

235. LONGITUDINAL PVDOMICS BIOREPOSITORY CORE URINE SPECIMENS COLLECTION, PROCESSING AND SHIPPING

235.1 Purpose

Participants who enroll in the Longitudinal PVDOMICS (L-PVDOMICS) study will be asked to provide biological specimens (blood and urine). The urine specimens will be used to assess urine biochemical biomarkers to determine whether a panel of biomarkers, including nitrate and nitrate in conjunction with uBrY and urinary F2isoP, can be used as a surrogate for PAH classification. If participants agree to storage of their biological samples for use in possible future studies, a separate repository informed consent will be signed.

Among those participants who consent for storage of biological specimens, serum, plasma, and **urine samples will be collected once for participants who agree to Level 1 participation**. This document describes the collection and processing of urine.

235.2 Biorepository Contacts

The Biorepository under supervision of Suzy Comhair, PhD, is part of the DCC and is located at the Cleveland Clinic, Cleveland, Ohio. Laura Peterson is the Biorepository manager. Their contact information can be found in the PVDOMICS Address Directory. For general questions and biosample shipment notification, e-mail pvd-biorepos@bio.ri.ccf.org.

235.3 Collection Schedule, Type and Volume of Samples

Urine will be collected at the L-PVDOMICS visit for Level 1 participants only.

235.4 Personnel Training Requirements

Study personnel certified for blood collection and processing in the parent study is automatically certified for L-PVDOMICS. Persons collecting and processing specimens for L-PVDOMICS who have not been certified for the parent study, will be certified by written exam to ensure samples are uniformly handled prior to analyses. Each PVDOMICS center will designate a study staff member who will be responsible for training the appropriate personnel.

Each person shipping specimens to the DCC L-PVDOMICS Biorepository needs to be certified according to Department of Transportation (DOT) and International Air Transportation Authority (IATA) regulations.

Each L-PVDOMICS center will designate a study staff member who will be responsible for training the appropriate personnel and maintaining the certifications locally.

235.5 Supplies for Urine Collection and Shipment

The Biorepository will provide sample collection/shipping kits (Tables 235.1-235.2). Ten collection/shipping kits will be shipped to each center at the beginning of the study. Additional kits

will be shipped to centers monthly or as needed. The number of the additional kits will be determined by the actual site recruitment.

The Biorepository provides the following materials for sample collection and shipment:

Table 235.1 L-PVDOMICS Urine Collection Kit

Item number	Description	Quantity
Fisher: NC9867141 Sarstedt:75562105CS200	SC Urine Cup 57x76mm 100ml PP Label Sterile	1
Fisher: 02-681-343	2 ml cryovial	6
Corning: 430656 Thermo Scientific: 339650	Corning® 5 mL Internal Threaded Polypropylene Cryogenic Vial, Self-Standing with Round Bottom	1
Fisher: 12-565-270 Thermo Scientific: 339652	Thermo Scientific™ Nunc™ 50mL Conical Sterile Polypropylene Centrifuge Tubes	1

Table 235.2 L-PVDOMICS Blood / Urine Shipping Kit: Dry Ice

Description	Notes
2 Big specimen foam box (16" x 16" x 16") with inner box	
4 Biohazard bags	
UN 3373 Category B label	
Class 9 miscellaneous dangerous goods label	
Blue UPS Dry Ice for Diagnostic or Medical Purposes sticker	

In addition, sites should have the following supplies and equipment for sample collection and shipment:

- o Refrigerated centrifuges with capacity of spinning at 2200g
(recommended: Allegra™ X-12R Centrifuge, Beckman Coulter)
- o -70°C/-80°C freezer
- o -20°C freezer
- o Laboratory supplies such as biohazard bags, gloves, sterile pipettes, pipettors, and sterile pipette tips
- o Pipetman set: 1000ul
- o Collection cleansing wipes
- o Dry ice pellets
- o Packing tape
- o Barcode scanner
(recommended: Datalogic Gryphon GD4430-BK-HD and can be purchased through:
RACO Industries

Contact – Judith Maria
888-433-1274
jmaria@racoindustries.com

Each lab must compute the appropriate rpm using this target xg force and the radius of their centrifuge rotor. This can be done by using the website: http://insilico.ehu.es/mini_tools/rcf_rpm.php, which calculates the RPM from input of target xg and centrifuge rotor radius.

It is recommended to calibrate pipettes used in the study twice per year.

All tubes and cryovials included in the collection kit are pre-labeled. The label is a preprinted barcode (8 alpha numeric digits), Kit ID (9 alpha numeric digits), and sample type (Figure 35.1)



Figure 235.1: Example of a urine cryovial / tube label

235.6 Preparation for L-PVDOMICS Urine Collection

Several days prior to collection the study coordinator prepares the urine collection kit for the participant and the data collection forms.

The coordinator contacts the participant the day prior to the collection to remind them to avoid all food and liquids except water, for 8-10 hours prior to collection only if the urine collection will occur on the day of blood collection. Every effort should be made to schedule the urine collection in the morning to avoid potential diurnal effects of measures. Time of collection will be recorded for all samples collections.

235.7 Specimen Storage Log Overview

All aliquoted urine samples must be logged into the Biological Sample Tracking System (BST) in the study database before stored in -70°C/-80°C freezer. Instructions for creating a specimen storage log are provided in Section 235.9.3.

235.8 Specimen Shipping Log Overview

Before urine samples are shipped to Biorepository, a shipping log must be created in the BST. Instructions are provided in Section 235.10 of the MOP.

235.9 L-PVDOMICS COLLECTION 1: URINE COLLECTION, PROCESSING, STORING

235.9.1 Collection

Ideally blood and urine collection should occur in the same day. Urine must be collected at the time of visit in a clinical setting only and cannot be collected prior to coming to visit. Encourage the participant to fill up cup about 1/2 - 2/3 full. Urine collection will not be repeated at another time irrespective of quality and quantity of collected specimen. Specimen collection instruction templates for females and males are provided in Appendix 235.1 and 235.2 respectively. If your institution already has established guidelines, you can follow those.

1. To obtain urine, the participant will void into the provided sterile collection container labeled with participant's study ID number.
2. Make sure to obtain a "clean catch" sample.
3. Follow "Universal Precautions" throughout the collection procedure; discard all contaminated material in biohazard container.
4. Complete Form 820 to document the urine collection.
5. Transport the urine container to the lab for processing.

235.9.2 Processing

1. Transfer at least 25 ml of urine into a 50 ml conical tube with sterile transfer pipette.
2. Centrifuge the 50 ml conical tube at 2200xg at 4°C for 10 minutes for Swing-Bucket Centrifuge or 15 minutes for Fixed Angle Centrifuge (balance the tube in centrifuge).
3. After centrifugation of the 50 ml conical tube:
 - o Immediately pipet 1.5 ml of urine into 5 pre-labeled 2 ml "Urine" cryovials with orange O-ring screw caps (Fisher Scientific; cap# 02-681-364).
 - o Transfer 5ml of urine into a 5ml pre-labeled "Urine" conical tube with sterile transfer pipette. Do not fill to the top of the tube, urine expands when frozen.
 - o Pour off most of the urine supernatant (leave a proximal 500ul of urine behind).
 - o Re-suspend the pellet in the urine that is left behind.
 - o Transfer the re-suspended pellet into pre-labeled 2 ml "Urine Pellet" cryovials with orange O-ring screw caps labeled "P" on the top (Fisher Scientific; cap# 02-681-364).
 - o Centrifuge at 16,000g (or top speed on microcentrifuge) for 4 minutes at room temperature.
 - o Pipet off remaining urine supernatant.
4. Place cryovials and 5 ml conical tube immediately on dry ice.

Complete Form 821 to document the details about urine processing. Use MOP Appendix 235.1 for quick reference for urine processing.

235.9.3 Creating Storage Log

All samples will be logged into the L-PVDOMICS Biological Sample Tracking (BST) system as part of the study database before storing in -70°C/-80°C freezer.

Access the L-PVDOMICS Biological Sample Tracking (BST) system online and input the participant ID and urine collection date in Form 830 in order to connect the specimens to the correct participant ID and collection. Scan the barcodes of all cryovials and tubes to insert a record for each of them. Once you have entered the information, print a copy of the specimen storage log for the participant's study records to keep a record of the barcode associated with each cryovial/tube.

235.9.4 Storage of Urine Cryovials and Tubes

After scanning the samples into L-PVDOMICS Study BST, the samples must be stored at -70°C/-80°C. Store all cryovials in an upright position in the appropriate size cardboard boxes with 9 x 9 cell dividers provided by the Biorepository.

Place in -70°C/-80°C freezer. Place 5 ml tubes in cardboard box without cell divider.

235.10 SHIPPING of Frozen Aliquots and Tubes to Biorepository

SHIPPING of Frozen Cryovials and PAXgene® to Biorepository

All samples stored at the centers should be shipped to the Biorepository on Monday – Wednesday as long as neither of the following two days is a holiday.

Email pvd-biorepos@bio.ri.ccf.org PRIOR to shipping to confirm the lab is able to receive packages the next day. See Section 235.11 for details.

NOTE: L-PVDOMICS biospecimens **MUST be shipped SEPARATELY from the PVDOMICS parent study's biospecimens.**

235.11 Shipping of All Collected Biospecimen Samples on Dry Ice to Biorepository

NOTE: Since the shipping instructions for biospecimens refer to blood and urine specimens, MOP Chapter 234.9 and MOP Chapter 235.11 are identical.

All frozen cryovials, PAXgene® and urine stock tubes must be shipped to the Biorepository Core according to the instructions below. Shipping can occur on Monday-Wednesday as long as neither of the following two days is a holiday. Email pvd-biorepos@bio.ri.ccf.org PRIOR to shipping to confirm the lab is able to receive packages the next day.

Personnel shipping samples must adhere to IATA and Dept. of Transportation shipping regulations. Please check with your institution for guidelines. Failure to comply may result in fines.

The *PAXgene*® tubes will be taken out of the racks, placed in the labeled “*PAXgene*®” cardboard box and covered with an absorbent sheet to minimize moving of tubes.

Shipping log instructions:

A shipping log must be created on the day the samples will be shipped. Access the L-PVDOMICS Biological Sample Tracking (BST) system online and scan the barcodes of all cryovials in Form 836 to insert one record for each. A shipment inventory will be generated that contains: date of shipment, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers, Kit ID and participant information (participant ID number, collection number, specimen collection date). Please note that L-PVDOMICS Study serum, plasma and urine shipments for several participants, blood draws and urine collections can be combined in the same large shipping box, but a separate shipping inventory must be created for each large box. A maximum of 7 boxes (9x9) can go into one large shipping box. Once 7 (9x9) boxes have been scanned), save the log, make note of the auto-populated Shipment Box ID and start a new shipment log if necessary. Print 2 copies of the shipment inventory (ies), one to include in each shipment box, and one to retain for the study records at the originating site.

Once the shipment information is completed in the BST system, an e-mail will automatically be sent to the Biorepository notifying them that a shipment should be expected. A copy of that e-mail will sent to the originating site. The e-mail message will include the originating site, the name of the person preparing the shipment and the shipment box ID in the body and a complete shipment inventory as an attachment.

Complete Form 803 and enclose a copy with the shipment.

Packing instructions:

1. Wrap rubber band around each specimen box and place inside biohazard bag. Up to 2 boxes can be in each bag. Seal the bag when full.
2. Place a thin layer of dry ice pellets in the bottom of the shipper.
3. Place up to 7 boxes in the plastic biohazard bag containing specimen on the dry ice.
4. Fill the rest of the space in the shipper with dry ice pellets. Leave 3.5” (height) empty in the top of the shipper for the lid’s foam insert.
5. Place the foam insert on top of the dry ice. Place a copy of the shipment log. Close and tape the outer box.
6. Affix the Biorepository “**To:**” address label to the side of the box in the upper left corner.
7. Affix the dry ice label (**UN1845**) to the same side of the box in the upper right corner. Enter the weight of dry ice on the label in kilograms.
8. Affix the “UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B” label to the left of the dry ice label.

9. Affix the blue UPS “Dry Ice for Diagnostic or Medial Purposes Only” sticker. Enter weight of dry ice in kg, must be the same as on the dry ice label
10. Create a UPS airbill to ship specimens to BIOREPOSITORY at Cleveland Clinic.
11. Send a shipment notification to the Biorepository via email at pvd-biorepos@bio.ri.ccf.org on the day the package is dropped for UPS pickup. Include the UPS tracking number in the notification.

Note: You should be able to setup your UPS account to send an automated message to pvd-biorepos@bio.ri.ccf.org when a shipment label is created.