

	BWH/Harvard Cohorts Biorepository Blood Processing Procedure		Pg. 1 of 14
	Doc Number: 2014 –006	Rev: 001	

TABLE OF CONTENTS

1. PURPOSE	2
2. SCOPE.....	2
3. DEFINITIONS	2
4. RESPONSIBILITIES	2
5. MATERIALS.....	2
6. PROCEDURE.....	2
5.1 PROCESS FLOW.....	3
5.2 PROCEDURE.....	3-13
7. APPLICABLE REFERENCES	14
8. QUALITY RECORDS.....	14

 	BWH/Harvard Cohorts Biorepository Blood Processing Procedure		Pg. 2 of 14
	Doc Number: 2014 –006	Rev: 001	

1. PURPOSE

The purpose of this document is to outline the steps for processing the Blood samples in the lab for a study in the collections lab.

2. SCOPE

This Standard Operating Procedure (SOP) will cover how to process blood samples collected for all studies and store them. This SOP is designed to be utilized to sort and process kits from each of the studies currently running in the lab. The SOP will direct how to arrange and prioritize the samples arriving each day. On any given day of sample receiving, varying numbers of each arm will arrive in the lab for processing. All kits must be processed on the same day as they arrive in the laboratory.

3. DEFINITIONS

- Aliquot - A sample or a portion of a larger whole
- Hemolysis – Destruction of red cells
- Desiccant – A substance that is used as a drying agent to absorb moisture from the air
- PPE – Personal Protective Equipment.
- SOP– Standard operating procedure. A written procedure for repetitive use as a practice, in accordance with agreed upon specifications aimed at obtaining a desired outcome.

4. RESPONSIBILITIES

- Project Manager – The role of the project manager is to oversee the work in the laboratory and deal with any deviations from the protocol.
- Research Assistants – The role of the research assistants is to perform the physical tasks of the lab bench



5. MATERIALS

PPE:

- Lab Coat
- Gloves
- Closed toe shoes
- Face shield/ goggles

Supplies:

- Centrifuge- Eppendorf Centrifuge 5810R, 15amp version
- Pipettors - 1000µL and 500µL
- Transfer pipette
- Tube rack
- Tray of dry ice
- Vial caps

 	BWH/Harvard Cohorts Biorepository Blood Processing Procedure		Pg. 3 of 14
	Doc Number: 2014 –006	Rev: 001	

- Labeled 4.5 mL and 1.8mL aliquot vials
- Vial trays

Centrifuge Settings

Temperature	4°C (allow centrifuge to pre-chill before using)
Speed (RCF)	1530
Time	20 Minutes

6. ENVIRONMENTAL HEALTH AND SAFETY

- 6.1 Assume universal biological agent handling precautions. Always treat all specimens as though they are infectious.
- 6.2 Always wash hands before and after contact with specimens.
- 6.3 Always wear gloves and protective clothing

7. PROCEDURE



7.1 Process Flow

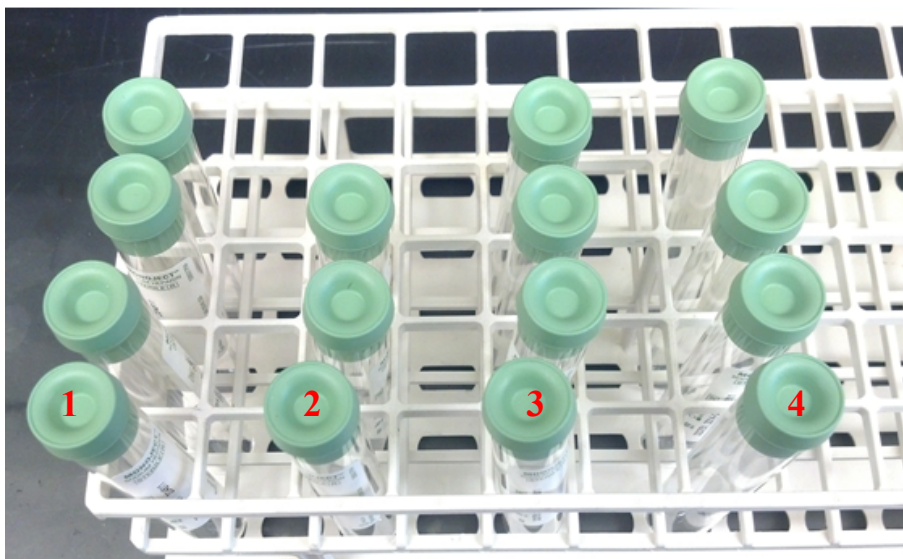
TBD

7.2 Procedure

7.2.1 Place blood tubes into racks

- After all the kits are received in LIMS, place the samples into blood racks by study in alias ID order working from left to right.
- Arrange the blood samples in the racks by alias ID as shown below. Once this is done hand off racks to the processing RA.

		BWH/Harvard Cohorts Biorepository Blood Processing Procedure		Pg. 4 of 14
		Doc Number: 2014 –006	Rev: 001	



***Place the fullest tube at the front and the shortest tube at the back. This is done for two reasons: 1) To distinguish the tubes for loading and balancing the centrifuge; 2) very short tubes have a tendency to contain grossly hemolyzed sample and therefore should not be allocated for a lab-ready aliquot.*

7.2.2 Centrifugation

- The Centrifuges used in the lab are: Eppendorf Centrifuge 5810R, 15amp version.
- Load the blood tubes from the blood trays into the buckets in the pre-chilled centrifuge:
- Place the blood tubes into the slots, ensuring that the buckets are distributed evenly or diametrically around the rotor.
- Load the blood tubes in alias ID order; however, tubes may need to be shuffled to balance out any tubes that are markedly short in volume.
- Weigh the loaded buckets with the aerosol cap on to ensure that buckets on the opposing ends of the rotor are balanced. Some redistribution of the tube or addition of tubes that are empty or filled with a small amount of water may be necessary for proper balance.
- Close the aerosol cap on the buckets and load them into the centrifuge as shown below



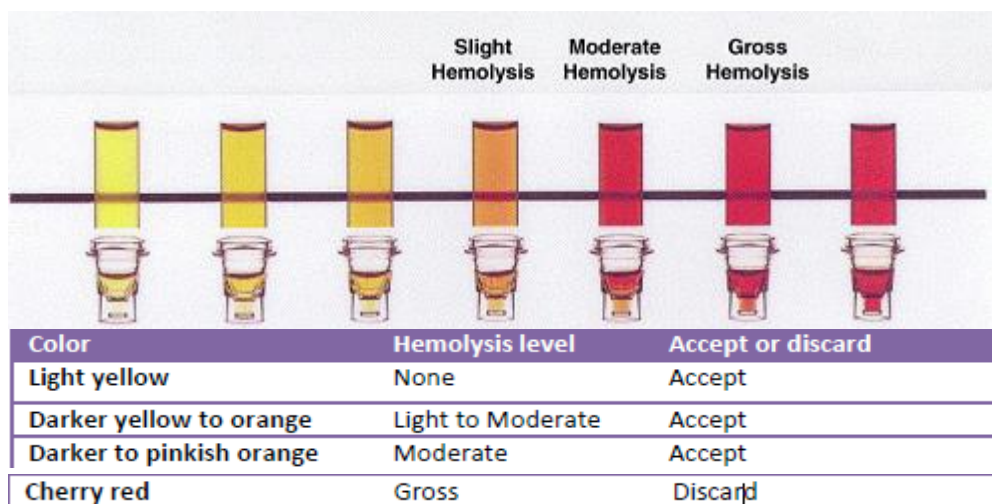
Example of a loaded centrifuge, where a yellow dot indicates a shorter volume tube

- Start the centrifuge and allow to run for 20 minutes at 1530 RPM at 4°C. Watch and listen for signs of imbalance while the centrifuge is starting up; if the buckets are not properly balanced, the centrifuge will shake or alarm.
- When the centrifuge stops, remove the tubes from the buckets and place them back into the blood tube rack in the same alias ID order, ensuring that short tubes are again placed in the back.
- Place the blood tubes in a 4°C refrigerator until ready to aliquot the samples.
- When samples are in the centrifuge, retrieve the labeled aliquot vials from their bins. They are stored by the kit ship date in alias ID order.
- Arrange the vials according to the **aliquot rack layout** reference sheets for each study.

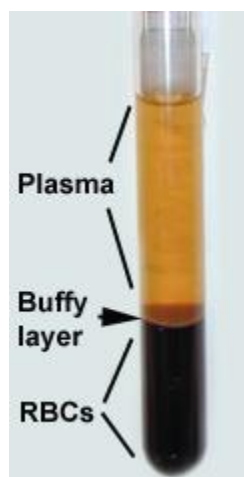
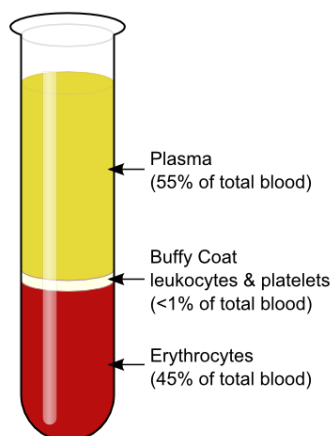
7.3 Set Up and Preparation for Aliquoting Plasma, WBC and RBC Samples

- Set up on a clean lab bench:
 - 1000µL pipettor & tips, 500µL pipettor & tips, transfer pipettes, aliquot vials on aliquot trays, discard tube, tray of dry ice with storage boxes
- Process samples in each arm in the racks as shown in 7.2.1 above in alias ID order.
- Place tray of aliquot vials onto the tray of wet ice. Ensure that the vials are arranged according to the **aliquot rack layout** reference sheet.
- Double check that the volume of the aliquots vials are arranged correctly in the tray.
- Fetch the blood tubes to be aliquoted from the refrigerator and place them into a new rack. Keep the blood tubes that are not being aliquoted in this batch in the refrigerator.
- At this time, verify that the alias ID on the blood tubes match those on the aliquot vials. Make note of hemolysis levels.

- Hemolysis may interfere with a future assay. Some blood tubes will arrive hemolyzed. Refer to the figure and table below for a description of hemolysis and whether to accept or reject the sample.
- The sample often appears darker in the collection tube. If unsure how to categorize the level of hemolysis, try aliquoting the sample into an aliquot tube. If in doubt, ask a supervisor.
- It is possible for a participant's set of blood tubes to have varying degrees of hemolysis. In this case, discard any samples that are grossly hemolyzed. Allocate samples from the least hemolyzed tube to create lab-ready aliquots.



- Place the rack of blood tubes in wet ice to keep the samples cold. Keep the tubes upright; tilting will disturb the separated layers.
- The centrifugation process will separate out the blood into plasma, WBC (buffy coat) and RBC components as shown below. The aliquots will be created in that order top to bottom
- WBC often presents as a thin layer that contains streaks of opaque material. The consistency is thicker and chunkier than plasma or RBC.



7.3.1 General rules for aliquoting

- Aliquot one participant set at a time. To prevent contamination, uncap, aliquot, and cap one participant's sample at a time.
- Check at each step that the alias IDs match between the parent tube and aliquot vial and that the correct volume aliquots are created.
- Lab-ready aliquots are priority. If there is insufficient sample to create all aliquots, always prioritize the lab-ready aliquots. Then, fill the permanent vials in the order that they are arranged in the tray, filling one vial completely before proceeding to the next; do not attempt to distribute the volume among all the aliquot vials.
- If at any point in the aliquot process that the lab-ready aliquot is lost (e.g. tray was knocked over), re-allocate any available volume from the permanent storage aliquot to create the lab-ready vial.
- Collect the sample, not the volume. Only aliquot as much component as there is. For example, be careful not to contaminate vials with other sample type (such as aliquoting RBC into WBC vials) just to fill up the vial.
- On 1.8mL and 4.5mL tubes, only fill to the fill line. Topping off the vial with extra volume may cause the sample to over expand in the freezer and crack.
 - Recommended pipette for each sample type is shown below:

Sample types	Pipette
--------------	---------

Plasma aliquots < 1.8 mL	Narrow orifice tip, <1000mL pipette
Plasma aliquots >1.8 mL	Transfer pipette
WBC aliquots < 1.8 mL	Wide orifice tip with filter, <1000 mL pipette
WBC aliquots > 1.8 mL	Transfer pipette
RBC aliquots < 1.8 mL	Wide orifice tip with filter, < 1000 mL pipette
RBC aliquots > 1.8 mL	Transfer pipette



7.3.2 Aliquot plasma

- Uncap the aliquot vials for the first alias ID and place the caps on a clean, dry surface. Ensure that the inside of the cap does not touch any surface.
- Remove the rubber stopper from the blood tubes using gauze so as not to soil your gloves
- Verify that the pipettes are set to the correct volume setting/s.
- Use a narrow orifice pipette tip and a pipette for any volumes <1.8mL and a transfer pipette for volumes >1.8mL.
- Aliquot plasma
- Do not pump samples with the pipette.
- Carefully push down on the plunger (or the bulb of the transfer pipette)
- Insert the pipette tip into the clear plasma layer and take up the volume.
- Release the volume into the aliquot vial.
- Remove as much of the translucent plasma layer as possible without taking up the opaque WBC layer below, holding the tube to eye level as necessary.
- Discard the pipette tip or transfer pipette into the biohazard bin when done using.
- Repeat the above until all plasma aliquots have been made.
- Cap the plasma aliquot vials.

7.3.3 Aliquot WBC

- Use the discard tube to discard miniscule amounts of plasma if it is difficult to aliquot down to the WBC without disturbing the layers.
- Verify that the pipette/s are set to the correct volume setting/s.
- Press down on the plunger or the bulb of the transfer pipette.
- Insert the tip of the pipette tip 2-3mm into the WBC layer.
- Swirl the tip along the edge of the tube, keeping the tip in the 2-3mm layer.
- Carefully take up the appropriate volume and release into the aliquot vial.
- Make the lab-ready aliquots first then make the perm aliquots.
- Repeat until all WBC aliquots have been created.
- Cap the aliquot vials.

7.3.4 Aliquot RBC

 	BWH/Harvard Cohorts Biorepository Blood Processing Procedure		Pg. 9 of 14
	Doc Number: 2014 –006	Rev: 001	

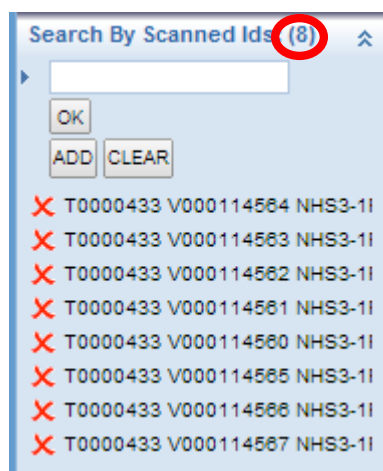
- Verify that the pipette/s are set to the correct volume setting/s.
- Press down on the plunger or the bulb of the transfer pipette and insert the tip into the RBC layer.
- Take up the RBC into the pipette and release into the aliquot vial.
- Repeat until all RBC aliquots have been created.
- Cap the aliquot vials.
- Typically, there will be some RBC left over.
- Only fill the aliquot vials to the fill line.
- Recap the tube with the rubber stoppers and throw into the biohazard trash.

7.3.5 Record volume and deviations on Aliquot sheet

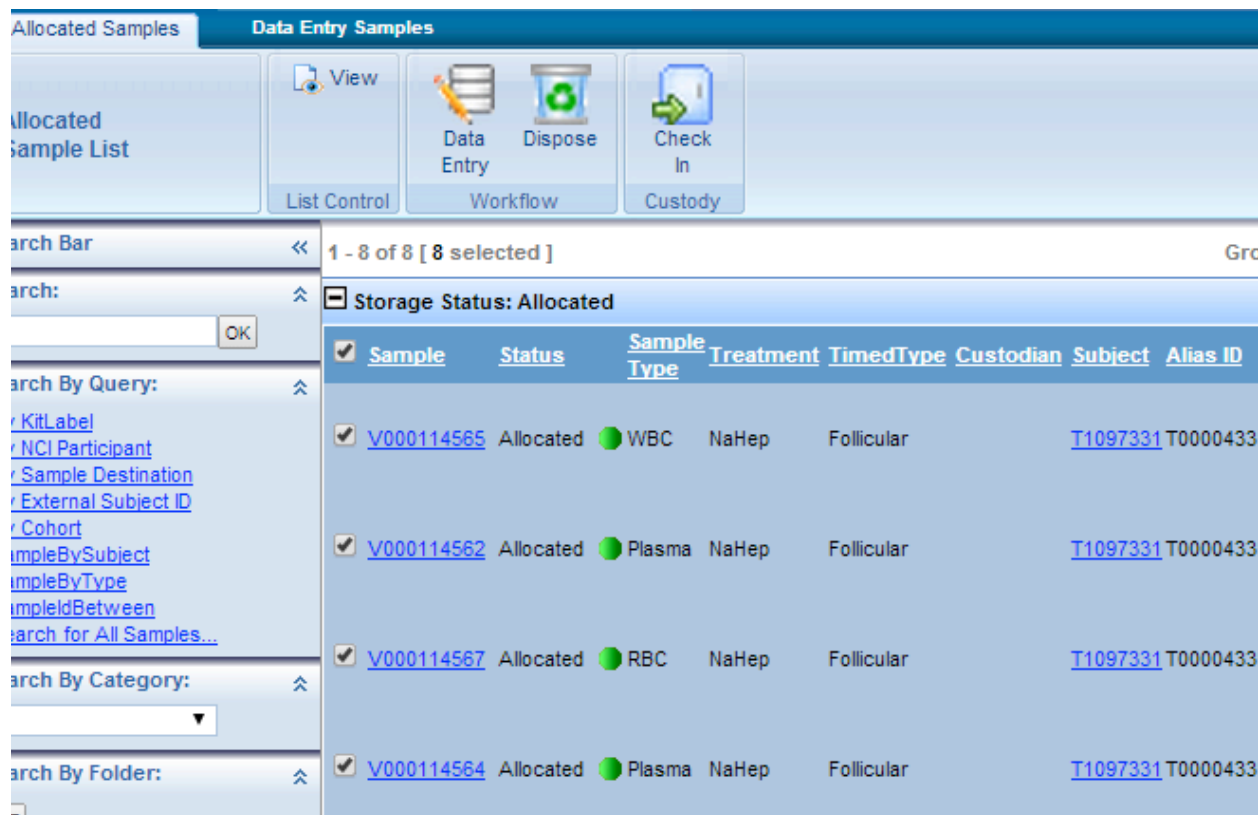
- Update aliquot sheet by marking off each aliquot in the comments column.
- Record estimated actual volume if the aliquot has less volume than expected.
- Note any deviations such as lipemic (fatty and turbid).
- Mark any aliquots that were not created (due to issues or short samples) with a 0.
- If no special notations are needed, mark off the vial with a check mark.
- If any vial was left empty, mark as 0 on the aliquot sheet and place the vial in a work tray. The vial will be
 - used to “discard” the vial record in LIMS
- Place the created aliquots in the tray of ice.
- Follow the steps in 7.3 above to process all the remaining blood samples.

7.3.6 Aliquot Data Entry in LIMS

- After aliquoting of all samples is complete, place the first set of aliquots in the wet ice tray.
- Also, place the box that the aliquots will be filed into a tray of dry ice.
- Log into LIMS and navigate to Sample Management/Allocated Samples.
- Scan the barcode from each vial into the “Search by Scanned IDs” field on the left panel.
- Verify that the number of vials scanned into LIMS (circled in red in image below) match the number of actual vials scanned as shown below:



- Click OK to pull the record list. Verify that the number of records that are pulled up match the number of records that were scanned. If the numbers do not match, clear all scanned IDs in the “Search by Scanned Ids” bar and rescan all the aliquots again.
- Click the check box on the records to select.
- Click on the Data Entry icon shown below.



- Fill in the Processing Date field.

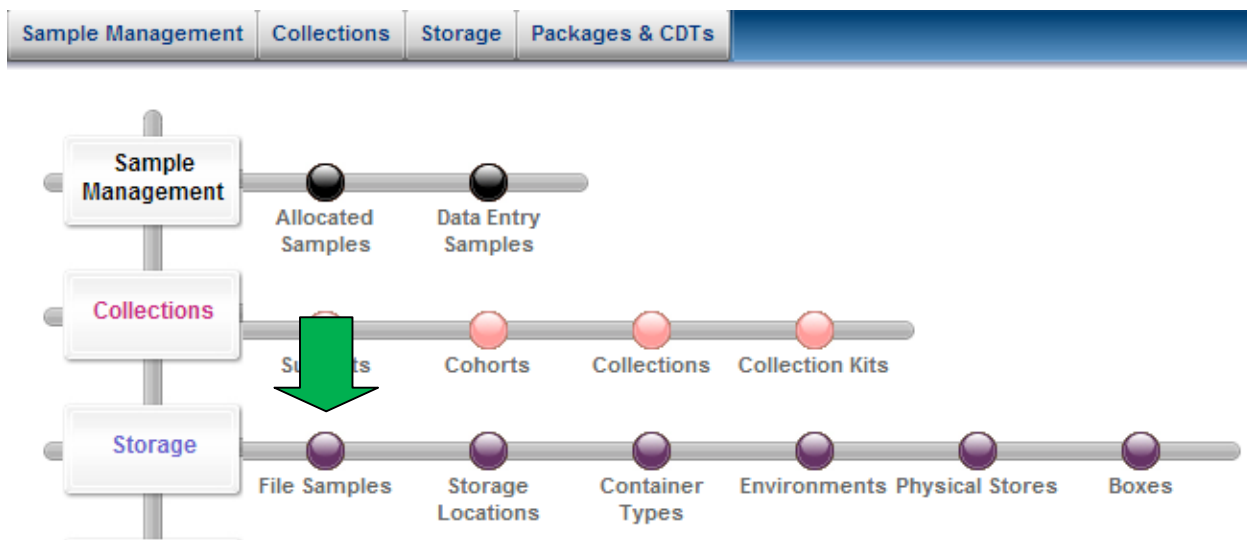
- Typing n will time stamp the record after the Save button is clicked.
- To batch-fill, enter n on the first line, hit shift and scroll to the last record on the page,
- If the processing date of the sample is not today's date, manually data enter the correct processing date instead of typing n.

Main Info		Sample Info			
Sample	Sample Type	Storage Status	CA	GLP	Processing Date
<input type="checkbox"/> V000005196	Urine, 24 hour	Received	No	No	n
<input type="checkbox"/> V000005197	Urine, 24 hour	Received	No	No	n
<input type="checkbox"/> V000005198	Urine, 24 hour	Received	No	No	n
<input type="checkbox"/> V000005199	Urine, 24 hour	Received	No	No	n
<input type="checkbox"/> V000005200	Urine, 24 hour	Received	No	No	n
<input type="checkbox"/> V000005201	Urine, 24 hour	Received	No	No	n
<input type="checkbox"/> V000005202	Urine, 24 hour	Received	No	No	n

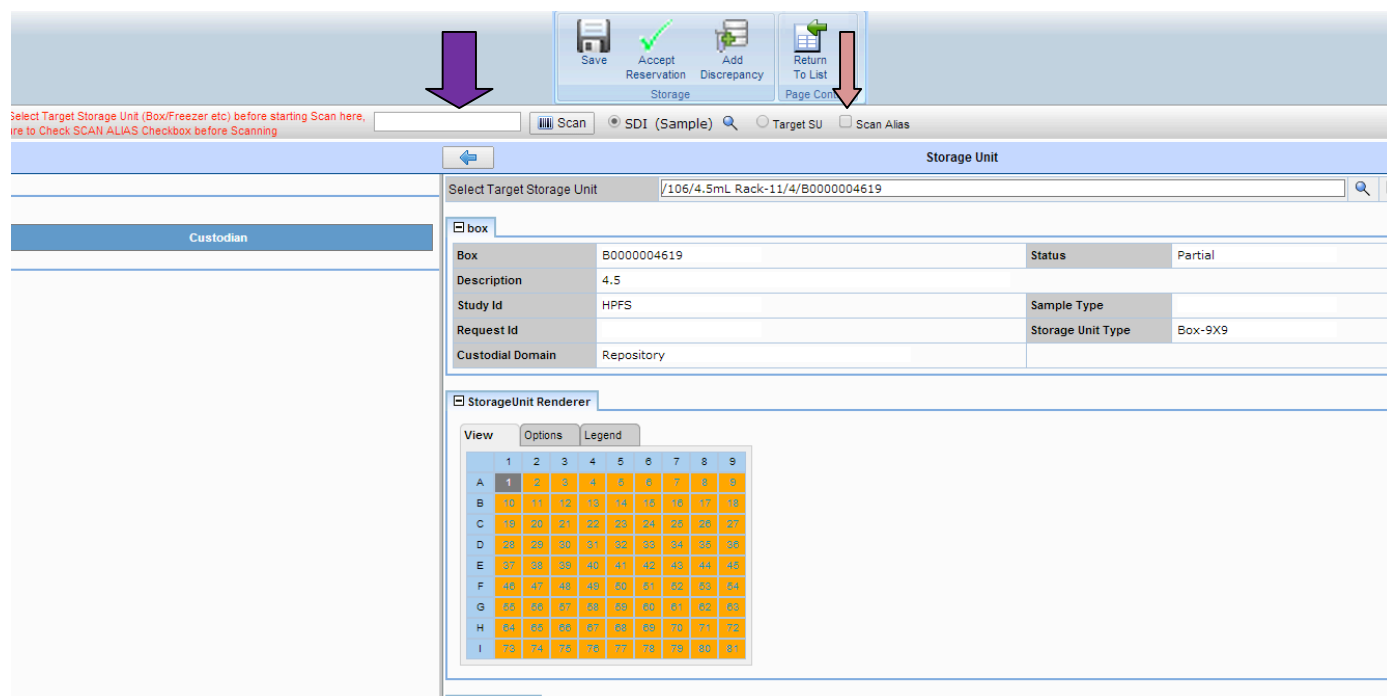
- Hit Save. Wait until the page refreshes and verify that the processing date field has been time stamped with the day's date and time. (This step may take time, but be patient; if you exit this page before the changes are applied, you will have to do this process all over!)
- In the "Initial Volume/Wt" field, fill in the actual volume of each aliquot. It is autopopulated to the volume that is expected for each aliquot. If the actual volume is the same as the expected volume, there is no need to make a change here.
- Hit the Return to List button.
- Hit CLEAR on the Search by Scanned IDs box and repeat steps 1-10 until all samples have been scanned and logged with a processing date.
- Repeat this until you have data entered all the aliquot racks into LIMS.
-

7.3.7 Filing Samples Into Storage Locations

- Once data entry has been performed on all the aliquot samples, Click on the Storage, File Samples tramline shown below with a green arrow



- On the screen that opens, click on the magnifying glass next to “Select Target Storage Unit” Field.
- Select the box where you will file the samples by scanning the anchor vial.
- Click OK.
- The Box will populate on the screen showing how many spots are filled in green and how many are empty in yellow as shown below:



Select Target Storage Unit (Box/Freezer etc) before starting Scan here, or to Check SCAN ALIAS Checkbox before Scanning

Save Accept Reservation Add Discrepancy Return To List Page Cont

Storage

Scan SDI (Sample) Target SU Scan Alias

Storage Unit



Select Target Storage Unit /106/4.5mL Rack-11/4/B0000004619

Box	B0000004619	Status	Partial
Description	4.5	Sample Type	
Study Id	HPFS	Storage Unit Type	Box-9X9
Request Id			
Custodial Domain	Repository		



StorageUnit Renderer

View Options Legend

	1	2	3	4	5	6	7	8	9
A	1	2	3	4	5	6	7	8	9
B	10	11	12	13	14	15	16	17	18
C	19	20	21	22	23	24	25	26	27
D	28	29	30	31	32	33	34	35	36
E	37	38	39	40	41	42	43	44	45
F	46	47	48	49	50	51	52	53	54
G	55	56	57	58	59	60	61	62	63
H	64	65	66	67	68	69	70	71	72
I	73	74	75	76	77	78	79	80	81

 	BWH/Harvard Cohorts Biorepository Blood Processing Procedure		Pg. 13 of 14
	Doc Number: 2014 –006	Rev: 001	

- Check the “Scan Alias” box shown with a pink arrow above and then click in the box before the “Scan” icon shown with a purple arrow above.
- Next, scan the barcode on the first aliquot vial. You should see it populate in the box in LIMS.
- Place the scanned vial into the box in the corresponding space to the spot in LIMS.
- Continue to scan the remaining vials until you have scanned all the vials into the box.
- Click the Save icon at the top of the Screen.
- Repeat this procedure until you have filed all the aliquots into all the boxes.
- Next, click on the Storage Locations tab at the top of the screen. It is the next stop on the tramline.
- Select the Freezer that you are going to file the box into.
- If the box is not filled, select the Transfer Freezer that you will store the boxes into temporarily.
- Once QA has been performed on the full boxes, move the boxes from the Transfer Freezer into the permanent freezer locations.

 	BWH/Harvard Cohorts Biorepository Blood Processing Procedure		Pg. 14 of 14
	Doc Number: 2014 –006	Rev: 001	

8. QUALITY RECORDS

List of applicable Quality records.

•

APPROVALS

<i>Approver</i>	<i>Approval Date</i>
<i>S.Sawyer</i>	<i>5/30/14</i>

REVISION HISTORY

<i>AUTHOR</i>	<i>REVISED SECTION/PARAGRAPH</i>	<i>REV</i>	<i>RELEASED</i>
Gakii Masunga	Initial Release		5/30/14

Draft and Archived/Obsolete revisions are not to be used.