

2020

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

Clinical Pharmacology Laboratory
San Francisco General Hospital
University of California, San Francisco
Peyton Jacob III, Ph.D. Neal L. Benowitz, M.D.

SHIPPING INSTRUCTIONS

PRINCIPAL INVESTIGATOR OR ASSIGNED PERSONNEL

Please notify Lisa Yu or Tina Won at Clinical Pharmacology Laboratory two days before planned shipment.

SAMPLE SHIPMENT INFORMATION

Fill out the Sample Shipment Information (see attached SampleShipmentInfo&List.xlsx) 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: Yu, Lisa lisa.yu@ucsf.edu
cc: Won, Tina tina.won@ucsf.edu

SAMPLE LABELING

For each specimen or sample, the identification should be printed or written on a self-adhesive label (preferably Avery® White WeatherProof™ Labels for Laser Printers 5520, 1" x 2-5/8" or of similar 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 further secured on the container with transparent tape. Even with the tape, the label may peel off if the container has direct contact with dry ice.

Sample names should have a maximum of 10 characters. Please use the minimum number of characters possible to uniquely identify the samples. Actual sample labels must match with the sample names on the Sample Shipment Form.

Sample labels should have the following (if applicable): Subject #, Session, Collection Date & time, and type of sample/matrix (saliva, plasma, etc).

IMPORTANT: PLEASE PROVIDE A SAMPLE SHIPMENT FORM PER MATRIX

PACKAGING & STORING SAMPLES FOR SHIPPING

Specimens should be grouped into freeze-safe zip-lock bags (20 samples per bag or less) or put in order into a storage box (this method is preferred if there are more than 25 samples). All zip-lock bags should be shielded from the dry ice by either wrapping the dry ice or wrapping the bags in newspaper. *Note: zip-lock bags may tear upon direct contact with dry ice during transit.* Storage boxes for shipping can be purchased from Fisher Scientific (ex. Cat# 11-678-24B). Dividers can also be purchased from Fisher Scientific (ex. Cat# 13-992-33). There are various box and divider sizes available depending on your needs.

SAMPLE 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

Samples can be shipped to the Clinical Pharmacology Laboratory by **overnight carrier** using the name and address on the first page of this document. It is preferable to ship early in the week (not on Friday), since our lab is closed on weekends. *Shipments will not be delivered to our lab during the weekend.* Suggested shipping container can be purchased at Fisher Scientific (Cat# 03-530-32). There are various container sizes available depending on your needs.

DRY ICE FOR SHIPPING

Crushed dry ice should be used for shipping.

SHIPPING COMPANY

Principal Investigator may use FedEx or any reliable shipping company, as long as the samples are delivered frozen with crushed dry ice. Please check with the shipping carrier for packaging and shipping guidelines.

NOTE

Specimens should be collected in polypropylene containers (Corning Cryogenic Vials #430490 or equivalent) with secure caps. Clear plastic containers (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 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 the collected blood sample for about 15 minutes to separate the blood from plasma.
4. Transfer plasma of samples into 4 mL Corning Cryogenic Vials (#430490).
5. Label plasma samples with Subject #, Session, Collection date & time, and sample type (plasma).

NOTE: Use caution during collection and transfer of plasma into cryogenic vials. Patients must not be smoking at the time of blood collection and the 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 and the surrounding air while collecting to minimize contamination.

6. Store the collected plasma samples in a -20°C freezer until time of shipment.
7. Follow the sample shipment instructions on first and second page of this document (also see SampleShipmentInfo&List.xlsx).

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: This amount of acid is for a 24 hour urine collection of about **1000 mL**. Use proportionally less for smaller sample volumes (ex. for a urine collection of 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 polyethylene vials (Fisher Cat# 03-337-23C) and cap.

3. Label urine samples with Subject #, Session, Collection date & time, and sample type (urine). Record the 24 hour urine sample volume.
4. Store the collected urine samples in a -20°C freezer until time of shipment.
5. Follow the sample shipment instructions on first and second page of this document (also see SampleShipmentInfo&List.xlsx).

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# 13-374-16)
3. Disposable funnel (Fisher Cat# 10-500-1) – Use if the vial or collection tube opening is too small.

INSTRUCTIONS FOR SUBJECTS:

1. Rinse mouth prior to collection using water. Subjects must remove lipstick or any lip-care product.
2. Chew parafilm as needed to generate saliva.

NOTE: This should be done **ONLY** if the subjects have difficulty producing saliva. Cotinine concentration in stimulated saliva may be slightly lower than in unstimulated saliva.*

3. Fill ¼ of a 20 mL vial (approximately 5 mL).

*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.

INSTRUCTIONS FOR RESEARCH TECHNICIANS:

1. Label saliva samples with Subject #, Session, Collection date & time, and sample type (saliva).
2. Cap collected saliva samples and let stand at room temperature for approximately one hour or until saliva and foam have settled.

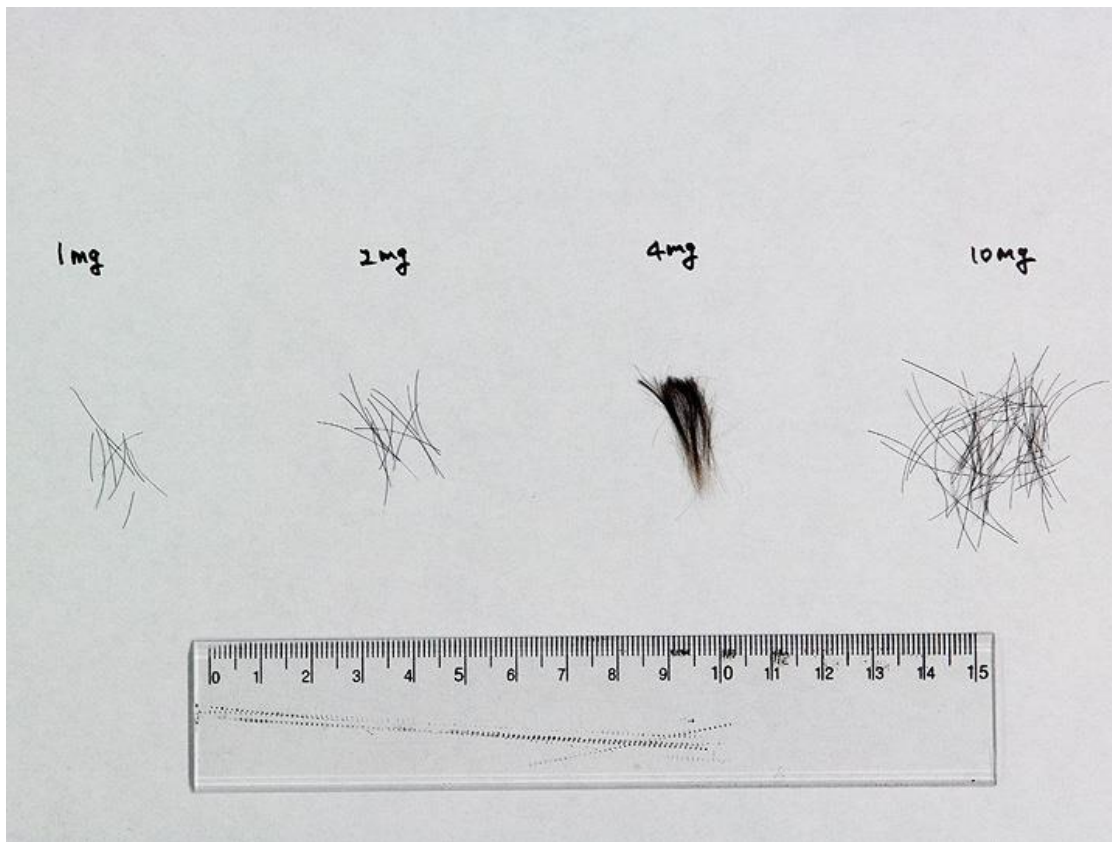
3. Store the collected saliva samples in a -20°C freezer until time of shipment.
6. Follow the sample shipment instructions on first and second page of this document (also see SampleShipmentInfo&List.xlsx).

D. HAIR (Nicotine)

1. Collect a 50mg hair sample.
2. Record the length of the hair. This step is especially crucial 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 or equivalent).
4. Seal the envelope with a Scotch Magic tape and store at room temperature.
5. Follow the sample shipment instructions on first and second page of this document (also see SampleShipmentInfo&List.xlsx).

NOTE: We have three suggestions to estimate 50mg of hair if a micro balance is not accessible.

- a. 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.
- b. We can send you some pre-weighed hair packets at 2, 10, 20, and 50 mg for comparison to estimate the amount.
- c. Use this slide for visual reference.



E. Urine (NNAL, Mercapturic Acids (VOC Biomarkers), and PAH Metabolites)

1. Collect about 15-20 mL of urine into 30 mL Nalgene 21040001 HDPE bottle (Fisher Cat# 02-893-5A) and cap. There is no need to add preservatives but **keep cold or freeze as soon as possible**.

NOTE. If collection is only for NNAL **or** PAH (not both assays), 10 mL will be sufficient. If only collecting 10 mL, please use 20 mL polyethylene vials (Fisher Cat # 03-337-23C)

2. Label urine samples with Subject #, Session, Collection date & time, and sample type (urine).
3. Store the collected urine samples in a -20°C freezer until time of shipment.
4. Follow the sample shipment instructions on first and second page of this document (also see SampleShipmentInfo&List.xlsx).