National CADDRE Study
Summary of Biosample Shipping, Processing and Storage

Shipping

Buccal Swab

After completion, swabs are returned to their packing tube and then placed in the mailer for shipping. Samples will be sent directly by the participant to the study Core Lab and Biosample Repository (CLBR) where they will be stored at -80ºC until DNA is extracted. CLBR staff, Data Center staff and study site staff will communicate on the receipt status of buccal samples. Participants whose samples are not received by the time of the clinic visit will be given an opportunity to complete the sample at the time of the clinic visit. In addition, as discussed below under sample processing, subjects providing samples that fail to amplify on a test PCR will be asked to supply another sample (this could be done at the clinic visit or may require another mailing).

Blood

Table 1 outlines the local processing of each sample, sample labeling and shipping.

Table 1. Venous blood samples

Draw Order	Storage mode	Amount	Local Processing	Local Storage	Label	Ship
1	EDTA purple top tube	3 ml	Invert and keep at room temp	Store at room temp* until shipped	Barcode & Subject ID #	Ship that day
2	SST red top tube	6 ml	Invert and keep at room temp	Centrifuge and store at room temp* until shipped	Barcode & Subject ID #	Ship that day
3	ACD yellow top tube	6 ml	Invert and keep at room temp	Store at room temp* until shipped	Barcode & Subject ID #	Ship that day
4	ACD yellow top tube	6ml	Invert and keep at room temp	Store at room temp* until shipped	Barcode & Subject ID #	Ship that day
5	4-circle Guthrie card	<1ml	Air dry and store in paper envelop	Dry, store at room temp until shipped	Barcode & Subject ID #	Batch and ship
*Exception being Saturday blood draw – see text						

Blood tubes will be retained at ambient temperature and shipped via Federal Express to the CLBR the same day they are drawn. Attempts will be made to schedule the majority of blood draw on Monday through Thursday. Should Friday visits be needed to accommodate potential participants' schedules, arrangements will be made with the CLBR to have staff to receive sample on Saturday. In the event that Saturday visits are needed, tubes will be refrigerated at the study site 4° C until they can be shipped on Monday.

Guthrie cards, which will also bear study ID and barcode label, will be air-dried for a minimum of 3 hours. Cards will be placed in gas-permeable, zip-closure plastic bags with desiccant and humidity indicator strips and sent to the CLBR in the next possible overnight shipment (because of drying time it may be that cards cannot be sent with the blood tubes but would have to go with another days specimens). Samples will batched and shipped.

Hair

Hair samples will be shipped to CLBR either in the package containing the blood samples or batched and sent under separate cover.

Biosample Labelling And Management

Biosample labeling and management will be a joint effort of the Data Coordinating Center (DCC), study site staff, and the CLBR. A barcode system will be used on all biosamples to minimize human error and allow semi-automated sample tracking and inventory. Barcodes will include coding for person ID, sample type, and study site. The CADDRE Information System (CIS) being developed by the DCC will be used for barcode generation. The DCC will also integrate with the Freezerworks software used by the CLBR to manage stored biosamples. This integration will allow for remote access to the specimen-tracking database. Upon receipt of a sample from a participant or study site, the CLBR will cross-check contents with the shipping form and then scan the barcodes into the tracking database and store the shipping form. The study site will be notified by the CLBR of receipt and any discrepancies between the shipping form and the contents will be addressed. Records of all removal of sample (i.e., for shipment to collaborating laboratories for analysis) will be created in the data system and procedures and fields relevant quality control parameters for remaining sample, such as number of freeze thaw cycles, will also be tracked. The CLBR will inventory biosamples quarterly and issue regular reports.

Biosample Processing And Storage At The Central Repository

Buccal Swab

After receiving and logging in cytobrushes, the CLBR will store at -80°. DNA will be extracted from 1 of the 3 cytobrushes per subject within 30 days of the postmark using standard methods. The laboratory will test amplification of extracted DNA for a moderate-length fragment. If there is amplification failure, DNA from a subsequent brush will be extracted and tested for amplification. If the second brush does not amplify, participants will be recontacted to provide another sample. Failure rate for moderate-length fragments are around 2.5% (Garcia-Closas, 2001).

DNA will be divided into equal aliquots in 0.25ml cryovials labeled with the barcode and study ID and frozen at -80°. The other two cytobrushes for each individual will be retained at -80°.

The Sample Tracking database will be updated to show DNA quantity available and document location of samples.

SST Tube Blood Samples

After receiving and logging in SST tubes, serum samples will be saved in 0.5ml aliquots and stored at -80°.

Yellow-top Tube Blood Samples

After receiving and logging in yellow-topped tubes, lymphocytes will be separated and cryopreserved for potential future immortalization. This will involve saving cells in RPMI with fetal bovine serum, adding DMSO, distributing into four aliquots, storing in liquid nitrogen in a programmable freezer. Cryovials will be labeled with ID numbers and bar codes and location and volumes will be noted in the Sample Tracking database. The extracted plasma will be saved in 0.5ml aliquots in labeled tubes and frozen at -80°.

Purple-top Tube Blood Samples

Purple-topped tubes will be logged in upon receipt. One aliquot of 0.5ml of whole blood will be frozen at -80°. Another aliquot of 0.5ml blood will go immediately to DNA isolation. Extracted DNA will be frozen in 0.25ml aliquots at -80°. The remaining sample will be spun down and plasma will be saved in 0.5ml aliquots and stored at -80°. Half of the PBMCs will be saved in 1.0ml aliquots in DMSO and stored at -120° and the remaining PBMCs will be aliquoted and stored as dry pellets in 0.25ml tubes at -80°. All storage tubes will be labeled with ID numbers and bar codes and locations and volumes will be noted in the Sample Tracking database.

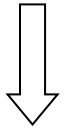
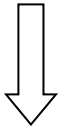
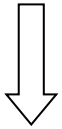
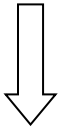
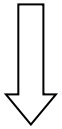
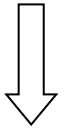
Guthrie Card Blood Samples

After receiving and logging in Guthrie cards, the CLBR will store cards at -20°.

Hair

After receiving and logging in hair samples, the CLBR will store the samples in their envelops at ambient temperature.

BIOSAMPLE STORAGE SUMMARY

BIOSAMPLE AS COLLECTED:	3 Cytobrush Buccal Swabs	3ml of Venous Blood in Purple top Tubes	12ml of Venous Blood in Yellow Top Tube	6ml of Venous Blood in SST Top Tube	<1ml of Venous Blood on 4 Circle Guthrie Card	Approximately 100 hairs from the occipital region
						
BIOSAMPLE AS STORED:	• 2 brushes at -80° • 0.25 ml aliquots of DNA from one brush (approx. 4µg) at -80°	• 0.5ml whole blood at -80° • 0.25ml aliquots of DNA from 0.5ml blood at -80° • 0.5 ml red blood cells • Plasma in 0.3ml aliquots at -80	• Plasma in 0.5ml aliquots at -80° • 2 equal aliquots of PBMCs in DMSO at -120° • 2 equal aliquots of PBMCs in 1 ml DMSO with RPMI and FBS in liquid nitrogen • Dry-pelleted PBMCs at -80°	• 0.5ml aliquots of serum at -80°	• Cards stored at -20° with desiccant	Complete sample stored in individual envelop at ambient temperature