

PROCEDURES FOR COLLECTING AND SHIPPING SPECIMENS FOR MEASUREMENT OF NICOTINE, COTININE AND OTHER NICOTINE METABOLITES, TOBACCO ALKALOIDS, AND CARCINOGEN BIOMARKERS

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SHIPPING INSTRUCTIONS:

PRINCIPAL INVESTIGATOR:

Principal Investigator (PI) or assigned personnel, notify Lisa Yu or Pura Tech at Clinical Pharmacology Laboratory two days before planned shipment.

SAMPLE SHIPMENT INFORMATION:

Fill out the Sample Shipment Information (see attached SampleShipmentForm.xls) and email or send to:

Attn.: Lisa Yu
San Francisco General Hospital
Building 100, Room 235
1001 Potrero Avenue
San Francisco, CA 94110

Phone: 415-282-9495
Fax: 415-206-5080
Email: lyu@medsfgh.ucsf.edu
cc: techp@medsfgh.ucsf.edu
cc: WilliamsV@medsfgh.ucsf.edu

SAMPLE LABELLING:

For each specimen or sample, the identification should be written on a self-adhesive label with a water-resistant marker, such as black color fine point Sharpie marker (red color is not recommended since it tends to rub off). The label should be secured on the container with transparent tape as well. Even with tape, the label may come off if the container has direct contact with dry ice.

Samples should be numbered sequentially with a maximum of 10 characters. Use the minimum number of characters to uniquely identify the samples if possible.

If applicable, samples labels should have the following: Subject #, Session, Collection date & time.

IMPORTANT: Include type of sample, e.g. plasma, urine, saliva.

PACKAGING & STORING OF SAMPLES FOR SHIPPING:

Specimens should be grouped into freeze-safe zip lock bags (20 samples per bag preferably or less) or put in order into a storage box, especially if the shipment is more than 25 samples. All zip-lock bags should be shielded from the crushed dry ice by wrapping either the dry ice or the bags in newspaper. Note: bags may tear with direct contact with the dry ice. A suggested storage box can be purchased from Fisher Scientific (800-766-7000 Cat#11-678-24B with dividers-Cat#13-992-3).

SAMPLE SHIPPING/DELIVERY:

Samples can be delivered to the Clinical Pharmacology Laboratory at SFGH (Bldg 100, Room 235) by research staff, or shipped by overnight courier.

SHIPPING BY COURIER:

It is preferable to ship early in the week (not on Friday), since our lab is closed on weekends. Samples should not arrive to our lab during the weekend. Suggested shipping container can be purchased at Fisher Scientific (800-766-7000) Cat# 03-530-3.

DRY ICE FOR SHIPPING:

Crushed dry ice should be used for shipping.

SHIPPING COMPANY:

PI may use FedEx or any reliable shipping company as long as the sample will be delivered frozen with crushed dry ice. Please check with shipping carrier for their packaging & shipping guidelines.

NOTE:

Specimens should be collected in polypropylene containers (Corning Cryogenic Vials #430490 or equivalent) with secure caps. Clear plastic (polystyrene) and glass tubes must not be used since they often crack or break in dry ice during shipping.

SAMPLE DISPOSAL:

Unless prior arrangement has been made, all samples will be disposed of one month after the final report is sent out to the Principal Investigator. For sample disposal, we follow the UCSF OEH&S (Office of Environmental Health & Safety) guidelines.

COLLECTION INSTRUCTIONS:**A. PLASMA Nicotine, Cotinine, and/or *trans*-3'-Hydroxycotinine**

1. Collect approximately 7 ml blood using 10 ml B-D vacutainer, green top (B-D #7874 containing 143 USP units Sodium Heparin-Fisher Cat#02-689-6).
2. Invert the collected blood sample several times to mix with the anticoagulant
3. Centrifuge collected blood sample for about 15 minutes to separate the blood with plasma
4. Transfer plasma sample into 4 mL Corning Cryogenic Vials (Fisher Cat#03-374-23)
5. Label plasma samples with Subject #, Session, Collection date & time, type of sample

NOTE: Use caution during collection and transfer of plasma into cryogenic vials: Patients must not be smoking at the time of blood collection. Collection should be done in a room where smoking has not recently occurred. If the nature of experiment requires that blood be collected during smoking, a plastic barrier should be placed between the syringe while collecting the blood and the surrounding air to minimize contamination.

6. Store the collected plasma samples into a -20 degrees C freezer until shipment
7. Follow sample shipment instructions. (see SampleShipmentForm.xls)

B. URINE Nicotine, Cotinine, and/or *trans*-3'-Hydroxycotinine or Minor Alkaloids Anabasine and Anatabine:

1. 24 hour urine samples should be collected into a container containing 7.5 ml of concentrated hydrochloric acid or about 1 tbsp of sodium bisulfate*, so that the final pH is about 2-3.

NOTE: *The amount of acid is for a 24 hour urine collection of about 1000 ml only. Use proportionally less for smaller sample volumes (ex.: for random urine collection of about 100-200 ml, 1.5 ml of hydrochloric acid or ¼ tbsp of sodium bisulfate will be sufficient).

2. Collect about 10 ml of the 24 hour urine into a polyethylene vials (Fisher Cat#03-337-23C) and cap.

3. Label urine samples with Subject #, Session, Collection date & time, type of sample also record the 24 hour urine sample volume as needed for the study.
4. Store the collected urine samples into a -20 degrees C freezer until shipment
5. Follow sample shipment instructions. (see SampleShipmentForm.xls)

C. SALIVA Nicotine, Cotinine, and/or *trans*-3'-Hydroxycotinine:

MATERIALS FOR SALIVA COLLECTIONS:

1. 20 ml polyethylene vials (Fisher Cat#03-337-23C)
2. Parafilm (Fisher Cat#1337416)
3. Disposable funnel (Fisher Cat#10-500-18) – use if the vial or collection tube opening is too small.

INSTRUCTION FOR SUBJECTS:

1. Rinse mouth prior to collection using tap water. Subjects must remove lipstick or any lips product.
2. Chew parafilm as needed to generate saliva.
NOTE: Do #2 only if the subjects have difficulty producing saliva
3. Fill 20 ml vial ~ ¼ or about 5 ml.

If needed, subject can chew on parafilm to stimulate saliva flow, but investigator should be aware that cotinine concentration in stimulated saliva may be slightly lower than in unstimulated saliva.*

*Saliva Cotinine Levels as a Function of Collection Method. N. G. Schneider, P. Jacob III, F. Nilsson, J. J. Leischow, N. L. Benowitz and R. E. Olmstead. *Addiction* (1997) 92 (3), 347-351.

INSTRUCTION TO RESEARCH TECHNICIANS:

1. Label collected saliva samples with Subject #, Session, Collection date & time, type of sample.
2. Cap collected saliva samples and let stand at room temperature for approximately one hour or until saliva and foam have settled; sample may require shaking.
3. Store the collected saliva samples into a -20 degrees C freezer until shipment.
4. Follow sample shipment instructions. (see SampleShipmentForm.xls)

D. HAIR Nicotine:

1. Collect about 50 milligram (mg) hair sample.
2. Record the length of the hair, especially if the time course of exposure is important. Hair grows at the rate of about 1 cm per month.

3. Place hair in a labeled envelope (Whatman, Inc., Env.Glassine Item#10548236, size 3-1/4 x 4-7/8 in.; Fisher Cat#50853572)
4. Seal the envelope (with a Scotch Magic tape, cat. 810) and store at room temperature.
5. Follow sample shipment instruction.

Note. To estimate how much hair is 50 mg, if a micro balance is not accessible, we have three alternatives as suggestions.

1. Count the hair strands. On average a single strand of 1 cm hair weighs about 0.1 mg or 10 cm (about 4 inches) hair weighs about 1 mg. Thus, about 50 strands of 10 cm hair will weigh 50 mg.
2. We can send you some pre-weighed hair packets at 2, 10, 20, and 50 mg for comparison to estimate the amount.
3. Use this slide for visual reference.



E. Urine: NNAL and PAH Metabolites

1. Collect about 15-20 mL of urine into Nalgene 2104-0001 size 30 mL HDPE bottle (Fisher Cat#02-893-5A) and cap. No need to add preservatives but **keep cold or freeze as soon as possible**. NOTE. If only collect for NNAL or PAH (not both assays), about 10 mL will be sufficient.

2. Label urine samples with Subject #, Session, Collection date & time, type of sample.
3. Store the collected urine samples into a -20 degrees C freezer until shipment.
4. Follow sample shipment instructions. (see SampleShipmentForm.xls)