

Appendix C: Lab Processing Flow Sheet/Template for Specimen Collection

(Version 0.9)

In the attached diagrams, we show how the collected biospecimens are processed and packaged in the field for transport to the Central Laboratory (Appendix C1); how personnel at the central laboratory will further process the samples once they have been received (Appendix C2); and how personnel at Environmental Pathology Laboratories (EPL), the NIEHS Repository, will process the received samples prior to placing them into liquid nitrogen (LN2) vapor phase ($\sim -140^{\circ}\text{C}$), -80°C mechanical freezers, -20°C walk-in freezers, or into temperature and humidity controlled ambient storage. Note that wherever possible aliquots of the various specimen types are divided into two separate storage locations to ensure that at least part of the sample will survive in the very unlikely event that one storage device should fail catastrophically.

Appendix C1:

This diagram schematically illustrates processing of hair, blood, urine, toenails, and dust samples by the home health agent (HVA) while in the study participant's home.

- Only the two red top blood collection tubes need processing in the field. After clotting for 30 minutes, the HVA will centrifuge the samples for 10-15 minutes at $2500 \times g$, and removed the serum supernatant into two 5 ml aliquot tubes. The stopper will be replaced on the red top tubes with the residual clots and these are placed into biospecimen bag #1 along with the remaining 6 blood collection tubes (2 lavender, 1 royal blue, 1 yellow, and one PAXgene tube). The 2 ml lavender top tube will be placed in Biospecimen bag #2 for diagnostic testing (CBC with WBC differential) at the central laboratory. Biospecimen Bag #1 is placed in the foam shipper along with a frozen icepack.
- The HVA will use a BD transfer straw to remove 8 ml of urine from the urine collection cup and place it into an 8 ml urine transfer tube. The urine transfer tube will be placed in Biospecimen Bag #3 along with the 2 ml lavender top tube. This sample will be used for the dip stick urinalysis at the central lab. Biospecimen Bag #2 is placed in the foam shipper.
- The remaining urine sample (in the tightly re-sealed original collection cup) is placed into Biospecimen Bag #3. Biospecimen Bag #3 is also placed in the foam shipper. Once all three biospecimen bags are in the foam shipper along with the frozen ice pack the lid is placed on the shipper and it is inserted into the outer cardboard shipping box.
- Hair and nail samples are sealed in labeled manila envelopes and placed on top of the foam shipper lid in the exterior cardboard shipping box.
- Dust is collected using the NCS study protocol. This dust sample will be placed in the ZipLoc™ bag, which is also placed on top of the foam lid in the outer cardboard box.

The Cardboard shipping box is sealed and either labeled for overnight shipping via FedEx, or the HVA can transport the box to a local Central Laboratory Patient Service Center for further processing and shipping.

Appendix C2.:

The Central Laboratory will follow the steps outlined in this appendix.

- ***Biospecimen Bag #1:*** The various blood samples in Biospecimen Bag #1 will be processed as shown.
 - Serum will be divided into two sets of five 1 ml aliquots in 1 ml cryovials. Half of the serum aliquots will be placed in freezer storage Box A and the other half in Box B.
 - The two EDTA (lavender top) and one ACD (yellow top) tubes will undergo a discontinuous Percoll gradient separation to isolate the white blood cells (buffy coat) from the plasma and red blood cells (RBC). The EDTA and ACD plasma and RBC fractions will be aliquotted into two different sets of 1 ml cryovials as shown. The EDTA and ADC buffy coat pellets will be stored in separate 1 ml cryovials. The plasma, buffy and RBC aliquots will be divided and half stored in Box A and half in Box B. Boxes A and B will be stored at -80° C in the Central Laboratory until they are shipped to EPL.
 - The two specialty tubes (royal blue-topped trace metals and PAXgene mRNA) are placed in freezer storage Box C along with the two red topped tubes with the red cell clots. Box C will be placed in a -20° C freezer until shipped to EPL.
- ***Biospecimen Bag #2:*** The samples in Biospecimen Bag #2 are sent directly to the testing area of the laboratory for analysis. The purple-topped whole blood specimen will be sent to the hematology section where it will undergo a complete blood count (CBC) along with a white blood cell (WBC) differential enumeration. The urine sample will be sent to chemistry for a dipstick urinalysis. The results of both assays will be reported electronically to SRA.
- ***Biospecimen Bag #3:*** The urine sample from Biospecimen Bag #3 will be aliquotted into four 5 ml aliquot tubes and two tubes will be placed in Box D and two in Box E. These samples will be stored at -80° C until shipped to EPL.
- ***Hair, Nail and Dust Samples:*** The envelopes containing the hair and nail samples will be placed in a cardboard Box G and stored at ambient temperature until sufficient samples have accumulated to be shipped to EPL. Dust samples will be placed in Cardboard Box H until sufficient samples have accumulated to send to EPL.

Appendix C3.

The top of this diagram illustrates how the Central Laboratory will package the various specimens for shipment to the repository at Environmental Pathology Laboratories.

- **Foam Shipping Box #1 and Box #2** will contain the biospecimens contained in Freezer Storage Boxes A and D, plus Boxes B and E respectively. These samples are divided in case one or the other of the boxes is damaged or delayed in shipment to the point that the samples thaw. Both boxes will be packaged with ~10 lbs of dry ice for overnight shipment to EPL.
- **Foam Shipping Box #3** will contain Boxes C, F, and I, which will be transported to EPL on frozen ice packs (not on dry ice).
- **Cardboard Shipping Box #4** will contain nail and hair samples. After a sufficient number of these samples have been accumulated, the Central Laboratory will ship these samples to EPL at ambient temperature.
- **Cardboard Shipping Box #5** will contain the dust samples. After a sufficient number of these samples have been accumulated, the Central Laboratory will ship these samples to EPL at ambient temperature.

Once shipments have been prepared, the Central Laboratory will send them to EPL via overnight FedEx shipment. Foam Shipping Box #1 and Box #2 will never be sent in the same shipment to preclude total loss of samples from a given set of subjects.

Once the samples have been received at EPL, each frozen sample will have a BSI ID label (Biological Specimen Inventory System, <http://www.bsi-ii.com/>) cryolabel applied and each sample will be logged into the BSI database. The BSI system will track the exact location of each sample while in storage. The boxes of frozen samples will be stored as shown. Care will be taken so that all samples from one study participant will never be stored in one single storage device.

- Samples from Boxes A, D, B, and E can be stored in LN2 vapor phase or at -80° C in mechanical freezers.
- Samples in Boxes C, F, and I will be stored in EPL's -20° degree walk in freezer.

Hair, nail and dust samples will be stored under ambient conditions in a secure temperature and humidity controlled conditions (~+20° C and 50% humidity) room at EPL.