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Laboratory Protocol

Aliquotting Plasma and Serum Samples into Multiple Portions

Date: 3 August 2017

Version Number: 1.2

Brief Summary of Procedure:

This SOP describes the procedure by which CCL creates a group of aliquots from a frozen primary (“Master”) source sample. The Master sample is first thawed and remixed thoroughly, and then defined volume portions (“aliquots”) are removed from the Master sample and dispensed into specified number of pre-labeled cryovials. These vials are then capped, arranged into a standard storage box, then placed into a -80 °C freezer for long term storage or until retrieved. Any remaining portion of the Master sample is discarded.

Expected Outcome of Procedure’s Use:

At the end of this SOP, the reader should thoroughly understand the process by which aliquots are created beginning with acquiring and subsequently thawing a Master Sample, then through the aliquot generation process and ending with the storage of the aliquots and the remaining Master sample. We will also include a detailed protocol for the shipment of samples.

Signature:

Technical Director:

Theodore E. Mifflin, Ph.D.

Date

SOP Training and Awareness Signoff List CCL Staff Training Records

My signature below indicates that I have read and understand the contents of this document as part of the CCL training process. If I locate or find errors and items to be corrected, I will notify my supervisor or the laboratory director as soon as possible.

Distribution and Signoff List

Name of Laboratory Personnel	Training Date	SOP Version	Signature

Comments _____

CCL Procedure for Creating Aliquots from Master Sample

A. Introduction --- General text description of procedure

1. This SOP describes the procedure by which CCL creates a series of labeled aliquots beginning with a source container ("Master Sample"). The Master Sample is first thawed and remixed thoroughly, then defined volume portions ("aliquots") are removed from the Master sample and delivered into specified number of pre-labeled vials. These vials are then capped, arranged into a standard storage boxes and placed into a -80 °C freezer for long term storage or until retrieved. All of the Master sample is consumed in the creation of the aliquots and none is retained.
2. The SOP also describes how the aliquots in cryovials with various sample volumes will be distributed into sample storage boxes. These are labeled and then distributed into CCL's -80 °C freezers, sent to analysis laboratory(s) or sent for storage to NIDDK Biorepository in their -80 °C freezers.
3. The SOP also describes how a series of labeled aliquots are defined as 'Blinded Replicates' and then distributed into the main collection of unknown samples. These aliquots are destined to be sent to service laboratories for analysis.
4. The basic premise of the Blinded Aliquot Generation process is that a series of vials have distributed among them samples that originate from the Master Sample that are 100% representative of the Master Sample. Adhering to this concept is essential of the contents in the Aliquot vials are presumed to be exact replicas of the Master Sample from which they were created.

B. Procedure

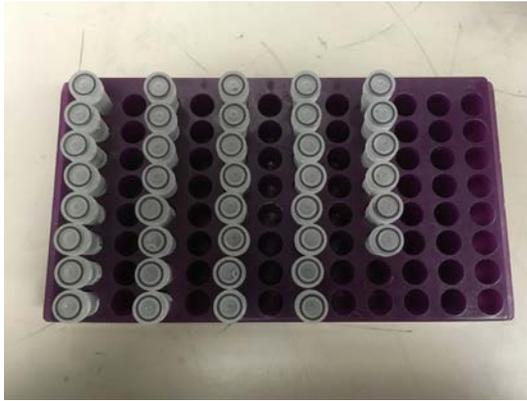
This procedure will be performed in CCL laboratory space, Rm 1-177. All workspaces will be cleaned prior to starting the procedure. Also ensure that the dry block incubator is operating at 25.0 °C. (Please check temperature of dry block(s) before starting procedure using a digital thermometer)

1. **SETUP Procedures for Aliquotting Protocol**
 - a. Disinfect and clean all equipment to be used before and after use. The equipment can be cleaned using Conflit laboratory spray and/or sterile alcohol prep pads.
 - b. Prior to processing, lay a sheet of bench paper (or equivalent) onto the work surface area
 - c. The samples will be handled in accordance with existing CCL policies regarding observance of Universal Precautions.
 - d. Aliquotting will be performed using manual equipment (e.g., multichannel pipettors) and sterile disposable pipet tips.
 - e. Aliquots and Master Sample vials are temporarily stored in CCL at -80 °C in accordance with client instructions for that group of samples.

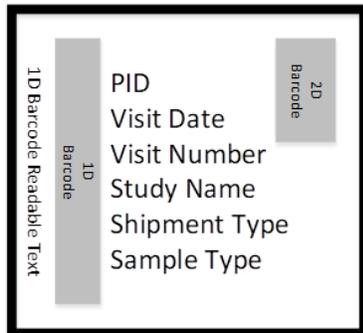
2. **Check-in and verification of Master Samples**
 - a. If Master Samples are from another source outside of CCL, then each Master Sample Vial needs to be verified against the shipping manifest.
 - b. If Master Samples are from within CCL, then each Master Sample Vial needs to be verified against the Pulling manifest.
 - c. Aliquotting will be performed using manual equipment (e.g., multichannel pipettors) and sterile disposable pipet tips.
 - d. CCL will confirm Master Sample IDs with sample manifest upon samples arrival into lab.
 - e. An annotation is to be added to the manifest if the Master Sample vial has any discrepancies such as damaged or unreadable label, it is underfilled or the Master Sample vial is damaged and /or leaking its contents.

3. **Thawing of Master Sample vials**
 - a. Master Sample vials should be kept in -80 °C freezer until the day they are to be used for creation of aliquot vials.
 - b. Once the entire group of Aliquot Vials have been arranged in to the 8x12 sample racks, then corresponding source samples (e.g., Master Samples) can be removed from the -80 °C storage and placed into the dry block set at 25.0 °C for thawing.
 - c. Thaw all of the Master Samples to be aliquotted in a dry block (@~25.0 °C) and remove promptly when the material has thawed.
 - d. To ensure complete thawing of each Master Sample, visually inspect each vial for completeness of thawing.
 - i. Slowly invert vial COMPLETELY 3 times and observe for ice pieces / unthawed sample) still present in the vial—
 1. Samples with ice are returned to block for **3 minutes** then rechecked and this process is continued until sample is completely thawed.
 2. Invert vial and observe color uniformity across the sample--dark gradient pooled towards one part of the vial indicates incomplete thaw—samples with irregular color uniformity are inverted and returned to block for **1 minute** then rechecked and this process is continued until sample is completely thawed.
 - ii. Once a Master Vial has been thawed completely, it should be placed into an ice bath / refrigerator where it is maintained while it awaits processing.
 - e. Thawed specimens are then mixed by vortexing on low for 30 seconds. Samples should not be allowed to stand more than 10 minutes to lessen risk of settling of precipitates that occurred upon the thawing process. If samples sit more than 10 minutes, samples must be vortexed on low for 30 seconds an additional time.

4. **Labeling Aliquot Vials for each Master Sample**
 - a. Arrange the Master Samples into 8x12 sample racks as pictured below. The order in which the Master Samples were in the source box should be maintained.



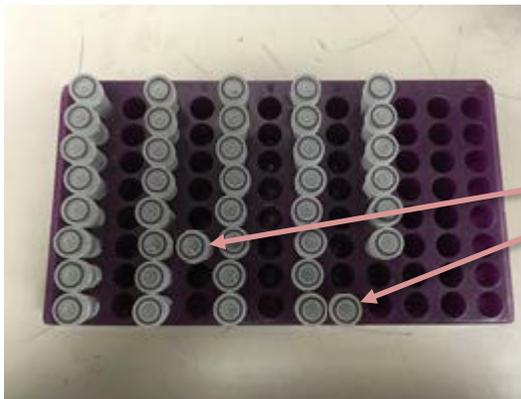
- b. Log on to Sample Processing System (SPS), CCL Sample Processing Software. Using the Identity of the first Master Sample vial ID in Row #1 (Vial # A1), use SPS to create a series of Aliquot Vial labels by switching the SPS function to “Store.”
- c. Scan the barcode for the Master Sample source box. The Master Sample source box is, in most cases, a box of CRIC samples from the CCL sample population. These should be labeled with a 2D barcode that when scanned into CCL Sample Processing Software (SPS) will display the entire contents of the box. An image of the source box will be pictured. Scan the SPS “Aliquot Box” barcode. This feature will generate a new barcode for each aliquot in one long strand in the order in which they appear in the box. The labels will maintain the same format as the original labels- patient ID (PID), visit date, visit number, study name, shipment type, and sample type. Each aliquot has a universally unique ID (UUID), which is automatically assigned by SPS. The UUID is a 28-digit barcode, which is fully encoded as a 2D barcode on each label. A 1D version, a linear barcode, is also included on each label for use in labs that do not have 2D barcode scanners. This barcode only encodes the first 8 characters of the UUID.



- d. Once the Aliquot Vial labels have been printed for all Master vials, verify correct order by comparing against the order of the Master vials.
- e. Once the Aliquot Labels have been verified, then they should be attached to the corresponding (empty) Aliquot vials (0.5mL or 2mL are available tube sizes).
- f. Arrange the labeled Aliquot vials into standard 8x12 sample racks in a linear arrangement to allow for proper alignment with corresponding Master Sample.

5. Creation of Blinded Replicate Vials

- a. The number of Blinded Replicates is typically set at 5% of the total number of Master Vials. This can be changed to a fixed number if 5% of the Master Vial population would not be significant population of Blinded Replicates.
- b. In the “Store” feature of SPS, scan the box barcode containing the Master Vials. Scan the SPS barcode “Obfuscate.” SPS will randomly select samples to be blinded. They will be marked in the box image pictured in SPS, being colored black.
- c. A blinded aliquot label will be automatically printed at the time of creation. The format will match that of the aliquot vials. However, the PID will be altered to blind the sample. The non-existent CRIC site “0901” is used for blinded samples. The “0901” PID is linked to the actual CRIC PID in the database. This linkage is only viewable to the user at the time of creation, a table will appear on the SPS screen indicating which PIDs are linked. The linkage is transferred to SDCC nightly, in the CCL data transfer. CCEB Biostats will have access to the actual PID as well as CCL administrators.
- d. Label a 2.0 mL aliquot vial with the blinded sample label. Confirm that the linked PIDs are next to each other, referring to the linkage table on SPS, placing the blinded sample tube next to the Aliquot vial, on the right in the empty column, in the 8x12 sample rack as arranged in step 4-a.



Blinded samples are placed in the 8x12 sample rack, next to the aliquot tube that is labeled with the actual PID. The blinded PID and the actual PID are linked in SPS. Arranging samples in this way allows for both aliquots to be created from the Master Vial at the same time.

6. Filling of Aliquot Vials and Blinded Sample Vials from Master Sample

- a. Remove the Master Sample vial cap and place the cap on the bench paper on the opposite end of the Aliquot Vials.
- b. Samples should not be allowed to stand more than 10 minutes to lessen risk of settling of precipitates that occurred upon the thawing process. If samples sit more than 10 minutes, samples must be vortexed on low for 30 seconds an additional time.
- c. Adjust a single volume dispense pipet to pipet the desired aliquot volume and attach a pipet tip to it.
- d. Remove the designated volume of sample from the Master Sample container and then dispense that volume into the first labeled Aliquot vial.
- e. Repeat steps a. through c. using a clean tip for subsequent Aliquot vials until all of the Aliquot vials designated to be created from that Master Sample have been filled with plasma or serum sample. Samples should be aliquoted from left to right until all aliquoting is completed.

- f. For those samples which have been designated as a source for blinded samples, remove the designated volume for the blinded sample from the Master sample container. Dispense that volume into the labeled blinded sample vial, at the same time that its linked partner sample is dispensed. Creating these aliquots at the same time, on the same thaw, helps to ensure that there are no variables between the two samples, they should be true duplicates.
- g. In case there is inadequate volume of sample from Master Sample to create all of the desired number of Aliquot Vials, then create as many full aliquots as possible. Using the last remaining (partial) aliquot, dispense it into the next empty vial.
- h. Annotate this partial fill volume for this aliquot vial so it can be recorded into SPS later.
- i. Replace the cap back onto the Master Sample container and place the caps onto all of the correspondingly filled Aliquot Vials.

7. Randomization of Blinded Replicate Aliquot Vials for Incorporation Aliquot Vial Population

- a. After all of the Master Samples have been aliquotted, then the collection of blinded samples need to be randomly incorporated into sets of corresponding Aliquot vials that will be sent to analysis laboratories.
- b. Combine all of the sealed Blinded Vials (BRVials) into a large clear plastic "Randomizer" bin with a secure lid.
- c. When all of the BRVials have been added into the clear plastic bin, close the lid and gently but completely invert the box 3x.
- d. Return the bin to the upright position and visually inspect the vials to establish that there had been complete mixing.
- e. Repeat steps c. and d. another time.
- f. Remove the bin lid and combine all of the sealed Aliquot vials into the large clear plastic bin with a secure lid so that it now contains all of the BRVials and Aliquot vials.
- g. Close the lid and gently but completely invert the box 3x.
- h. Return the bin to the upright position and visually inspect the vials to establish that there had been complete mixing.
- i. Repeat steps g. and h. another time.
- j. Remove the lid and remove the randomized collection of vials into new 2" storage boxes. Completely fill as many boxes as is required to empty the plastic bin. Each 2" storage box will now contain a random mixture of BRVials and Aliquot vials.
- k. Visually inspect the Randomizer bin for any remaining vials, then replace the lid, invert, and check for any noise created by one (or more) vials that were not removed from the bin. If no noise is heard, then the filling of the UPENN/CCL boxes is complete.
- l. Once each 2" storage box has been filled or there are no more Aliquot Vials to be stored, then the filled box(s) should be scanned into SPS so that their ultimate destination is recorded by registration into SPS.
- m. Be sure to record the volume of liquid in each vial as required by SPS.
- n. Once the samples vial boxes have been registered into SPS, they can be transferred to a CCL -80 °C freezer for storage or placed into the CCL Shipping Freezer awaiting outbound shipment to their ultimate destination.

8. Shipment of Samples to Outside Laboratories

- a. For those samples that will be tested at labs other than CCL, samples must be shipped overnight on dry ice. This maintains the samples in a frozen condition.
- b. Communicate with receiving lab and confirm availability to accept a shipment of samples.
- c. Once confirmation from receiving lab is established, remove the samples from CCL -80 °C freezer.
- d. Locate a Thermosafe shipping container. The shipping container is an insulated Styrofoam cooler with a cardboard box exterior. Ensure that the shipper is in good condition, taking particular care to inspect the interior cooler, checking for cracks and wear.
- e. Fill the bottom of the cooler with a layer of dry ice.
- f. Log on to SPS and open the “Store” module. Select create new container and select a 30 slot shipper as your container. Begin scanning the box barcodes into the shipper until all boxes are scanned into the shipper in SPS. Export the SPS manifest of the shipper contents. This manifest includes the sample 1D barcode, 2D barcode, PID, visit date, visit number, volume, number of thaws, box 1D barcode, box 2D barcode, and the placement of each sample within the box.
- g. Place a rubberband around each box of samples.
- h. Place each box inside of a biohazard plastic bag, along with an absorbent square to soak up any liquid in the event of an unintentional thaw.
- i. Place all banded and bagged boxes into the shipper.
- j. Surround all of the boxes with dry ice, on the top and sides of the stacks of boxes.
- k. Place the cooler lid on top and seal the outer cardboard box with packing tape. Do not seal all of the seams entirely. There must be room for dissipating dry ice gas to escape from the box.
- l. Affix a dry ice UN1845 label on the outside of the package, making note of the amount of dry ice included in the package.
- m. Ship the package via UPS or FedEx overnight and call for a pickup
- n. The package must be picked up directly from the lab. Do not leave the package at public drop stations. If the pickup does not occur before lab closing, the package must be disassembled and the contents must be stored back in a CCL -80 °C freezer. A new attempt at shipping can be made on the next day.
- o. Send the tracking number and shipping manifest to the responsible party at the receiving lab. Be sure to copy the data coordinating center.
- p. The next day, track the package and confirm receipt and condition of shipment with the receiving lab.

C. Problems that may appear during use of procedure

1. Several problems could occur during the execution of this SOP. They range from being minor in impact to ones with major consequences that need substantial attention to resolve.
2. These problems will be noted in the Attached Appendix (Appendix A - Problems and Resolutions).

D. Comments or Notes on Procedure

1. This protocol was derived from one that was established to use an automated liquid handler (HAMILTON Star) to create aliquots. This protocol has been edited to reflect the manual nature of the procedure.
2. A key objective has to be that all samples are completely thawed and thoroughly remixed to 100% homogeneity before the aliquots are created. If remixing is not done completely, aliquots of irregular composition will be created.

E. Cited References or Documents for Referral

1. CCL SOP on Processing TRANSCOLD and TRANSDRY Samples.
2. Automated Aliquot Generation Protocol, Fisher Biosciences, Rockville, MD.

F. Equipment Required for Thawing Samples

1. 4 Block dry bath incubator, adjustable temperature, Fisher Isotemp. Fisher Scientific, Pittsburgh, PA
2. Manual (RAININ) pipettors capable of dispensing multiple aliquots of urine.
3. Cryovials, 0.50 and 2.0 mL, sterile with graduations and screw cap with O-ring. Denville Scientific, Somerville, NJ.
4. FrezrBond Labels, 1x1" to label 2.0 mL vials and 2' freezer boxes. BRADY Corporation.

APPENDICES

Appendix - A

Problems and Resolutions Associated with Performance of this SOP

1. **Problem:** There is insufficient volume of a Master Sample container from which to create the requested number of Aliquot Vials (e.g., total = 14). There is only one resolution for this problem.

Resolution: Solution is to consume the entire volume to create as many Aliquot Vials as can be produced at the desired volume / aliquot. Since the vials are filled left ⇒ right (i.e., smallest volume to largest aliquot volume), then the last vials filled are expected to contain 1.0 mL volume /vial. Any vial that is filled to < 1.0 mL of sample should have an annotation stating “fill volume is 0.XX mL” when that vial is scanned into SPS.

2. **Problem:** The MASTER Sample Vial is cracked and or leaking fluid. There is no acceptable resolution to this problem as the composition of the material remaining inside the vial is likely different compared with other MASTER Sample Vials created from the original same sample.

Resolution: CCL should ask for another Master Sample vial of the same ID #. If this is not available, then aliquots cannot be created for this sample.

3. **Problem:** The Dry block incubator is not on or does not display the correct thawing temperature of 25.0 °C. The incubator needs to be operating at the correct temperature at least for 4 hours prior to placing vials into it for thawing.

Resolution: Check the unit is plugged into the electrical outlet and is switched on. If display doesn't illuminate, connect the unit to another outlet and try again. If no operation, obtain another dry block incubator and adjust it operate at 25.0 °C.

Appendix – B

Symbol Scanner Adjustment Barcodes

1. Barcodes to Disable and Enable 1D Barcode Scanning



***Enable Code 128**



Disable Code 128

2. Barcodes to Adjust Scanner Focus



***Far Focus**



Near Focus



Smart Focus

Appendix - C

Project Specific Aliquotting Plans

Project name: BioCon Metabolomics

Parent study samples to be pulled and aliquoted: CRIC

Sample type: Plasma

Shipment type: TRANSDRY

Study visit(s): All samples are from V5 (Year 1)

File name of sample pull list: 7.7.17 BioCon Eugene Rhee EDTA

The table below describes the number of aliquots to be created from each Master sample as well as the volume and destination of each aliquot.

Aliquot #	Volume	Destination laboratory	Planned assays for this aliquot
1	200 uL	Broad Institute	Metabolomic profiling
2*	200 uL	Metabolon	Metabolomic profiling
3**	200 uL	Broad Institute	Metabolomic profiling (blind duplicate)

*90 samples are identified in the sample pull list as requiring a second aliquot which will be sent to Metabolon. All other samples require only Aliquot #1 to be sent to the Broad Institute.

** 5% of samples will be randomly selected by the CCL for creation of blind duplicate samples to be sent to the Broad Institute.

Box Labelling instructions:

All samples being shipped to the Broad Institute should be boxed together and the boxes should be labeled as BioCon Metabolomics samples from the CRIC study for the Broad Institute.

All samples being shipped to Metabolon should be boxed together and the boxes should be labeled as BioCon Metabolomics samples from the CRIC study for Metabolon.

