

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 programmed.
 - 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.

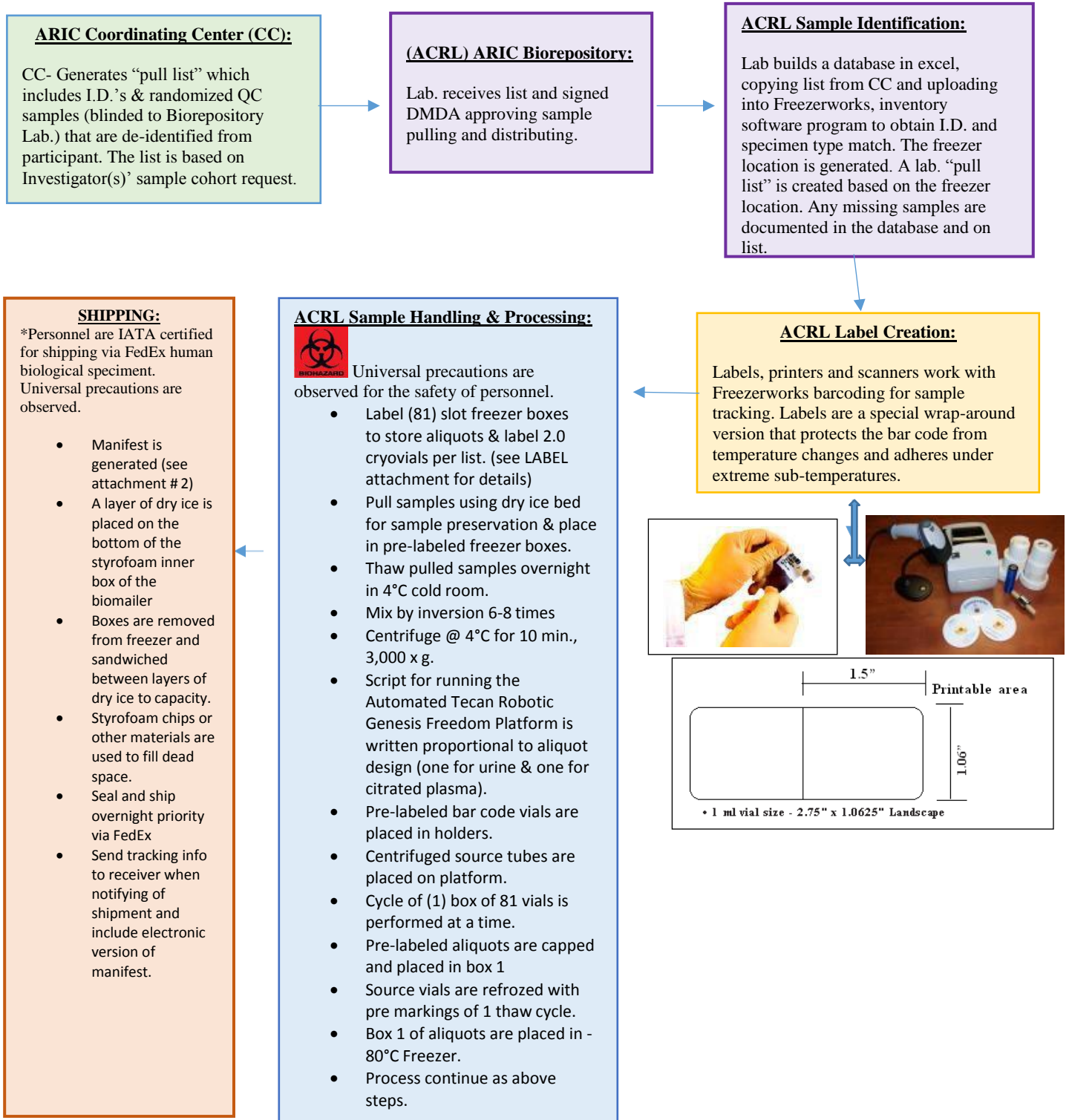
4. Packaging:

- 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



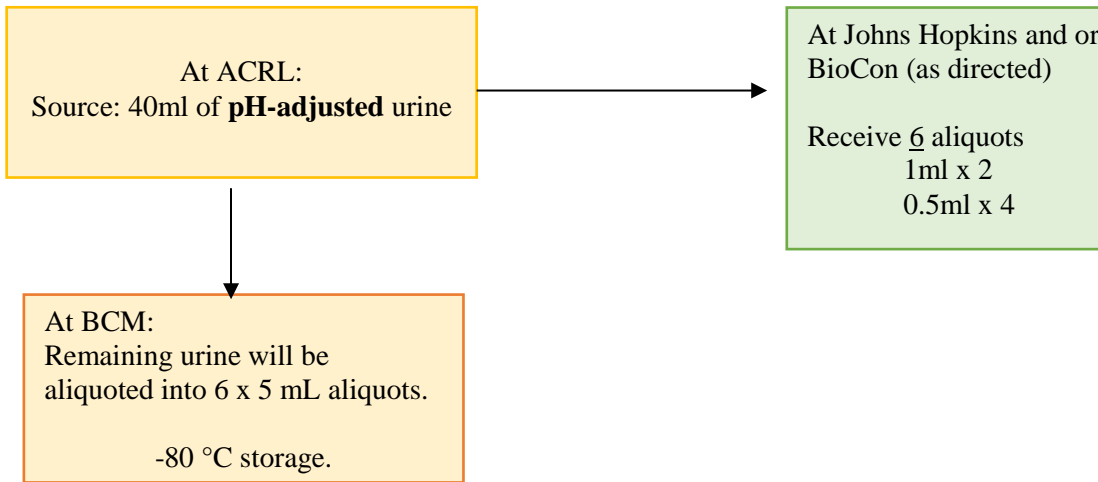
The Atherosclerosis Clinical Research Laboratory (ACRL) at the Baylor College of Medicine (BCM) has already re-aliquoted a large number of ARIC Visit 4 urine samples through funding from previous ancillary studies. The laboratory intends to continue this aliquoting protocol (as outlined below) with one 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

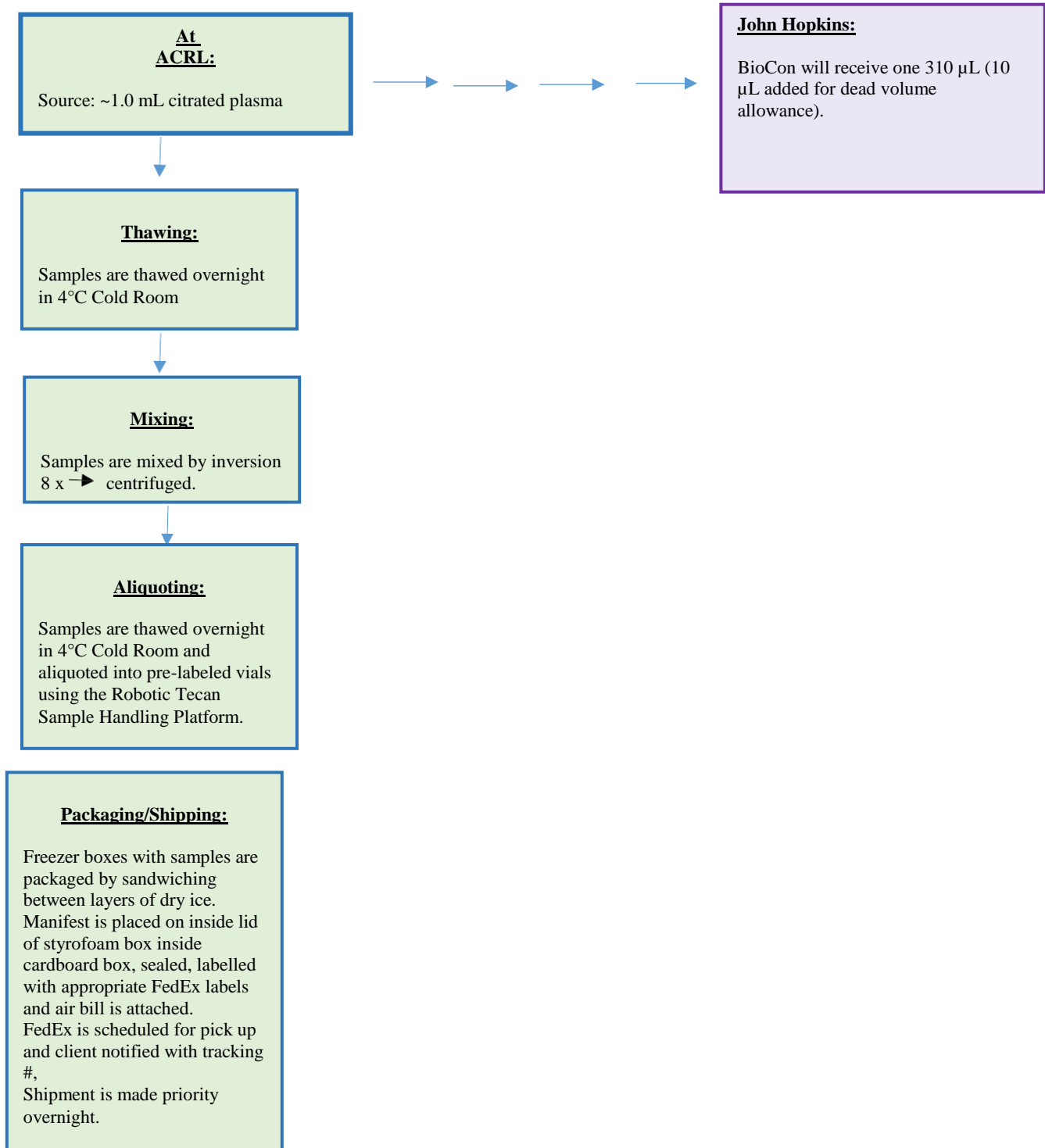
At BCM:

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





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

					BACK							
	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		
					FRONT							



ATTACHMENT # 3 – MANIFEST EXAMPLE

<i>ATHEROSCLEROSIS CLINICAL RESEARCH LABORATORY (ACRL)</i>										
ACRL Address					ACRL Contact					
6565 Fannin Street, M.S. F-701 Houston, Texas 77030					Joe Raya: jraya@bcm.edu Phone: (281) 216-5592					
Study Name: CKD BioCon Study Project Investigators: Casey Rebholz/Joe Coresh					Fax: (713) 798-7400					
					Recipients Use Only					
Ship To:					Date Received: _____ By: _____					
Address:					Condition Code: 01____(Good) Code: 02____(Thawed-Cool) Code: 03____(Thawed - Warm) Code: 06____(Short sample. or no sample.) Code: 07____(Other)					
Contact Person:		Fax:								
Phone:										
E-mail:										
Specimen Log:										
				Shipment Date:		Batch#		Initials: <u>LA</u>		
ARIC I.D.	Unique Accession I.D.	Sample Type	Sample Vol.	Study Visit	# Vials	Box #	Sample Position #	Thaw Cycles	Comment(s)	Recipients I.D. Scan Ck
F100920		citrate plasma	310 µL	v4	1			1		
F102224		citrate plasma	310 µL	v4	1			1		
F103140		citrate plasma	310 µL	v4	1			1		

<i>ATHEROSCLEROSIS CLINICAL RESEARCH LABORATORY (ACRL)</i>										
ACRL Address					ACRL Contact					
6565 Fannin Street, M.S. F-701 Houston, Texas 77030					Joe Raya: jraya@bcm.edu Phone: (281) 216-5592					
Study Name: CKD BioCon Study Project Investigators: Casey Rebholz/Joe Coresh					Fax: (713) 798-7400					
					Recipients Use Only					
Ship To:					Date Received: _____ By: _____					
Address:					Condition Code: 01____(Good) Code: 02____(Thawed-Cool) Code: 03____(Thawed - Warm) Code: 06____(Short sample. or no sample.) Code: 07____(Other)					
Contact Person:		Fax:								
Phone:										
E-mail:										
Specimen Log:										
				Shipment Date:		Batch#		Initials: <u>LA</u>		
ARIC I.D.	Unique Accession ID	Sample Type	Sample Vol.	Study Visit	# Vials	Box #	Sample Position #	Thaw Cycles	Comment(s)	Recipients I.D. Scan Ck
F100920		urine		v4				1		
F102224		urine		v4				1		
F103140		urine		v4				1		

Doc# _____



Atherosclerosis Clinical Research Laboratory Effective Date: 8/7/2011