	<b>Title:</b>	<b>Biospecimen Post Collection Processing</b>		
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<b>Data Collection Site (DCS)</b>	<b>Version:</b>	4.0	<b>Number of Pages:</b>	18

### 1.0 Purpose:

The purpose of this document is to describe the standardized procedure for processing and aliquotting blood and urine samples collected at the DCS visit into the Matrix tubes and GenPlates.

### 2.0 Scope:

This document is to be used by all DCS laboratory staff when processing and aliquotting blood and urine samples.

### 3.0 Responsibilities:

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

### 4.0 Related Documents:

- **MAN\_BCP\_0217** - Labware Guide
- **CHA\_BCP\_0003\_1** - CLSA Biospecimen Collection and Processing at a Glance
- **CHA\_BCP\_0003\_2** - Matrix Storage Box Chart – Mapping of Biospecimen Aliquots
- **SOP\_BCP\_0001** - Collection of Blood by Venipuncture SOP
- **SOP\_BCP\_0002** - Collection of Random Urine Specimens SOP
- **SOP\_BCP\_0005** - Processing of the CPT tube SOP
- **SOP\_BCP\_0014** - Coulter A<sup>c</sup>•T diff – Analyzing Whole Blood Samples SOP
- **SOP\_BCP\_0015** - Coulter A<sup>c</sup>•T diff – Analysis Evaluation SOP
- **SOP\_BCP\_0037** - Operation and Maintenance – Eppendorf 5702R Centrifuge SOP
- **SOP\_BCP\_0040** - Operation and Maintenance – BioCision CoolCell SOP
- **SOP\_BCP\_0080** - Packing and Shipping CRX Vapor Shipper from Site to BBC SOP
- **SOP\_BCP\_0406** – Site Specific - Waste Disposal SOP
- Beckman Coulter Data Transfer (Located on CLOUD website)

### 5.0 Definitions:

- **Acid Citrate Dextrose (ACD):** used as an anticoagulant in the yellow stoppered BD tube.
- **Buffy Coat (BC):** a layer of nucleated cells and platelets formed above the red cells during settling or centrifugation of anticoagulated whole blood.
- **Cell Preparation Tube (CPT):** a blue and black stoppered BD tube containing sodium citrate anticoagulant and Ficoll density gradient medium.

- **Ethylenediaminetetraacetic acid (EDTA):** used as an anticoagulant in the lavender stoppered BD tube.
- **Platelet poor plasma (PPP):** plasma with a platelet count of  $<10 \times 10^9$ .
- **Venipuncture:** the puncture of a vein for collecting blood specimens.

## 6.0 Equipment:

- BioCision CoolCell, 12 chambers;
- Centrifuge, Eppendorf 5702R with rotor A4-38;
- Fast Dryer, GenVault GVG6000;
- Freezer, Isotemp Ultra Low Temperature Undercounter;
- Pipetter, Eppendorf Model Research plus adjustable 100-1000  $\mu\text{L}$ ;
- Pipetter, Eppendorf Model Research plus multi 12-channel 0.5-10  $\mu\text{L}$ ;
- Pipetting Station, Argos Flexirack;
- Refrigerator, AGA Marvel Model 6CADM;
- Rotator, Labquake;
- 2D barcode box scanner, Thermo Fischer Scientific Visionmate;
- Individual tube scanner, Thermo Fischer Scientific Visionmate One;
- 8 Channel Decapper, Thermo Fischer Scientific; and,
- Brady Code Reader 3500 - Barcode Scanner.

## 7.0 Supplies:

- Specimen barcode labels;
- DMSO, 20% diluted with RPMI 1640 growth medium;
- 5 mL polypropylene round-bottom tubes with caps;
- Absorbent gauze pad;
- GenPlates and covers;
- Gloves, non-latex disposable;
- Lab coat;
- Pipette tips, disposable;
- 25 mL multichannel reagent reservoir (Pipette trough);
- Transfer pipettes;
- Sharps disposal container;
- Matrix storage box;
- 2D coded Matrix Screw Top storage tubes (0.5 mL, V-bottom);
- 15 mL conical tube; and,
- Resealable zipper-locked bag.

## 8.0 Procedure Steps:

### 8.1 Gather and Organize Supplies

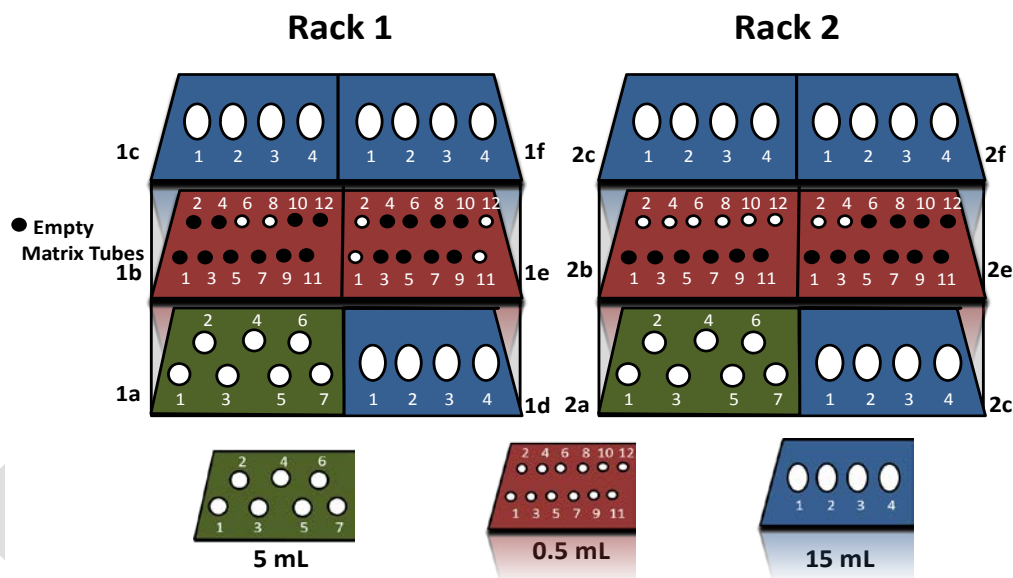
8.1.1 Gather the following items:

- 1 x column of specimen barcode labels
- 2 x 5 mL polypropylene round bottom tubes with caps
- 42 x 2D coded Matrix Screw Top storage tubes
- 1 x pipette trough, 1 mL multichannel
- Transfer pipettes
- 1 x GenPlate with plastic cover and foil bottom
- 1 x 15 mL conical tube

8.1.2 Set-up FlexiRack pipetting station. Use the appropriate inserts and 34 empty matrix tubes. Refer to [Figure 1](#).

8.1.3 Set-up the BioCision CoolCell. Refer to *SOP\_BCP\_0040 - Operation and Maintenance – BioCision CoolCell*.

**Figure 1.** Set-up of the FlexiRack Pipetting Station for Biospecimen Post Collection Processing.



### 8.2 General Instructions

8.2.1 When filling tubes hold pipette close to the bottom and draw it up as it fills to avoid splatter.

8.2.2 Use a small absorbent gauze pad to contain aerosols when removing caps/stoppers from all tubes.

8.2.3 Manually remove caps from 2D coded Matrix tubes as you require them.

8.2.4 Place caps removed on a clean surface, open end facing up. Be cautious not to contaminate them.




- 8.2.5 To prevent leakage and prevent desiccation of samples in the freezer, use the Thermo Scientific 8 Channel Capper-Decapper to tighten all of the caps on the 2D coded Matrix tubes.
- 8.2.6 Use a new pipette tip and transfer pipette for each sample type.
- 8.2.7 Ensure that the correct date and times are entered in the pop-up windows in LabWare.
- 8.2.8 All samples, except the CPT tubes, must be processed within 2 hours of collection.

**IMPORTANT:** Verify that deviations in aliquot volumes, hemolysis, lipemia or other specimen comments have been logged in LabWare (see *MAN\_BCP\_0217 – Labware Guide*).

### 8.3 Pre-Centrifugation

- 8.3.1 Review *CHA\_BCP\_0003\_1 CLSA - Biospecimen Collection and Processing at a Glance*.
- 8.3.2 Gather the filled tubes and urine container brought to the specimen processing bench post-venipuncture. Refer to *SOP\_BCP\_0001 - Collection of Blood by Venipuncture SOP* and *SOP\_BCP\_0002 - Collection of Random Urine Specimens SOP*.

**NOTE:** All samples must be labeled in LabWare for a current participant before starting the next step.

- 8.3.3 In LabWare, under the sample processing stage, select **Start** under **Tube Shaker** and scan the linear barcode for each of the following tubes:
  - x3 mL EDTA (lavender cap) 
  - 1 x 6 mL EDTA (lavender cap) 
  - x3 mL ACD (yellow cap) 

Ensure the correct date and time are entered in the pop-up window.

- 8.3.4 Place the tubes from step 8.3.3 on the rotator.
- 8.3.5 Place the remaining tubes in the back row of the FlexiRack Pipetting Station. It is recommended practice for the collection tube to be maintained in a vertical position when processing specimens for biobanking.

The CPT tubes will need to be batch processed within 6 hours of collection. Refer to *SOP\_BCP\_0004- Processing of the CPT Tube*.

The ACD tube may be batch processed within 1.5 hours of collection. Refer to section **8.14 Processing the ACD Tube** for further instructions.

### 8.4 Whole Blood Aliquots and Whole Blood to GenPlate *Whole Blood Aliquots*

- 8.4.1 Remove caps from the 2D coded Matrix tubes in rack 1, section 1b, positions 9 and 11. Refer to [Figure 3](#) and [Table 1](#).
- 8.4.2 The 6mL EDTA tube must stay on the rotator for at least 3 minutes. Then, take it and the 6 mL EDTA tube from the rotator and place it in rack 1, section 1a, position 7 of the FlexiRack Pipetting Station.

- 8.4.3 In LabWare, under the **Sample Processing** tab, click **Stop** under the **Tube Shaker**. Scan the linear barcode of the 6 mL EDTA tube into LabWare. Ensure the correct date and time are entered in the pop-up window.

**NOTE:** Use ONE of the THREE 6 mL EDTA tubes for the two whole blood aliquots.

- 8.4.4 Remove the stopper from the 6 mL EDTA tube.
- 8.4.5 Pipette 500 µL of blood into the uncapped 2D coded Matrix tubes, in Rack 1, section 1b, positions 9 and 11.
- 8.4.6 Place the lids back on the 2D coded Matrix Tubes.
- 8.4.7 In LabWare, under **Sample Aliquotting**, click on **0.5 mL Matrix Tube**.
- 8.4.8 Scan the linear barcode on the 6 mL EDTA tube. Then, as prompted, scan the 2D coded matrix tubes using the single tube 2D barcode scanner.

***Whole Blood to GenPlate***

- 8.4.9 Pipette approximately 1.5 mL of blood from the 6 mL EDTA tube into the multichannel reagent reservoir using a transfer pipette.

**NOTE:** Do not use Genplate that is missing sliver foil. Ship the damaged Genplate to the BBC.

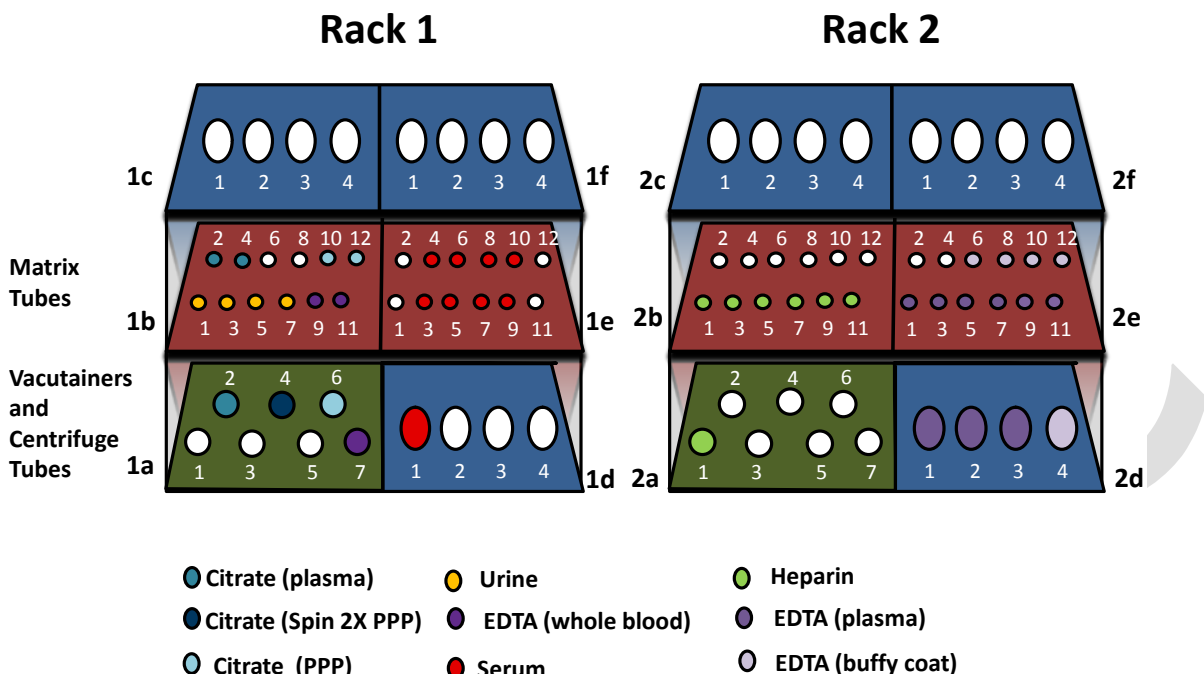
- 8.4.10 Replace the stopper.
- 8.4.11 In LabWare, under the **Sample Aliquotting** tab, click on **GenPlate**.
- 8.4.12 Scan the linear barcode of the 6 mL EDTA tube. Ensure the correct date and time are entered in the pop-up window.
- 8.4.13 Scan the linear barcode of the GenPlate.
- 8.4.14 In LabWare, select the horizontal section to be filled (i.e., a1-d24 or g1-j24 or m1-p24) from the pop-up window. Reference **Figure 2** for GenPlate layout.

**Figure 2.** GenPlate Layout for Whole Blood Aliquots from 3 Different Participants

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
a	Participant 1																							
b																								
c																								
d																								
e	Participant 2																							
f																								
g																								
h																								
i	Participant 3																							
j																								
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- 8.4.15 Use the 12-channel pipette to aspirate 10  $\mu$ L of blood and dispense into alternating empty wells of the GenPlate (one horizontal section per participant). Repeat to fill all 96 wells. See [Figure 2](#) for GenPlate layout.  
**NOTE:** This can be completed during the multiple samples centrifugation.
- 8.4.16 Place the GenPlate inside a clean resealable zipperlocked bag for temporary storage until all 3 horizontal sections of the plate are filled.
- 8.4.17 When all sections are filled, in LabWare, under **Sample Aliquotting**, click on **Start Drying**.
- 8.4.18 Scan the linear barcode of the GenPlate. Ensure the correct date and time are entered in the pop-up window.
- 8.4.19 Place the full GenPlate in the GenVault dryer and turn on the dryer.
- 8.4.20 After the Genplate has dried for a **minimum of 16 hours**, remove the GenPlate from the GenVault dryer. In LabWare, under **Sample Aliquotting**, click **Stop Drying**.
- 8.4.21 Scan the linear barcode of the GenPlate.
- 8.4.22 Seal the GenPlate with the adhesive film provided. Be careful to place the adhesive film on the plate, avoiding creases and without missing any sections.
- 8.4.23 Store the complete GenPlate at room temperature in the provided shipping bag containing the desiccant until ready to be shipped to the BBC. Refer to the **Preparing Genplates for shipment section** in *SOP\_BCP\_0080 - Packing and Shipping CRX Vapor Shipper from Site SOP*.

**Figure 3.** Layout of the FlexiRack Pipetting Station for Biospecimen Post Collection Processing






**Table 1.** Position of 2D coded Matrix tubes in the FlexiRack Pipetting Station during Biospecimen Post Collection Processing.

Rack	Section	Positions	Specimen type	Procedure step
1	1b	1, 3, 5, 7	Urine	8.10
1	1b	9, 11	EDTA Whole Blood	8.4
1	1b	2, 4	Citrate Plasma	8.11
1	1b	10, 12	Citrate PPP	8.11
1	1e	3, 4, 5, 6, 7, 8, 9, 10	Serum	8.12
2	2b	1, 3, 5, 7, 9, 11	Heparin Plasma	8.11
2	2e	1, 3, 5, 7, 9, 11	EDTA Plasma	8.11
2	2e	6, 8, 10, 12	EDTA Buffy coat	8.11

## 8.5 Multiple Sample Centrifugation

8.5.1 Gather the following:

- 2 x 2.7 mL Citrate (blue cap) 
- 1 x 6 mL Heparin (green cap) 
- 3 x 6 mL EDTA (lavender cap) 

8.5.2 Gently invert each tube 5–10 times by hand.

- 8.5.3 Check that the A4-38 rotor with round buckets and the 7 mL adaptors are installed in the centrifuge.
- 8.5.5 In LabWare, under the **Sample Processing**, click on **Start** below **Multiple Samples**. Scan each linear barcode from steps 8.5.1 into the LabWare. Ensure the correct date and time are entered in the pop-up window. Balance each tube in the centrifuge bucket after scanning.
- 8.5.6 Attach the bucket covers.
- 8.5.7 Close the centrifuge lid.
- 8.5.8 Centrifuge at 2.0 rcf (2000 g) for 10 minutes at 22°C with the SOFT brake. Using the soft brake will prevent disruption of the gradient.

**NOTE:** Aliquot urine while the centrifuge runs (may be done at any stage of processing). Refer to step 8.2 **General instructions** and step 8.10 **Aliquotting of the Urine Specimen**.

**NOTE:** It is also possible to aliquot ACD samples during this centrifugation (Refer to *8.14 Processing the ACDTube*)

- 8.5.9 When the centrifuge stops, open the centrifuge lid.
- 8.5.10 Remove the bucket covers.
- 8.5.11 In LabWare, under the **Sample Processing** stage, click **Stop** under **Multiple Samples**. Remove each tube without disturbing the blood layers and scan the linear barcode of each tube into the. Ensure the correct date and time are entered in the pop-up window.
- 8.5.12 Place the tubes in the FlexiRack pipetting station. Refer to [Figure 3](#).

## 8.6 Preparing Platelet Poor Plasma (PPP) for Centrifugation

- 8.6.1 Remove the stopper from one of the two citrate tubes.
- 8.6.2 Using the new transfer pipette, pipette plasma to the 5mL round bottom polypropylene tube labeled "PPP" in LabWare. Aspirate slowly by flowing the plasma down the side of the tube. Do not take all of the plasma to avoid transferring any cells. Take approximately  $\frac{3}{4}$  of plasma from the top.
- 8.6.3 Replace the citrate tube stopper.
- 8.6.4 Cap the labeled 5 mL polypropylene round bottom tube.

## 8.7 Centrifugation of Citrate Plasma

- 8.7.1 In LabWare, in **Sample Processing**, click **Start** under **Centrifuge Single Sample**.
- 8.7.2 Scan the linear barcode on the 5 mL polypropylene round bottom tube.
- 8.7.3 Balance in the centrifuge buckets.
- 8.7.4 Attach the bucket covers.



- 8.7.5 Close the centrifuge lid.
- 8.7.6 Centrifuge at 1.5 rcf (1500 g) for 5 minutes at 22°C with the SOFT brake.

**NOTE:** Complete hematology testing while the centrifuge runs (may be done at any stage of processing). Refer to step 8.9 **Hematology Testing**.

- 8.7.7 Once the centrifuge has stopped, open the centrifuge lid.
- 8.7.8 Remove the bucket covers.
- 8.7.9 Remove the 5 mL polypropylene round bottom centrifuge tube.
- 8.7.10 In LabWare, in **Start Processing**, click **Stop** under the **Centrifuge Single Sample** and open the centrifuge lid.
- 8.7.11 Open the centrifuge lid and scan the 5 mL polypropylene round bottom tube. Ensure the correct date and time are entered in the pop-up window.
- 8.7.12 Place in rack 1, section 1a, position 6 of the FlexiRack pipetting station.

## 8.8 Centrifugation of Clotted Blood (Serum)

- 8.8.1 After collection, the 10 mL serum tube must sit in the Flexirack for at least 30 minutes. Check that blood has clotted before going further with processing. An increased turbidity of the blood sample should be noticeable.

**NOTE:** If a soft clot is observed, the sample should be left longer to complete clotting.

- 8.8.2 Bring the unopened tube to the centrifuge.
- 8.8.3 In LabWare, in **Sample Processing**, click on **Start** under **Centrifuge and Single Sample**. Scan the linear barcode label on the serum tube. Ensure the correct date and time are entered in the pop-up window.
- 8.8.4 Balance the serum tube in the centrifuge buckets.
- 8.8.5 Attach the bucket covers.
- 8.8.6 Close the centrifuge lid.
- 8.8.7 Centrifuge at 2.0 rcf (2000 g) for 10 minutes at 22°C with the SOFT brake.

**NOTE:** Aliquot Plasma, Buffy Coat, and Platelet Poor Plasma while the centrifuge runs. Refer to step 8.2 **General Instructions** and step 8.11 **Aliquoting Plasma, Platelet Poor Plasma, and Buffy Coat**.

- 8.8.8 Open the centrifuge lid once the centrifuge has stopped.
- 8.8.9 Remove the bucket covers.

- 8.8.10 In LabWare, in the **Sample Processing**, click **Stop** under **Centrifuge and Single Sample**. Remove the serum tube without disturbing the blood layers and scan the linear barcode label.. Ensure the correct date and time are entered in the pop-up window.
- 8.8.11 Place the serum tube in the FlexiRack pipetting station rack 1, section 1d, position 1.
- 8.8.12 Proceed to step **8.12 Aliquotting Serum**.

## 8.9 Hematology Testing

- 8.9.1 Remove the 3 mL EDTA tube from the rotator. The sample must mix for a minimum of 30 minutes to allow for the blood and anticoagulant to equilibrate.
- 8.9.2 In LabWare, under the **Sample Processing** stage, click **Stop** under **Tube Shaker** and scan the linear barcode. Ensure the correct date and time are entered in the pop-up window.
- 8.9.3 Remove the stopper from the 3 mL EDTA tube.
- 8.9.4 Perform Hematology testing using the Coulter Ac•T diff. analyzer. Refer to *SOP\_BCP\_0014 - Coulter Ac•T diff – Analyzing Whole Blood Samples* and *SOP\_BCP\_0015 - Coulter Ac•T diff – Analysis Evaluation*.
- 8.9.5 Re-cap and return the 3 mL EDTA tube to the back of the Flexirack until discarded.
- 8.9.6 Launch the Microsoft Excel sheet by double clicking on the Excel Macro File entitled “Beckman Coulter Act Data Collection.xlsm”. This file should be located on the Desktop of the BCP computer.
- 8.9.7 Once the results are transferred, the computer will ask ‘Do you want to copy the clip?’ Click ‘Yes.’
- 8.9.8 In LabWare, in **Sample Processing**, click on **Ac•T diff. Analyzer** icon. Scan the linear barcode label on the 3mL EDTA tube and press **OK**. A window entitled “Paste Beckman Coulter Result Data” will appear. Paste the results from the clip into the window.

## 8.10 Aliquotting of Urine Specimen

- 8.10.1 Remove the caps from the 2D coded Matrix tubes in rack 1, section 1b, positions 1, 3, 5 and 7.
- 8.10.2 Take the urine container and check that the lid is tightly closed.
- 8.10.3 Mix the urine container 5 times by gentle inversion.
- 8.10.4 Observe the characteristics of the urine sample. In LabWare, in **Sample Aliquotting**, click on **Sample Characteristic Comments**. Fill in the fields required as described in *MAN\_DCS\_0217 – LabWare Guide*.
- 8.10.5 Open the urine container lid carefully to avoid spillage.

- 8.10.6 Pipette 500 µL of urine into the uncapped 2D coded Matrix tubes in rack 1, section 1b, positions 1, 3, 5, and 7.
- 8.10.7 Cap the 2D coded Matrix tubes and the urine collection container.
- 8.10.8 In LabWare, in **Sample Aliquotting**, click on the **0.5 mL Matrix Tube**. Scan the linear barcode of the original urine container.
- 8.10.9 Scan each of the 2D coded Matrix tubes for the urine sample using the single tube 2D barcode scanner.

## 8.11 Aliquotting Plasma, Platelet Poor Plasma, and Buffy Coat

### *Citrate Plasma*

- 8.11.1 Remove caps from 2D coded Matrix tubes for citrate plasma, in rack 1, section 1b, positions 2 and 4. Refer to [Figure 3](#) and [Table 1](#).
- 8.11.2 Remove the stopper from the remaining 2.7 mL citrate plasma tube.
- 8.11.3 Pipette 500 µL of plasma into the uncapped 2D coded Matrix tubes, in rack 1, section 1b, positions 2 and 4.
- 8.11.4 Replace the 2.7 mL citrate plasma tube stopper.
- 8.11.5 Screw the caps back onto the 2D coded Matrix tubes.
- 8.11.6 In LabWare, in the **Sample Aliquotting** stage, click on the **Aliquot Plasma**. Scan the linear barcode of the 2.7 mL citrate tube.
- 8.11.7 Scan each of the 2D coded Matrix tubes for citrate plasma using the single tube 2D barcode scanner.

### *Citrate Platelet Poor Plasma (PPP)*

- 8.11.8 Remove caps from 2D coded Matrix tubes for citrate PPP in Rack 1, section 1b, positions 10 and 12. Refer to [Figure 3](#) and [Table 1](#).
- 8.11.9 Remove the cap of the 5 mL polypropylene round bottom tube labeled "PPP".
- 8.11.10 Pipette 500 µL of PPP into the uncapped 2D coded Matrix tubes in Rack 1, section 1b, positions 10 and 12.

**NOTE:** Do not use the last 1/4 of plasma at the bottom of the tube. Do not disturb the cells at the bottom of the tube, regardless if they are visible or not.

- 8.11.11 In LabWare, under the **Sample Aliquotting** stage, click on **0.5 mL Matrix Tube** button. Scan the linear barcode of the 5 mL polypropylene round bottom tube labeled PPP.
- 8.11.12 Scan each of the 2D coded Matrix tubes for citrate PPP using the single tube 2D barcode scanner.

### *Heparin Plasma*

- 8.11.13 Remove caps from 2D coded Matrix tubes for heparin plasma in rack 2, section 2b, positions 1,3,5,7,9 and 11. Refer to [Figure 3](#) and [Table 1](#).
- 8.11.14 Remove the stopper from the 6 mL heparin tube.
- 8.11.15 Pipette 500 µL of plasma into the uncapped 2D coded Matrix tubes, in rack 2, section 2b, positions 1,3,5,7,9 and 11.
- 8.11.16 Replace the 6 mL heparin tube stopper.
- 8.11.17 Screw the caps back onto the 2D coded Matrix tubes.
- 8.11.18 In LabWare, under the **Sample Aliquotting** stage, click on the **Aliquot Plasma**; scan the linear barcode of the 6 mL heparin tube.
- 8.11.19 Scan each of the 2D coded Matrix tubes for heparin plasma using the single tube 2D barcode scanner.

***EDTA Plasma and Buffy Coat (BC)***

- 8.11.20 Uncap the 5 mL polypropylene round bottom tube labeled BC in rack 2, section 2d, position 4 of the FlexiRack.
- 8.11.21 Remove the caps from the 2D coded Matrix tubes for EDTA plasma in rack 2, section 2e, positions 1, 3, 5, 7, 9 and 11. Refer to [Figure 3](#) and [Table 1](#).
- 8.11.22 Remove the stoppers from all the 6 mL EDTA tubes.
- 8.11.23 Pipette 500 µL of plasma into the uncapped 2D coded Matrix tubes in rack 2, section 2e, positions 1, 3, 5, 7, 9, and 11.
- 8.11.24 Leave ~1 mL layer of plasma in each 6 mL EDTA tube.
- 8.11.25 Screw the caps back onto the 2D coded Matrix tubes.
- 8.11.26 In LabWare, under the **Sample Aliquotting** stage, click on **Aliquot Plasma**. Scan the linear barcode of ONE of the three 6 mL EDTA tubes.

**NOTE: Use ONE of the TWO remaining 6 mL EDTA tube for the EDTA Plasma aliquots. Do NOT reuse the 6 mL EDTA tube that was previously used for whole blood.**

- 8.11.27 Scan each of the 2D coded Matrix tubes for EDTA plasma using the single tube 2D barcode scanner.
- 8.11.28 Using a new transfer pipette, aspirate the white cell layer into the 5mL polypropylene round bottom tube identified for "buffy coat". The portion of the sample transferred will be enriched with white cells but will contain a thin layer of overlying plasma and underlying red cells. Repeat for the remaining two EDTA tubes.
- 8.11.29 Gently mix the pool of buffy coat using the transfer pipette.
- 8.11.30 Replace the 6 mL EDTA tube stoppers.

- 8.11.31 Remove caps from 2D coded matrix tubes in rack 2, section 2e, positions 6,8,10,12. Refer to [Figure 3](#) and [Table 1](#).
- 8.11.32 Pipette 200 µL of pooled buffy coat into the uncapped 2D coded Matrix tubes in rack 2, section 2e, positions 6, 8, 10 and 12.
- 8.11.33 Screw the caps back onto the 2D coded Matrix tubes.
- 8.11.34 In LabWare, under the **Sample Aliquotting** stage, click on **0.5 mL Matrix Tube** button. Scan the linear barcode of the 5 mL polypropylene round bottom tube labeled "Buffy Coat" (BC).
- 8.11.35 Scan each of the 2D coded Matrix tubes for buffy coat using the single tube 2D barcode scanner.

### 8.12 Aliquotting Serum

- 8.12.1 Remove caps from the 2D coded Matrix tubes for serum in rack 1, section 1 positions 3-10. Refer to [Figure 3](#) and [Table 1](#).
- 8.12.2 Remove the stopper of the 10 mL serum tube.
- 8.12.3 Pipette 500 µL of serum into the uncapped 2D coded Matrix tubes in rack 1, section 1e, positions 3-10.
- 8.12.4 Replace the 10 mL serum tube stopper.
- 8.12.5 Screw the caps back onto the 2D coded Matrix tubes.
- 8.12.6 In LabWare, under the **Sample Aliquotting** stage, click on **0.5 mL Matrix Tube** button. Scan the linear barcode of the serum tube.
- 8.12.7 Scan each of the 2D coded Matrix tubes for serum sample using the single tube 2D barcode scanner.

### 8.13 Recording of Specimen Inventory and Short Term Storage

- 8.13.1 Obtain a permanent Matrix storage box for the study participant. This may be obtained from storage or the Isotemp Ultra Low Temperature Undercounter Freezer depending if this is the first, second, or third participant for the box.
- 8.13.2 Transfer all filled 2D coded Matrix tubes from the FlexiRack pipetting station to the Matrix box.
- 8.13.3 Cover the Matrix box with the lid and lock it.
- 8.13.4 In LabWare, in **Sample Aliquotting**, click on **Freeze Samples**. Enter the Interview ID. LabWare will list the sets of aliquots that are ready for freezing. Verify that the number of aliquots for each sample is accurate. Select all if there is no discrepancy and press **OK**
- 8.13.5 Once you've selected all the samples you are about to freeze, press OK.

- 8.13.6 If there is any discrepancy between the numbers of aliquots or if any other issue arises with the time and date entered in LabWare, immediately contact [lims@clsa-elcv.ca](mailto:lims@clsa-elcv.ca) for assistance and open a WebIssue.
- 8.13.7 In LabWare, in **Sample Aliquotting**, click on **Scan Matrix Box**; scan the Matrix Box linear barcode. Press **OK**. Refer to *8.15 Scanning the Matrix box with the 2D barcode scanner for storage*. Copy the contents of the Matrix Box from the Thermo software and paste it into the pop-up window.
- 8.13.8 Verify that deviations in aliquot volumes, hemolysis, lipemia or other specimen comments in LabWare (refer to *MAN\_BCP\_0217 – LabWare Guide*).
- 8.13.9 Place the Matrix Box in the Isotemp Ultra Low Temperature Undercounter Freezer.

#### 8.14 Processing the ACD Tube

**NOTE:** After ACD tube has been on the rotator (see step 8.3.3 and 8.3.4) for a minimum of 3 minutes and up to 1.5 hours, process the ACD tube. In order to maximize BioCision CoolCell usage, batch the specimens of 2 participants if available.

- 8.14.1 Obtain two 2D coded Matrix tubes and place in the FlexiRack pipetting station.
- 8.14.2 Remove the caps from the 2D coded Matrix tubes.
- 8.14.3 Take the DMSO/RPMI solution from the laboratory refrigerator.
- 8.14.4 Pipette 250 µL of the DMSO/RPMI solution into the 2D coded Matrix tubes.
- 8.14.5 If the ACD tube has been on the rotator for at least 3 minutes then, remove the ACD tube from the rotator.
- 8.14.6 In LabWare, in **Sample Processing**, click **Stop** under the **Tube Shaker** and scan the linear barcode. Ensure the correct date and time are entered in the pop-up window.
- 8.14.7 Remove the stopper from the ACD tube.
- 8.14.8 Pipette 250 µL of blood into the uncapped 2D coded Matrix tubes from 8.14.6.
- 8.14.9 Replace the tube stopper on the ACD tube.

**NOTE:** If processing more than one ACD tube repeat steps 8.14.2 to 8.14.10 until all ACD tubes are processed.

- 8.14.10 In LabWare, under the **Sample Aliquotting** stage, click on the **0.5 mL Matrix Tube**, scan the linear barcode of the ACDtube.
- 8.14.11 Screw the caps back onto the 2D coded Matrix tubes.
- 8.14.12 Scan the 2D coded Matrix tubes for the ACD samples from 8.14.10 using the single tube 2D barcode scanner.
- 8.14.13 Gently mix the 2D coded Matrix tubes 5 times by inversion.

- 8.14.14 In LabWare, under **Sample Aliquotting**, click **Start** under **1° C/min Freezing** and scan the linear barcode of the ACD tube. Ensure the correct date and time are entered in the pop-up window.
- 8.14.15 Place the 2D coded Matrix tubes in the BioCision CoolCell freezing container and place in the -80°C freezer.

**Very Important:** The BioCision CoolCell and cooling ring must be at room temperature at the start of use. It should warm to room temperature within 10-15 minutes with the centre solid cooling ring removed.

- 8.14.16 Remove the 2D coded Matrix tubes from the BioCision CoolCell after a **minimum** of 4 hours. Insufficient cooling will damage cells and reduce live cell yield.
- 8.14.17 In LabWare, in **Sample Aliquotting**, click **Stop** under the **1° C/min Freezing** button and scan any of the linear barcodes of the two 2D coded Matrix tubes for the ACD samples. Ensure the correct date and time are entered in the pop-up window.
- 8.14.18 In LabWare, in **Sample Aliquotting**, click the **Freeze Samples** button. Scan the 2D barcode of the ACD aliquots using the single tube scanner. Verify and select the ACD sample you are freezing and press OK. Ensure the correct date and time are entered in the pop-up window.
- 8.14.19 Repeat procedure steps 8.14.14 to 8.14.18 for all ACD 2D coded Matrix tubes, and prepare the BioCision CoolCell to be reused. Refer to *SOP\_BCP\_0040 Operation and Maintenance – BioCision CoolCell*.
- 8.14.20 Transfer all filled 2D coded Matrix tubes from the BioCision CoolCell to the Matrix box.
- 8.14.21 Place the Matrix Box in the Isotemp Ultra Low Temperature Under Counter Freezer.

### **8.15 Scanning the Matrix box with the 2D barcode scanner for storage.**

- 8.15.1 Ensure that the Thermo software is open on the computer. Make sure it is on rack mode.
- 8.15.2 Defrost the bottom of the Matrix Box to be sure the barcodes are readable. To do this, place the bottom of the box on a sponge that is soaked in 100% alcohol, and wipe off condensation with lint free cloth. This should remove the ice and frost formed under the box.
- 8.15.3 Place the box on the scan. When prompted, scan the box label and press enter.
- 8.15.4 Check the computer screen to ensure that there are no missing barcodes in the file at the bottom of the screen. If there are samples in the spots indicated as missing re-wipe the bottom of the Matrix Box and re-scan following steps 8.15.1-8.15.3.
- 8.15.5 In the Thermo software, use the "Copy All" button to capture all the barcodes in the box.

- 8.15.6 Go back to LabWare in **Sample Aliquotting** stage, and scan the linear barcode of the matrix box into the **Scan Matrix Box** dialogue box and click OK.  
**NOTE:** The file should import automatically. If it fails, copy the contents from the Matrix Box scanner software (Thermo scan).
- 8.15.7 A new dialogue box will appear. Paste the contents of the Thermo scan into the **Paste Matrix Box Data** dialogue box and click **OK**.
- 8.15.8 Return to the matrix box to the -80°C 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 WebIssue at <https://clsacloud.clsa-elcv.ca/webissues/client>.

### 8.16 Waste Disposal

- 8.16.1 In LabWare, in **Sample Processing**, click on the **Dispose Sample** button and scan the linear barcodes of all samples to be discarded.
- 8.16.2 Click **OK** when finished scanning all the linear barcodes and a list of samples to be disposed of will appear.
- 8.16.3 Refer to *SOP\_BCP\_0009 Waste Disposal SOP* to dispose of all used tubes, 15 ml conical tubes, 5 ml polypropylene round bottom tubes, whole blood, plasma, serum, and used consumables.
- 8.16.4 Dispose of remaining urine in the toilet and discard the empty container.

### 9.0 Documentation and Forms:

- **CRF\_BCP\_0002** - Urine Collection Case Report Form
- **CRF\_BCP\_0001** - Blood Collection Case Report Form
- **DOC\_BCP\_0058\_9** - Downtime - Daily Time Zero Participant Specimen Log
- **DOC\_BCP\_0067\_10** - Downtime - Daily Matrix Box Storage Time

### 10.0 References:

- Clinical and Laboratory Standards Institute (CLSI) H18-A4 Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Fourth Edition. Vol. 30 No. 10.
- Imagen Standard Operating Procedure (SOP) Work Package 8 Sample Collection and Processing. May 2008

### Baseline Revision History:

New Version #	Revision Date	Revision Author	Content Approver
4.0	2015-APR-13	Chetna Naik	Cynthia Balion
<b>Summary of Revisions</b>			
Formatting changes throughout the document			
All use of the word 'Vacutainer' deleted. Now simply refer to tubes.			



Purpose, Scope, and Responsibilities sections reviewed and clarified.
Moved reference to the CRFs and DOCs to the 'Documentation and Forms' section.
In <b>4.0 Related Documents</b> , reference to Waste Disposal SOP changed to <i>SOP_DCS_0406 - Site-Specific Waste Disposal SOP</i> .
In <b>7.0 Supplies</b> : "Germicidal disposable cloths, CiDecon wipes" removed from list; gloves are now called 'non-latex disposable' rather than 'nitrile'.
Grammatical and wording changes made to steps throughout section <b>8.0</b> .
<b>8.2.8</b> - Step added: All samples, except the CPT tubes, must be processed within 2 hours of collection.
Under <b>8.2.8</b> , in IMPORTANT note box, reference made to <i>MAN_BCP_0217 – LabWare Guide</i> .
<b>NOTE under 8.2.8 deleted</b> : If, for some reason, there are not enough aliquots for a certain test, press ENTER on the keyboard to skip entering the remaining aliquot barcodes when prompted. References have been added to the comments module in LabWare in section <b>8.13</b> .
<b>8.3.3</b> : 3 mL lavender tube lid colour changed
<b>8.4.3 Note added</b> : Use ONE of the THREE 6 mL EDTA tubes for the two whole blood aliquots.
<b>8.4.8: Scan method clarified</b> : Scan the linear barcode on the 6 mL EDTA tube. Then, as prompted, scan the 2D coded matrix tubes using the single tube 2D barcode scanner.
<b>8.4.14</b> : Pop up window information added
<b>8.4.15: Note added</b> : This can be completed during the multiple samples centrifugation.
<b>8.5.1</b> : List of vacutainers combined into one; not split into 'unopened' and 'previously used'.
<b>8.5.8: Additional note added</b> to existing note: It is also possible to aliquot ACD samples during this centrifugation.
<b>8.6.2</b> : Wording of second sentence clarified: Aspirate slowly by <b>flowing</b> the plasma volume down the side of the tube.
<b>8.7.6 and 8.8.7</b> : Sentence deleted: Using the soft brake will prevent disruption of the gradient.
<b>8.8.1</b> : Step reworded.
<b>8.9.3 moved to 8.9.8</b> . Steps renumbered. Step clarified.
<b>8.9.6</b> : Details added.
<b>8.9.7 added</b> : Once the results are transferred, the computer will ask 'Do you want to copy the clip?' Click 'Yes.'
Added <b>8.10.4</b> : Observe the characteristics of the urine sample. In LabWare, in <b>Sample Aliquotting</b> , click on <b>Sample Characteristic Comments</b> . Fill in the fields required as described in <i>MAN_DCS_0217 – LabWare Guide</i> .
<b>8.11.10</b> : Note clarified.
<b>8.11.26: New note added</b> : Use ONE of the TWO remaining 6 mL EDTA tubes for the EDTA Plasma aliquots. Do NOT reuse the 6 mL EDTA tube that was previously used for whole blood.
<b>8.11.28</b> : Rewritten for accuracy.
<b>8.13.5: Deleted reference to pop-up window</b> : Ensure the correct date and time are entered in the pop up window.
<b>8.13.6</b> : Clarified step and added reference to opening a WebIssue.
<b>8.13.8</b> : Added reference to making comments in LabWare.
<b>8.14.11 &amp; 8.14.12</b> : steps reordered
<b>8.15.3</b> : Clarified step.
<b>8.15.8: "Very Important" note added</b> : 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, return to the respective section in the SOP. If the issue cannot be resolved, file a WebIssue at <a href="https://clsacloud.clsa-elcv.ca/webissues/client">https://clsacloud.clsa-elcv.ca/webissues/client</a> .			
<b>New Version #</b>	<b>Revision Date</b>	<b>Revision Author</b>	<b>Content Approver</b>
3.0	2012-Aug-30	Lori Dewar	Cynthia Balion
<b>Summary of Revisions</b>			
Updated section 3.0, 4.0 and 6.0			
Added; Note and Very Important text boxes under step 8.2.7; note under step 8.4.9; step 8.4.12, 8.4.18, 8.4.21, 8.7.10, 8.10.2, 8.13.4-8.13.6, 8.14.3, 8.14.17-8.14.21, 8.15.5; waste disposal section			
Added document numbers throughout document			
Deleted original step 8.3.3-8.3.5, 8.5.3, 8.14.1-8.14.3, 8.15.1-8.15.3,			
Wording clarification throughout document			
Updated step 8.5.5, section 8.6, step 8.7.1, 8.7.11, 8.8.3, 8.8.10, 8.9.2-8.9.4, 8.9.7, 8.11.9, 8.11.14-8.11.34, 8.14.6-8.14.16, 8.15.1-8.15.3,			
<b>New Version #</b>	<b>Revision Date</b>	<b>Revision Author</b>	<b>Content Approver</b>
2.0	2012-Aug-10	Nicole Brooks	Cynthia Balion
<b>Summary of Revisions</b>			
Updates section 4.0, 5.0 and 6.0			
Added supplies to step 8.1.1			
Wording clarification throughout the SOP			
Added step 8.2.5, 8.3.4, 8.3.5, Note under step 8.3.5, 8.4.1-8.4.3, 8.4.5-8.4.8, 8.4.10-8.4.12, note under 8.4.12, 8.4.16, 8.4.17-8.4.19, note under 8.4.20, 8.5.14, 8.6.9, 8.9.2, 8.9.3, 8.10.6, 8.11.6-8.11.7, 8.11.11-8.11.12, 8.11.21-8.11.24, 8.11.30-8.11.31, 8.12.6-8.12.7, 8.14.2, 8.15.3, 8.15.4, 8.15.11-8.15.13, 8.15.20, section 8.16			
Clarified/updated step 8.3.6, 8.3.8, 8.4.4, 8.4.17, 8.4.20, 8.5.6, 8.5.7, 8.5.12, 8.5.15, 8.5.16, 8.6.1-8.6.10, 8.7.1, 8.7.8, 8.7.10, 8.7.11, 8.8.1, 8.8.3, 8.8.12, 8.9.4, 8.9.6, 8.11.14, 8.13.5-8.13.7, 8.14.1, 8.14.3, 8.15.7, 8.15.16			
Deleted original steps 8.3.5, 8.3.6, 8.4.6, 8.4.7, 8.4.17-8.4.19, 8.7.2			