SAMPLE PROCESSING SOP

Purpose: To standardize a processing procedure for sample aliquoting in the ACRL.

Procedure:

1. Processing:

- a. One unit (or vial) of the sample type is thawed overnight in the walk-in cold room for processing.
- b. Bar-coded labels are prepared and printed. (See attachment #1 label template).
 - The labels are 1-2 ml vial size Portrait Wraparound (self laminating, thermal, strongly adhesive in low temperatures). Catalogue #LBL-02WR from Dataworks.
 - Printer is a Zebra brand, model # TLP-2824 Plus
- c. Labels are affixed to 2.0 mL cryovials for aliquoting.
 - One label for each aliquot to be made.
- d. Unit or vial is removed from the 4°C walk-in cold room, mixed by inversion 8x, and centrifuged in a refrigerated centrifuge for 10 minutes at (3,000 x g).
- e. Remove centrifuged source vial.
- f. The Tecan Robotic Liquid Handling System (Tecan Genesis Freedom 200 model), is used to aliquot desired volumes of the sample from the centrifuged source tube into the pre-labeled cryovials.
 - Source sample vials and pre-labeled vials are placed in the racks on the platform.
 - ➤ A script is written denoting the sample volumes to dispense from the source vials using the Tecan software.
 - An aspirate/dispense sequence with tip changes between samples is programed.
 - > Start is then entered for the aliquoting process to begin.
 - ➤ Upon completion of the aliquoting sequences, the aliquots are capped and placed into freezer boxes (See #2 Storage illustration, 81 slot freezer boxes are used).
 - Place freezer boxes of aliquots in freezer racks.
 - Place racks in assigned freezer to ship out.

- g. Manual Backup Procedure (non-automated): Affix bar-code labels to 1.0 mL or 2.0 mL cryovials and place in racks on a wet ice bed dispense desired amount of sample into the vials using an appropriate Eppendorf Repeater Pipette.
 - Cap ans place in 81 slot freezer boxes, continue from this point as described for automated procedure.

2. Storage: (Relative to Specific Project)

- a. 2 inch cardboard freezer boxes are labeled as Box 1 of x ,
 Sample-Type, Sample 01 Sample x.
- b. Freezer boxes are marked to indicate a start position.
- c. Freezer boxes are placed into freezer racks.
- d. Freezer boxes/racks are stored in a -80°C Freezer.

3. Inventory: (Relative to Specific Project)

- a. Storage location is logged manually in an excel spreadsheet kept on the master PC (F740).
- b. Storage location and inventory are entered and managed in the FreezerWorks Inventory Software.
- c. Immediately refreeze source sample after aliquoting and update storage inventory. Be sure and mark tube as 1 x thaw, 2 x thaws cycle, etc.

4. Packaging:

- a. Use a double box (styrofoam inner box and cardboard outer box) biomailer.
- b. Attach manifest enclosed in a zip lock plastic bag to lid of styrofoam box.
- c. Place a layer of dry ice on bottom of shipping biomailer, add labeled freezer boxes containing samples followed by another layer of dry ice and continue sandwiching process to capacity.
- d. Place styrofoam chips or other appropriate materials to fill dead space.
- e. Close the lid and seal the outer lid.
- f. Attach appropriate labels to box (dry ice, etc.)
- g. Schedule FedEx for pick up and print air bill.

5. Shipping:

- a. Attach manifest (Attachment 3) which consist of Investigator and Study Project name, contact name, email, telephone, shipping address, sample list distributed from CC, scan verification, sample type, sample volume, number of aliquots, sample placement illustration, areas for receiver to log information and sender contact information and laboratory address.
- b. Notify receiving site of shipping date and provide FedEx tracking number.
- c. Ship on Monday Wednesday priority overnight.

Visit 4 Sample Handling for Citrated Plasma & Urine Procedures Flowsheet

ARIC Coordinating Center (CC):

CC- Generates "pull list" which includes I.D.'s & randomized QC samples (blinded to Biorepository Lab.) that are de-identified from participant. The list is based on Investigator(s)' sample cohort request.

(ACRL) ARIC Biorepository:

Lab. receives list and signed DMDA approving sample pulling and distributing.

ACRL Sample Identification:

Lab builds a database in excel, copying list from CC and uploading into Freezerworks, inventory software program to obtain I.D. and specimen type match. The freezer location is generated. A lab. "pull list" is created based on the freezer location. Any missing samples are documented in the database and on list.

SHIPPING:

*Personnel are IATA certified for shipping via FedEx human biological speciment. Universal precautions are observed

- Manifest is generated (see attachment # 2)
- A layer of dry ice is placed on the bottom of the styrofoam inner box of the biomailer
- Boxes are removed from freezer and sandwiched between layers of dry ice to capacity.
- Styrofoam chips or other materials are used to fill dead space.
- Seal and ship overnight priority via FedEx
- Send tracking info to receiver when notifying of shipment and include electronic version of manifest.

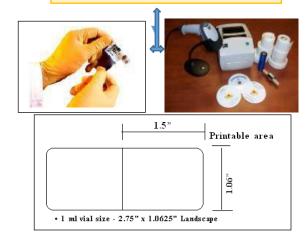
ACRL Sample Handling & Processing:

Universal precautions are observed for the safety of personnel.

- Label (81) slot freezer boxes to store aliquots & label 2.0 cryovials per list. (see LABEL attachment for details)
- Pull samples using dry ice bed for sample preservation & place in pre-labeled freezer boxes.
- Thaw pulled samples overnight in 4°C cold room.
- Mix by inversion 6-8 times
- Centrifuge @ 4°C for 10 min., 3,000 x g.
- Script for running the Automated Tecan Robotic Genesis Freedom Platform is written proportional to aliquot design (one for urine & one for citrated plasma).
- Pre-labeled bar code vials are placed in holders.
- Centrifuged source tubes are placed on platform.
- Cycle of (1) box of 81 vials is performed at a time.
- Pre-labeled aliquots are capped and placed in box 1
- Source vials are refrozed with pre markings of 1 thaw cycle.
- Box 1 of aliquots are placed in -80°C Freezer.
- Process continue as above steps.

ACRL Label Creation:

Labels, printers and scanners work with Freezerworks barcoding for sample tracking. Labels are a special wrap-around version that protects the bar code from temperature changes and adheres under extreme sub-temperatures.



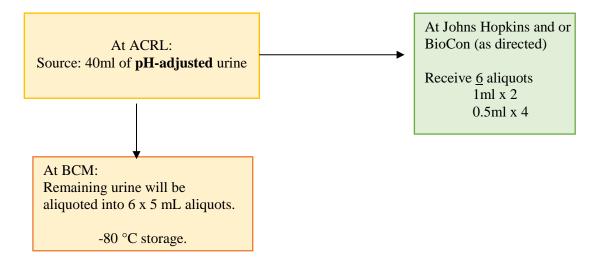
The Atherosclerosis Clinical Research Laboratory (ACRL) at the Baylor College of Medicine (BCM) has already re-aliquoted a large number of ARIC Visit 4 urine samples through funding from previous ancillary studies. The laboratory intends to continue this aliquoting protocol (as outlined below) with one exception that filtration of urine samples will not be part of the protocol in order to save on sample processing costs for future ancillary studies.

Visit 4 Urine Aliquoting Protocol Design

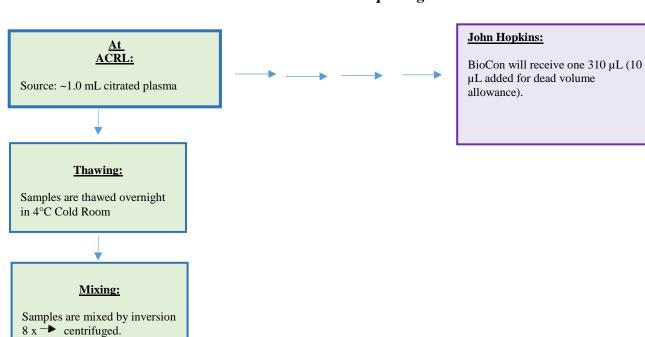
At BCM:

- 1. Urine specimen status
 - a. pH-adjusted urine
 - b. Volume: ~40mL
 - c. No freeze-thaw cycles
- 2. Protocol:
 - a. pH-adjusted 40ml vials
 - i. Identify, pull, and thaw samples (thaw overnight @ 4°C or 2-3 hours @ room temp)
 - ii. Invert 6-8 times per Baylor protocol
 - b. For BioCon: Using the thawed urine sample (~35ml) in the 40ml vials
 - i. Do NOT filter
 - ii. Aliquot (6 vials; total volume: 7.5 ml)
 - 1. 1 ml x 2
 - 2. 0.5ml x 4
 - iii. Ship (4) 0.5 mL aliquots and (2) 1.0 mL to Hopkins (Hagerstown) per Hopkins' protocol and/or some aliquots to BioCon as directed. Store others.
 - c. Aliquot the remaining urine sample (~30ml) from the original vials into 6 x 5mL aliquots & re-freeze -80°C storage at BCM (document one thaw cycle).

Visit 4 Urine Aliquoting Distribution Flowsheet



Visit 4 Citrated Plasma Aliquoting Distribution



Aliquoting:

Samples are thawed overnight in 4°C Cold Room and aliquoted into pre-labeled vials using the Robotic Tecan Sample Handling Platform.

Packaging/Shipping:

Freezer boxes with samples are packaged by sandwiching between layers of dry ice.

Manifest is placed on inside lid of styrofoam box inside cardboard box, sealed, labelled with appropriate FedEx labels and air bill is attached.

FedEx is scheduled for pick up and client notified with tracking #,

Shipment is made priority overnight.

ATTACHMENT # 1 -ALIQUOT LABEL(S) EXAMPLE

Citrate & Urine Label Example:

JXXXXXXCTR

Citrate Plasma ARv4 (0.310 mL)

JXXXXXXU1

Urine ARv4 1/4 (0.5 mL)

JXXXXXXU2

Urine Arv4 2/4 (0.5 mL)

JXXXXXXU3

Urine Arv4 3/4 (0.5 mL)

JXXXXXXU4

Urine Arv4 4/4 (0.5 mL)

JXXXXXXU5

Urine ARv4 1/2 (1.0 mL)

JXXXXXXU6

Urine ARv4 2/2 (1.0 mL)

Freezer box Label Example:

Study P.I.: Casey/Coresh ARIC v4 CKD BioCon Study (1.0 mL) pH Adj Urine Box # 1 of _____

Study P.I.: Casey/Coresh ARIC v4 CKD BioCon Study (0.5 mL) pH Adj. Urine Box # 1 of _____



ATTACHMENT # 2 – FREEZER BOX WITH SAMPLE LOCATION

						,,_					
					ВА	СК					
	R9	73	74	75	76	77	78	79	80	81	
	R8	64	65	66	67	68	69	70	71	72	
	R7	55	56	57	58	59	60	61	62	63	
	R6	46	47	48	49	50	51	52	53	54	
Left	R5	37	38	39	40	41	42	43	44	45	Right
	R4	28	29	30	31	32	33	34	35	36	
	R3	19	20	21	22	23	24	25	26	27	
	R2	10	11	12	13	14	15	16	17	18	
	R1	1	2	3	4	5	6	7	8	9	
		C1	C2	C3	C4	C5	C6	C7	C8	C9	
					FRO	TNC					

ATTACHMENT #3 – MANIFEST EXAMPLE

Address: Condition Code: 01(Good)	ATHEROSCLEROSIS CLINICAL RESEARCH LABORATORY (ACRL)													
Houston, Texas 77030		ACRL							ACRL Contact					
		•												
Date Received: By:	Study Name:	CKD BioCon St												
Condition Code: 01	Investigators:	Casey Rebholz/			Recipients Use Only									
Condition Code: 01(Good)	Ship To:		Date Re	ate Received:By:										
Contact Person:	Address:													
Contact Person:						-								
Contact Person:							Condition Code: 01 (Good)							
Contact Person: Fax:									,					
Specimen Log: Shipment Date: Batch# Initials: LA Recipients	Contact Person:													
Specimen Log:			Fax:				6(Short sample. or no sample.)							
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F103140 plasma 310 μL v4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	F102224			310 μL	v4	1			1					
ACRL Address	F103140			310 μL	v4	1			1					
ACRL Address														
	ATHEROSCLEROSIS CLINICAL RESEARCH LABORATORY (ACRL)													
Houston, Texas 77030		ACR	L Addres	ss							ACRL Conta	ct		
Study Name: CKD BioCon Study Project Investigators: Casey Rebholz/Joe Coresh			1											
Nestigators: Casey Rebholz/Joe Coresh														
Condition Code: 01(Good) Code: 02(Thawed-Cool) Code: 03(Thawed - Warm) Code: 06(Short sample. or no sample.) Code: 07(Other) Code: 08(Short sample. or no sample.) Code: 07(Other) Code: 08(Short sample. or no sample.) Code: 07(Other) Code: 08(Short sample. or no sample.) Code: 08(Short sample. or no sample.) Code: 08(Stort sample. or no sample.) Code: 08(Other) Code: 08(Other) Code: 08(Other) Code: 08(Other) Code: 08(Other) Code: 08(Short sample. or no sample.) Code: 08(Other) Code: 08(Short sample. or no sample.) Code: 08(Other) Code: 08(Other)	_													
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Code: 02(Thawed-Cool) Code: 03(Thawed - Warm) Code: 03(Thawed - Warm) Code: 04(Short sample. or no sample.) Code: 05(Other)											-			
Code: 02(Thawed-Cool)														
Code: 02(Thawed-Cool)														
Contact Person:														
Phone: Fax: Code: 06		Code: 02(Thawed-Cool)												
Code: 07(Other) Code: 07(Other)		Code	Code: 05(Inawed - warm)											
Specimen Log: Shipment Date: Batch# Initials: LA														
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