labeled, that you will **dispose of at the end of the day**.

**8.3.8** Transfer PBS from the urine container to the 15 mL conical tube.

**8.3.9** Cap each conical tube and gently invert once by hand to mix. This inversion is to mix the PBS as the cells do not need any agitation for washing.

#### 8.4 Second Centrifugation

**8.4.1** In LabWare, in **Sample Processing**, under **Centrifuge Single Sample** press **Start** and scan the linear barcode label on the 15 mL conical tube. Ensure the correct date and time are entered in the pop-up window. Repeat for each 15 mL conical tube.

**8.4.2** Place in the A-8-17 rotor in the centrifuge and balance..

**8.4.3** Close the centrifuge lid.

- 8.4.4** Centrifuge at 1.5 rcf (1500 g) for 10 minutes at 22°C with the SOFT brake turned **OFF**.
- 8.4.5** Open the centrifuge lid once the centrifuge has stopped. Remove the conical tubes. An opaque white cell pellet should be visible on the bottom of the conical tube.
- 8.4.6** In LabWare, in **Sample Processing**, under **Centrifuge Single Sample**, press **Stop** and scan the linear barcode label on the 15 mL conical tube. Ensure the correct date and time are entered in the pop-up window. Repeat for each 15mL conical tube.
- 8.4.7** Place the conical tube in the FlexiRack Pipetting Station. Refer to [Figure 3](#).
- 8.5 Second Cell Wash**
- 8.5.1** Uncap each conical tube.
- 8.5.2** Use a new transfer pipette and aspirate the supernatant of a conical tube down to within 1-2 mL of the pellet (the cone portion of the conical tube), slowly following the fluid volume down the side of the tube at a constant rate.
- 8.5.3** Discard supernatant into the liquid waste container.
- 8.5.4** Gently re-suspend the cells in the remaining supernatant by slowly aspirating and dispensing the remaining liquid at least 5 times to lift and break up the pellet.
- 8.5.5** Repeat procedure steps 8.5.2 to 8.5.4 for each conical tube.
- 8.5.6** Pour PBS from the urine container labeled as PBS into each labeled 15 mL conical tube up to the 14 mL mark.
- 8.5.7** Cap each conical tube and gently invert once by hand to mix.
- 8.5.8** In LabWare, in **Sample Processing**, under **Centrifuge Second Spin**, press **Start** and scan the linear barcode label on the 15 mL conical tube. Ensure the correct date and time are entered in the pop-up window. Repeat for each 15 mL conical tube.
- 8.5.9** Place in the A-8-17 rotor in the centrifuge and balance.
- 8.5.10** Close the centrifuge lid.
- 8.5.11** Centrifuge at 1.5 rcf (1500 g) for 10 minutes at 22°C with the soft brake turned off.
- 8.5.12** Open the centrifuge lid once the centrifuge has stopped.
- 8.5.13** Remove the conical tubes. An opaque white cell pellet should be visible on the bottom of the conical tube.
- 8.5.14** Observe the pellet. In LabWare, in **Sample Processing**, click on **Sample Characteristics**. Fill in the fields required as described in *MAN\_DCS\_0217 – LabWare Guide*.

**8.5.15** In LabWare, in **Sample Processing**, under **Centrifuge Second Spin**, press **Stop** and scan the linear barcode on the 15 mL conical tube \. Ensure the correct date and time are entered in the pop-up window. Repeat for each 15 mL conical tube.

## **8.6 Aliquotting and Freezing PBMC**

**8.6.1** Uncap each 15mL conical tube.

**8.6.2** Use a new transfer pipette to aspirate the supernatant of a 15 mL conical tube down to within 1 mL of the pellet (the cone portion of the conical tube).

**8.6.3** Discard supernatant into the liquid waste container.

**8.6.4** Using the 1 mL pipette carefully remove the remaining supernatant from one conical tube. All supernatant has to be removed before adding 610 µL of PBS in order to get maximum cell concentration.

**8.6.5** With a new pipette tip, add 610 µL of PBS and gently re-suspend. This is the PBMC cell suspension.

**NOTE:** Pipetting up and down is necessary to break up the pellet but if the cells are re-suspended with too much force then cell damage will occur.

**8.6.6** Repeat procedure steps 8.6.4 to 8.6.5 for each 15 mL conical tube.

**8.6.7** Uncap the matrix tubes and place the screw tops upside down on a clean surface to avoid contamination.

**8.6.8** Using a new pipette tip, add 400 µL of PBS to the 6 coded Matrix Screw Top storage tubes for the first participant.

**8.6.9** Pipette 100 µL of the PBMC cell suspension and transfer into the 6 coded Matrix Screw Top storage tubes. Repeat using a new pipette tip for each of the 6 matrix tubes uncapped in 8.6.8.

**8.6.10** In LabWare, in the **Aliquotting** stage, click on **0.5 mL Matrix Tube** and scan the 15 mL conical tube barcode label. Then, scan the 6 CPT PBS matrix tube aliquots with the individual tube scanner.

**8.6.11** Obtain a permanent Matrix storage box for the study participant. This may be obtained from storage or the freezer depending if this is the first, second, or third participant for the box.

**8.6.12** Transfer all of the filled matrix tubes from the FlexiRack Pipetting Station.

**8.6.13** Return any unused matrix tubes to bulk storage and record the aliquot as insufficient quantity in LabWare. *Refer to MAN\_BCP\_0217 – LabWare Guide.*

**8.6.14** Cover the storage box with the lid and lock the matrix box.

**8.6.15** In LabWare, in **Sample Aliquotting**, click on **Freeze Samples**. Scan the linear barcode label of the 15 mL conical tube. Select the CPT-PBS aliquots and check that the number of aliquots is correct.



- 8.6.16 Once you have selected all the samples you are about to freeze, press OK.
- 8.6.17 If there is any discrepancy between the numbers of aliquots you are going to store and number of aliquots listed in the dialog or if you mistakenly mark samples with the wrong time, then please contact [lims@clsa-elcv.ca](mailto:lims@clsa-elcv.ca) and create a Weblssue immediately.
- 8.6.18 In LabWare, in **Sample Aliquotting**, click on **Scan Matrix Box** and scan the Matrix Box linear barcode. Copy the contents of the Matrix Box data and paste the data into the pop-up window.
- 8.6.19 Place the Matrix Box in the freezer.

**Very Important:** Before finishing the participant, open the **Sample Folder** and ensure that beakers are full for all processed samples. In the event that the beaker is not full, complete the required steps outlined in the *MAN\_BCP\_0217 – LabWare Guide*. If the issue cannot be resolved, file a Weblssue at <https://clsacloud.clsa-elcv.ca/webissues/client>.

- 8.6.20 Verify that deviations in aliquot volumes, hemolysis, lipemia or other specimen comments have been logged in *LabWare*. Refer to *MAN\_BCP\_0217 – LabWare Guide*.

**9.0 Documentation and Forms:**

- CRF\_BCP\_0001 – Blood Collection Case Report Form
- LabWare

**10.0 References:**

- BD Vacutainer CPT Cell Preparation Tube with Sodium Citrate Product Insert

**Baseline Revision History:**

New Version #	Revision Date	Revision Author	Content Approval
4.1	2015-APR-13	Chetna Naik	Cynthia Balion
<b>Summary of Revisions</b>			
Correction made to <b>Step 8.6.9:</b> Pipette 100 µL of the PBMC cell suspension and transfer into the 15 ml conical tube <b>6 coded Matrix Screw Top storage tubes</b> . Repeat using a new pipette tip for each of the 6 matrix tubes uncapped in 8.6.8.			
New Version #	Revision Date	Revision Author	Content Approval
4.0	2015-JAN-21	Chetna Naik	Cynthia Balion
<b>Summary of Revisions</b>			
SOP formatting updated.			
Centrifuge speeds provided in relative centrifugal force (rcf).			
<b>8.0:</b> added: CPT tubes must be processed and frozen within 6 hours.			
<b>Figure 1:</b> image edited.			
<b>8.2.9: edited:</b> Open the centrifuge lid <b>five minutes after</b> the centrifuge has stopped, <b>in case there has been a breakage</b> .			
<b>Note added:</b> If you have heard a breakage during centrifugation, wait half an hour before opening the centrifuge.			
<b>Former step 8.3.1</b> now a <b>reminder</b> . Renumbered following steps.			
<b>8.3.6-8.3.7:</b> “Pour PBS...14mL mark” sentence moved from 8.3.6 to 8.3.7.			
<b>8.3.8: Note added:</b> Don’t pour PBS directly from the stock bottle. Instead, pour PBS into a sterile urine container, correctly labeled, <b>that you will dispose of at the end of the day</b> .			
<b>8.3.8 added:</b> Transfer PBS from the urine container to the 15 mL conical tube.			
<b>8.4.1, 8.4.6, 8.5.8, 8.5.15</b> sentence added: Repeat for each conical tube.			

<b>8.4.2 and 8.5.9:</b> Removed awkward sentencng explaining balancing the tubes.			
<b>8.5.6:</b> Removed sentence: <del>If particles from the gel barrier were dislodged during the aspiration procedure in Step 8.3.6 remove them now using a new transfer pipette.</del>			
<b>8.5.7 added:</b> Cap each conical tube and gently invert once by hand to mix.			
<b>8.5.14 added:</b> Observe the pellet. In LabWare, in <b>Sample Processing</b> , click on <b>Sample Characteristics</b> . Fill in the fields required as described in <i>MAN_DCS_0217 – LabWare Guide</i> .			
<b>8.6.5:</b> wording clarified.			
<b>8.6.9 clarified:</b> Pipette 100 µL of the PBMC cell suspension and transfer into the 15 mL conical tube. Repeat using a new pipette tip for each of the 6 matrix tubes uncapped in 8.6.8.			
<b>8.6.15: step clarified:</b> In LabWare, in Sample Aliquotting, click on Freeze Samples. <b>Scan the linear barcode label of the 15 mL conical tube.</b> <del>enter the interview ID.</del> <b>Select the CPT-PBS aliquots and</b> LabWare will list the sets of aliquots that are ready for freezing check that the number of aliquots <b>is correct for each sample and select all if there is no discrepancy.</b>			
<b>8.6.16:</b> removed sentence: <del>Ensure the correct date and time are entered in the pop up window.</del>			
<b>8.6.17:</b> Instructions on adding a WebIssue added.			
<b>8.6.19: Very Important note added:</b> Before finishing the participant, open the Sample Folder and ensure the beakers are full for all processed samples. In the event that the beaker is not full, complete the required steps outlined in the <i>MAN_BCP_0217 – LabWare Guide</i> . If the issue cannot be resolved, file a WebIssue.			
<b>8.6.21: step deleted:</b> <del>Place the Matrix Box in the Isotemp Ultra Low Temperature Undercounter Freezer.</del>			
New Version #	Revision Date	Revision Author	Content Approval
4.0	2015-JAN-21	Chetna Naik	Cynthia Balion
<b>Summary of Revisions</b>			
SOP formatting updated.			
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<b>Note added:</b> If you have heard a breakage during centrifugation, wait half an hour before opening the centrifuge.			
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<b>8.4.1, 8.4.6, 8.5.8, 8.5.15</b> sentence added: Repeat for each conical tube.			
<b>8.4.2 and 8.5.9:</b> Removed awkward sentencng explaining balancing the tubes.			
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<b>8.6.5:</b> wording clarified.			
<b>8.6.9 clarified:</b> Pipette 100 µL of the PBMC cell suspension and transfer into the 15 mL conical tube. Repeat using a new pipette tip for each of the 6 matrix tubes uncapped in 8.6.8.			
<b>8.6.15: step clarified:</b> In LabWare, in Sample Aliquotting, click on Freeze Samples. <b>Scan the linear barcode label of the 15 mL conical tube.</b> <del>enter the interview ID.</del> <b>Select the CPT-PBS aliquots and</b> LabWare will list the sets of aliquots that are ready for freezing check that			

the number of aliquots <b>is correct</b> for each sample and select all if there is no discrepancy.			
<b>8.6.16:</b> removed sentence: Ensure the correct date and time are entered in the pop up window.			
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<b>8.6.19: Very Important note added:</b> Before finishing the participant, open the Sample Folder and ensure the beakers are full for all processed samples. In the event that the beaker is not full, complete the required steps outlined in the <i>MAN_BCP_0217 – LabWare Guide</i> . If the issue cannot be resolved, file a WebIssue.			
<b>8.6.21: step deleted:</b> Place the Matrix Box in the Isotemp Ultra Low Temperature Undercounter Freezer.			
New Version #	Revision Date	Revision Author	Content Approval
3.0	2013-JUN-12	Chetna Naik	Cynthia Balion
<b>Summary of Revisions</b>			
Section 4.0 – Added related documents			
Section 7.0- Added plastic Safety-T-Flex Caps to supplies			
8.1.3 – Removed the labeling information to Venipuncture SOP			
8.2.3 – Added info to replace the Vacutainer stopper with plastic Cap.			
LabWare information added to entire document			
8.5.6 – Added details for collecting cells with proper procedure to get maximum concentration of cells			
8.5.8 to 8.5.12 – details for loading the A-8-17 rotor added			
Section 8.6 updated with information for freezing samples and capturing information in LabWare			
New Version #	Revision Date	Revision Author	Content Approval
2.0	2012-SEP-27	Lori Dewar	Cynthia Balion
<b>Summary of Revisions</b>			
Added document numbers throughout document			
Updated supplies in section 4.0 and 7.0			
Added step 8.2.3, 8.5.7, 8.5.9, 8.6.8			
Clarified wording in step 8.2.4, 8.2.11, 8.4.1, 8.4.7, 8.6.7			
Added note under step 8.3.1			