

34. PVDOMICS BIOSPECIMEN BLOOD PROCESSING AND SHIPPING

34.1 Purpose and Background

Participants who enroll in the PVDOMICS study will be asked to provide biological samples (blood and urine) for OMICS analysis (Genomics, Proteomics, Metabolomics, Coagulomics and Cell Biomics). If participants agree to storage of their biological samples for use in possible future studies, a separate Biorepository informed consent will be signed.

Among those participants who consent for storage of biological specimens, serum, plasma, buffy coat, and urine samples will be collected at various time points depending on required samples. This document describes the collection of blood specimens. Chapter 33 describes collection of blood specimens.

Chapter 35 describes collection, processing and shipment of urine specimens.

34.2 Logistics for Biorepository

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. The 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.

All samples collected at the specific centers will be shipped at room temperature or on dry ice to the Biorepository for processing, storage, and distribution of samples to PVDOMICS cores and investigators for genome, proteome, metabolome, coagulome, and advanced cell biome analyses upon approval by the Steering Committee. At the end of the study, any remaining biological samples will be transferred to a central biobank or to the National Heart, Lung and Blood Institute (NHLBI) Biological Specimen and Data Repository Information Coordinating Center (BioLINCC) biobank.

34.3 Collection Schedule, Type and Volume of Samples

Blood is collected twice during the study. Collection 1 (C1) should be at time of enrollment during Visit 1 and Collection 2 (C2) should be done at the time of the Right Heart Catheterization (RHC) with or without invasive Cardiopulmonary Exercise Testing (iCPET). Table 34.1 provides the blood volumes needed for the OMICS studies.

(see table on next page)

Table 34.1 PVDOMICS Blood Samples Volumes (number of tubes x tube size) Collected for OMICS

Vacutainer™ blood tubes	Collection 1 (C1)	Collection 2 (C2)	
		RHC with/without iCPET: Rest	RHC with iCPET: Rest, Peak and Post
Gold top (SST)	2 x 5 ml	1 x 5 ml	1 x 5 ml
Green top (Na-Heparin)	1 x 4 ml		
Lavender top (EDTA)	1 x 10 ml 1 x 4 ml	2 x 10 ml 3 x 4 ml	2 x 10 ml 7 x 4 ml
Red top (<i>PAXgene</i> ®)		4 x 2.5 ml	8 x 2.5 ml
Blue top (Na-Citrate)	1 x 4.5 ml		
TOTAL Blood Collected	32.5 ml	47 ml	73 ml

34.4 Personnel Blood Processing and Shipping Training Requirements

The Biorepository and the DCC will conduct a webinar with video and an in-person training session prior to the beginning of the study. In addition, persons collecting and processing specimens for PVDOMICS will be certified by written exam to ensure samples are uniformly handled to analyses.

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

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

34.5 Supplies for Processing, Storing and Shipment Blood

The Biorepository provides pre-labeled processing and shipping kits (Table 34.2-34.6). Five processing 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 additional kits will be determined by center recruitment. If kits are not received, or extra kits are needed, centers should enter an Emergency Supplies Request Form 340 in the study database.

Table 34.2 PVDOMICS Blood Collection 1 (C1) Processing Kit

Description	Quantity
0.5 ml Yellow top cryovial	14
0.5 ml Blue top cryovial	13
0.5 ml Lavender top cryovial	13

**Table 34.3 PVDOMICS Blood Collection 2 (C2) Processing Kit:
RHC with or without iCPET at Rest**

Description	Quantity
0.5 ml Yellow top cryovial	10
0.5 ml Lavender top cryovial	73
2 ml Clear top cryovial	1

**Table 34.3HC PVDOMICS Blood Collection 2 (C2) Processing Kit:
Healthy Controls**

Description	Quantity
0.5 ml Yellow top cryovial	10
0.5 ml Lavender top cryovial	40
2 ml Clear top cryovial	1

**Table 34.4 PVDOMICS Blood Collection 2 (C2) Processing Kit:
RHC with iCPET during Rest, Peak and Post**

Description	Quantity
0.5 ml Yellow top cryovial	10
0.5 ml Lavender top cryovial	117
2 ml Clear top cryovial	1

The catalog numbers for the Fisher scientific items above are as follow: 0.5ml cryovial 02-681-333; Yellow cap, 02-681-360, Purple cap, 02-681-366; Clear cap, 02-681-358; Blue cap, 02-681-359; 2ml tube, 02-681-343.

Table 34.5 PVDOMICS Storage Kit

Description	Quantity
Pre-labeled cardboard box with 9x9 cell dividers for cryovials with absorbent sheet for monthly shipping	12 for non-exercise centers 15 for exercise centers

Table 34.6 PVDOMICS Shipping Kit: Room Temperature

Description
Big specimen box (2.5" x 9.5" x 12.5") with insulated envelope inside
Gel pack
95kPa Specimen Transport Bag
Absorbent sheet tube holder
UN 3373 Category B label

Table 34.7 PVDOMICS Blood / Urine Shipping Kit: Dry Ice

Description	Notes
2 Big specimen foam box (16" x 16" x 16") with innerbox	
6 Biohazard bags	
Big specimen foam box (15" x 13" x 14") with innerbox	This additional box will be sent only to centers collecting blood at Peak and Post
2 Biohazard bags	These additional bags will be sent only to centers collecting blood at Peak and Post
UN 3373 Category B label	
Class 9 miscellaneous dangerous goods label	
Blue UPS Dry Ice for Diagnostic or Medical Purposes sticker	

Note that the big specimen shipment boxes will be reused and should not be discarded. In addition, centers 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 Dry ice pellets
 - o Permanent black marker
 - o Packing tape
 - o Clear parafilm
 - o Conical tube rack (Fisher: 14-791-6D) is highly recommended to avoid tube breakage
 - o Pipetman set: 1000ul and 200ul
 - 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

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

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.

All cryovials included in the collection kits are pre-labeled. The label is a preprinted barcode (8 alpha numeric digits), study participant ID number (6 alpha numeric digits), collection number (2 alpha numeric digits), sample type, and collection scenario (Figure 34.1).

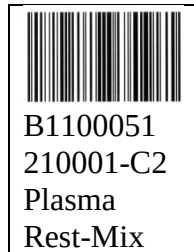


Figure 34.1: Example of a cryovial label

34.6. Specimen Storage Log Overview

All samples aliquoted during Collection 1 and Collection 2 must be logged into the Biological Tracking System (BST) in the study database before stored in $-70^{\circ}\text{C}/-80^{\circ}\text{C}$ freezer. Instructions for creating a specimen storage log are provided in Sections 34.8.1 and 34.9.1 respectively for each collection.

34.7 Specimen Shipping Log Overview

Before samples are shipped to Biorepository, a separate shipping log must be created in the Biological Tracking System (BST) in the study database for each box sent to the Biorepository. Instructions are provided in Section 34.10 of the MOP.

34.8 PVDOMICS COLLECTION 1: PROCESSING, STORING and SHIPPING

The blood draw for Collection 1 will generally occur at the first study visit (for details see MOP Chapter 33). The processing of all 6 Vacutainer™ blood tubes needs to start within 1 hour of collection.

Table 34.8 Vacutainer™ Blood Tubes Collection 1 (C1)

Description	Quantity
5 ml Gold top (SST)	2
4 ml Green top (Na-Heparin)	1
4 ml Lavender top (EDTA)	1
4.5 ml Blue top (Na-Citrate)	1
10 ml Lavender top (EDTA)	1

At arrival of Vacutainer™ blood tubes into the lab:

- o Check if participant ID number on the Vacutainer™ blood tubes matches with ID number on the screw cap tubes and ID sticker provided in processing kit. Coordinator must also check expiration date on Vacutainer™ blood tubes. Place ID sticker on Form 301: Center Blood Collection 1- Processing Sample.
- o Complete questions 1-6 on Form 301 before processing the Vacutainer™ blood tubes and the rest of the form as you process the tubes.

34.8.1 Processing

1. Two Gold tops “Serum CC-PRL”: Allow the blood to clot in an upright position at room temperature for 30 minutes.
2. Green top “Genomics” and Lavender top (10ml) “Cell Biomix”: Do not centrifuge. Do not refrigerate or freeze at any time. Leave at room temperature and ship **the same day** to Biorepository (see below).
3. Centrifuge Lavender top “Plasma” and Blue top “Plasma-Cit” at 2200g at 4°C for 10 minutes for Swing-Bucket Centrifuge or for 15 minutes if using a Fixed Angle unit. After centrifugation of Lavender top “Plasma”.
 - o Immediately pipet 200 ul of plasma into 13 pre-labeled “Plasma” cryovials (0.5ml) with purple screw cap.
NOTE: Place a black dot with permanent marker on the screw cap if the volume is less than 200 ul.
 - o Place samples on dry ice until all samples from Collection 1 are aliquoted
4. After centrifugation of Blue top “Plasma-Cit”:
 - o Immediately pipet 200 ul of plasma into 13 pre-labeled “Plasma-Cit” cryovials (0.5ml) with blue screw cap.

NOTE: Place a black dot with permanent marker on the screw cap if the volume is less than 200 ul.

- o Place samples on dry ice until all samples from Collection 1 are aliquoted
 - 5. 30 minutes after 2 Gold tops “Serum CC-PRL” are placed in upright position, centrifuge at 2200g at 4°C for 10 minutes for Swing-Bucket Centrifuge or for 15 minutes if using a Fixed Angle unit.
 - 6. After centrifugation of the two Gold tops:
 - o Immediately pipet **400 ul of serum** into 4 pre-labeled “Serum CC-PRL” cryovials (0.5ml) with yellow screw cap.
 - o Pipet **200 ul** of serum into 10 pre-labeled “Serum” (0.5ml) with yellow screw cap.
- NOTE: Place a black dot with permanent marker on screw cap in volume is less than 200ul.
- o Place samples on dry ice until all samples from Collection 1 are aliquoted.
 - 7. Access the PVDOMICS Biological Sample Tracking (BST) system online and input the participant ID and blood collection date in order to connect the specimens to the correct participant ID and collection in Form 330. Scan the barcodes of all cryovials to insert one record for each cryovial. Indicate whether the cryovial was marked with a black dot due to insufficient volume of blood. Once information is entered, 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.
 - 8. Store all cryovials in an upright position in the pre-labeled cardboard boxes with 9 x 9 cell dividers provided by the Biorepository and place in -70°C/-80°C freezer (Table 34.9). The 9 x 9 boxes need to be filled to capacity and could contain cryovials from more than one participant.

Table 34.9 Processing and Storage of Cryovials of Collection 1 (C1)

Cryovial label	Color Cryovial Screw cap	Volume	Number of cryovials	Storage box
Serum-CC-PRL	Yellow	400ul	4	SERUM/BC/Urine
Serum	Yellow	200ul	10	SERUM/BC/Urine
Plasma	Purple	200ul	13	PLASMA
Plasma-Cit	Blue	200ul	13	PLASMA

34.8.2 SHIPPING of Green top (4ml) “Genomics” and the Lavender top (10ml) “Cell Biomix” Plasma Vacutainers™ to Biorepository at ROOM TEMPERATURE

Creating a shipping log

Green top (4ml) “Genomics” and the Lavender top (10ml) “Cell Biomix” Vacutainers™ blood tubes are scanned into the PVDOMICS Biological Sample Tracking (BST) system. Access the BST system online and input the participant ID and blood collection date in order to link the specimens to the correct participant ID and collection in Form 335. Scan the barcodes of tubes to insert one record for each. Once information is entered, print two copies of the specimen log: one for the participant’s study records to keep a record of the barcode associated with each tube and one to be enclosed in the shipment box.

Shipping Vacutainers™ blood tubes to Biorepository

The Green top (4ml) “Genomics” and the Lavender top (10ml) “Cell Biomix” Vacutainers™ blood tubes must be shipped at room temperature **on the day of blood draw** for arrival to the Biorepository Core within 24 hours. Complete Form 302 and enclose a copy with the shipment.

To pack the specimen properly for shipment in the boxes provided as part of the shipping kit (Table 34.7).

1. Ensure rubber stopper of each filled specimen tube is tight.
2. Secure with parafilm.
3. Verify tube is labeled correctly and label is well adhered to tube surface. Secure with packing tape if necessary.
4. Verify tube label matches label placed on associated paperwork (shipping log, specimen collection form).
5. Place each tube into the absorbent sheet tube holder.
6. Place the tubes in the 95kPa Specimen Transport Bag.
7. Remove white adhesive liner from the bag and discard.
8. Place bag in gel wrap (the gel pack should NOT be frozen).
9. Place gel wrap into insulated envelope.
10. Snap close envelope.
11. Place envelope into box.
12. Include copy of the specimen shipment log as required by Federal regulations.
13. Close box.
14. Use package-sealing tape over the locking tabs of the mailer.
15. Place provided UN3373 diagnostic specimen sticker (IATA 650 compliance) on the top of the box on the upper right corner.

If shipping occurs during Monday through Thursday, **Priority Overnight UPS Courier** is the required shipping method and provided by the DCC:

Address: Cleveland Clinic
Lerner Research NB2-037
Attn: Laura Peterson
2111 E. 96th St.
Cleveland, Ohio 44106
(216) 445-6625

Study personnel should be aware when the last UPS pick up of the day is since the blood needs to be shipped on the day of collection.

Please provide advance notice as soon as possible by emailing pvd-biorepos@bio.ri.ccf.org if Collection 1 occurs before a non-working day in order to arrange staff to receive and process the samples. The package will need to be sent to a residential address since our dock is not open on the weekend or holidays.

If shipping occurs on Friday, email the DCC and we will create the UPS shipping bill. An orange UPS “Saturday Delivery” label needs to be affixed to the package. These stickers will be provided. Again, please notify the Biorepository as soon as possible if this occurs.

If shipping occurs the day before a holiday, Sterling Courier will need to be used. Contact the DCC and we will help to arrange the shipment. Please notify the Biorepository as soon as possible.

NOTE: If a Friday shipment (for Saturday delivery) will NOT occur, please notify the Biorepository as soon as possible so that lab personnel will not be required to come in to the lab on a Saturday.

34.8.3 SHIPPING of Frozen Cryovials to Biorepository

Frozen vials from Collection 1 and Collection 2 that are stored at local centers must be shipped to the Biorepository. They can be shipped Monday-Wednesday as long as the next day is not a holiday. Email pvd-biorepos@bio.ri.ccf.org and include the tracking number. See Section 34.10 for shipping instructions.

34.9 PVDOMICS COLLECTION 2: PROCESSING, STORING and SHIPPING

The blood draw for Collection 2 takes place during the Right Heart Catheterization (RHC). Some centers will perform a RHC without invasive Cardiopulmonary Exercise testing (iCPET) and some centers will perform a RHC with iCPET at rest, while others will perform a RHC with iCPET at rest, peak and post exercise. The amount of Vacutainer™ blood tubes and processing depends if RHC is performed during “rest” or “rest, peak and post” exercise.

During the RHC procedure, Venous blood, Systemic artery blood (Syst Art) (OPTIONAL), Pulmonary capillary blood (Wedge) and Pulmonary artery blood (Mix) will be collected.

Blood Processing Protocol A: RHC blood with or without CPET at rest

Blood Processing Protocol B: RHC blood with Invasive CPET (iCPET) at rest, peak and post exercise

Forms

Non-invasive CPET Clinical centers **21, 22, 31, and 61** complete Blood Processing Forms 305 and 306 for **all participants** who had C2 blood collected.

Invasive CPET Clinical centers **11, 41, and 51** complete Blood Processing Forms 308 and 309 for **all participants** who had C2 blood collected.

OMICS Blood Collection	CPET-type Center	Participant type	Biorepository Forms Used
Collection 1	All Centers	Everyone (PH, comparator, healthy control)	Use forms F300, F301 and F303.
Collection 2	Non-Invasive CPET Centers (21,22,31,and 61)	Everyone (PH, comparator, healthy control)	Use forms F304, F305 and F306
	Invasive CPET Centers (11, 41, and 51)	Everyone, <i>even if only ‘rest’ blood was obtained or non-invasive CPET was done or a healthy control</i> (PH, comparator, healthy control)	Use forms F307, F308 and F309

34.9.1 Blood Processing Protocol A: RHC with or without iCPET at rest

The blood draw for Collection 2 will generally occur at the second study visit (for details see MOP Chapter 33) during the RHC with or without iCPET. The systemic artery blood collection is optional and clinical center dependent.

The processing of the Vacutainer™ blood tubes needs to start within 1 hour of collection. Depending on the procedure time, some Vacutainer™ blood tubes may need to be brought to the laboratory for processing before the end of the collection procedure.

Table 34.10 Vacutainer™ Blood Tubes Collection 2: RHC with/without iCPET at Rest

Description	Quantity
Red top <i>PAXgene</i> ®	4
5 ml Gold top (gel barrier)	1
10 ml Lavender top	2
4 ml Lavender top (EDTA)	3

At arrival of Vacutainer™ blood tubes into the lab:

- o Check if participant ID number on the Vacutainer™ blood tubes matches with ID number on the screw cap tubes and ID sticker provided in processing kit.
- o Place ID sticker on Form 306.
- o Complete questions 1-5 on Form 305 and Form 306 before processing the Vacutainer™ blood tubes. Complete the rest of the forms as you process the samples.

34.9.1.1 Processing

1. Place all Red top *PAXgene*® tubes, i.e., Genomic Rest Venous, Genomic Rest Mix, Genomic Rest Wedge, and Genomic Rest Syst Art upright at room temperature for 2 hours.
2. Gold top “Rest Venous Serum”: Allow the blood to clot in an upright position at room temperature for 30 minutes.
3. Centrifuge the two Lavender tops (10ml) “Plasma Rest Venous” and the three Lavender top (4ml) i.e. Plasma Rest Mix, Plasma Rest Wedge, Plasma Rest Syst Art at 2200g at 4°C for 10 minutes for Swing-Bucket Centrifuge or for 15 minutes if using a Fixed Angle units.
4. After centrifugation of all Lavender tops, start processing as follows:

Two Lavender top (10ml) labeled with Plasma-Rest Venous

- o Pipet 200 ul of plasma into 40 pre-labeled “Plasma Rest Venous” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap in volume is less than 200ul.

- o Remove the buffy coat from two Lavender tops “Plasma Rest Venous” using a Pasteur pipette.
- o Ensure that you collect all of the white cells. You will also draw up a little of the plasma from above the cells and red blood cells from below – this is normal, but try to collect as little “extra” fluid as possible.
- o Place the two buffy coats in one pre-labeled “Buffy coat Rest Venous” cryovials (2.0ml) with clear screw cap.
- o Place cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Rest Mix

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Rest Mix” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap in volume is less than 200ul.

- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Rest Wedge

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Rest Wedge” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap in volume is less than 200ul.

- o Place cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender tops (4ml) labeled with Plasma Rest Syst Art

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Rest Syst Art” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.

- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

5. 30 minutes after the Gold top “Rest Venous Serum” is placed in upright position, centrifuge at 2200g at 4°C for 10 minutes for Swing-Bucket Centrifuge or for 15 minutes if using a Fixed Angle unit.
6. After centrifugation of the Gold top “Rest Venous Serum”:
 - o Immediately pipet 200 ul of serum into 10 pre-labeled “Serum Rest Venous” cryovials (0.5ml) with yellow screw cap.
 - o Place Samples on dry ice until all samples from Collection 2 are aliquoted.
7. 2 hours after all the Red tops PAXgene® tubes are placed in upright position
 - o Access the PVDOMICS Biological Sample Tracking (BST) system online and input the participant ID and blood collection date in order to connect the specimens to the correct participant ID and collection in Form 330. Scan the barcodes of all cryovials to insert one record for each cryovial. Indicate whether the cryovial was marked with a black dot due to insufficient volume of blood. Once information is entered, 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.
8. After scanning

- o Transfer the Red top *PAXgene*® tube upright to the -20°C freezer for 24 hours.
 - o Do not freeze tubes upright in a styrofoam tray as this may cause the tubes to crack. A 15ml Conical Tube rack (Fisher: 14-791-6D) is recommended.
 - o After 24 hrs, transfer the Red top *PAXgene*® tube upright to at -70°C/-80°C Freezer. A 15ml Conical Tube rack (Fisher: 14-791-6D) is recommended.
9. Store all cryovials in an upright position in the respective pre-labeled “PLASMA” and “SERUM/BC/Urine” cardboard boxes with 9 x 9 cell dividers provided by the Biorepository and place in -70°C/-80°C freezer (Table 34.11). The 9 x 9 boxes need to be filled to capacity and may contain cryovials from more than one participant.

34.9.2 Blood Processing Protocol B: RHC with iCPET at rest, peak and post exercise

Blood draw Collection 2 will generally occur at the 2nd visit (for details see MOP Chapter 33) during the RHC with iCPET. The systemic artery blood collection is optional and clinical center dependent.

The processing of the VacutainerTM blood tubes needs to start within in 1 hour of collection. Depending on the procedure time, some VacutainerTM blood tubes need to be brought to the laboratory for processing before the end of the collection procedure.

Table 34.11 VacutainerTM Blood Tubes Collection 2: RHC with/without iCPET at Rest

Description	Quantity
Red <i>PAXgene</i> ®	8
5 ml Gold top (gel barrier)	1
10 ml Lavender top	2
4 ml Lavender top (EDTA)	7

At arrival of VacutainerTM blood tubes into the lab:

- o Check if participant ID number on the VacutainerTM blood tubes matches with ID number on the screw cap tubes and ID sticker provided in processing kit.
- o Place ID sticker on Form 308.
- o Complete questions 1-5 on Form 308 and Form 309 before processing the VacutainerTM blood tubes. Complete the rest of the forms as you process the samples.

34.9.2.1 Processing

1. Place all eight Red top *PAXgene*® tubes, i.e., Genomic Rest Venous, Genomic Rest Mix, Genomic Peak Mix, Genomic Post Mix, Genomic Rest Wedge, Genomic Rest Syst Art, Genomic Peak Syst Art, and Genomic Post Syst Art upright at room temperature for 2 hours.
2. Gold top “Serum Rest Venous”: Allow the blood to clot in an upright position at room temperature for 30 minutes.
3. Centrifuge the two Lavender tops (10ml) “Plasma Rest Venous” and seven Lavender tops (4ml) collected during **Rest** (Mix, Wedge and Syst Art), during **Peak** (Mix and Syst Art), and during **Post** (Mix and Syst Art) at 2200g at 4°C for 10 minutes for Swing-Bucket Centrifuge or for 15 minutes if using a Fixed Angle unit.

Two Lavender tops (10ml) labeled with Plasma Rest Venous

- o Pipet 200 ul of plasma into 40 pre-labeled “Plasma Rest Venous” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap in volume is less than 200ul.

- o Remove the buffy coat from two Lavender tops (10ml) “Plasma Rest Venous” by using a Pasteur pipette.
- o Ensure that you collect all of the white cells. You will also draw up a little of the plasma from above the cells and red blood cells from below – this is normal, but try to collect as little “extra” fluid as possible.
- o Place the two buffy coats in one pre-labeled “Buffy coat Rest Venous” cryovials (2.0ml) with clear screw cap.
- o Place cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Rest Mix

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Rest Mix” cryovials (0.5ml) with violet screw cap.
NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.
- o Place all cryovials immediately on dry ice until all samples of Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Rest Wedge

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Rest Wedge” cryovials (0.5ml) with violet screw cap.
NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.
- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Rest Syst Art

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Rest Syst Art” cryovials (0.5ml) with violet screw cap.
NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.
- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Peak Mix

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Peak Mix” cryovials (0.5ml) with violet screw cap.
NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.
- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Peak Syst Art

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Peak Syst Art” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.

- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Post Mix

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Post Mix” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.

- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

Lavender top (4ml) labeled with Plasma Post Syst Art

- o Pipet 200 ul of plasma into 11 pre-labeled “Plasma Post Syst Art” cryovials (0.5ml) with violet screw cap.

NOTE: Place a black dot with permanent marker on screw cap if the volume of plasma is less than 200ul.

- o Place all cryovials immediately on dry ice until all samples from Collection 2 are aliquoted.

4. 30 minutes after the Gold top “Serum Rest Venous” is placed in upright position, centrifuge at 2200g at 4°C for 10 minutes for Swing-Bucket Centrifuge or for 15 minutes if using a Fixed Angle unit.
5. After centrifugation of the Gold top “Serum Rest Venous”.
 - o Immediately pipet 200 ul of serum into 10 pre-labeled “Serum Rest Venous” cryovials (0.5ml) with yellow screw cap.
 - o Place Samples on dry ice until all samples of Collection 2 are aliquoted.
6. 2 hours after all the Red tops *PAXgene*® tubes are placed in upright position
 - o Access the PVDOMICS Biological Sample Tracking (BST) system online and input the participant ID and blood collection date in order to connect the specimens to the correct participant ID and collection in Form 330. Scan the barcodes of all cryovials to insert one record for each cryovial. Indicate whether the cryovial was marked with a black dot due to insufficient volume of blood. Once information is entered, 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.
7. After scanning
 - o Transfer the *PAXgene*® Blood tube upright to the -20°C freezer for 24 hours.

- o Do not freeze tubes upright in a styrofoam tray as this may cause the tubes to crack. A 15 ml Conical Tube rack (Fisher: 14-791-6D) is recommended.
 - o After 24 hours, transfer the *PAXgene*® Blood tube upright at $-70^{\circ}\text{C}/-80^{\circ}\text{C}$ freezer. A 15 ml Conical Tube rack (Fisher: 14-791-6D) is recommended.
8. Store all cryovials in an upright position in the respective pre-labeled “PLASMA” and “SERUM/BC/Urine” cardboard boxes with 9 x 9 cell dividers provided by the Biorepository and place in $-70^{\circ}\text{C}/-80^{\circ}\text{C}$ freezer (Table 34.11). The 9 x 9 boxes need to be filled to capacity and may contain cryovials from more than one participant.

Table 34.11 Storage of Cryovials of Collection 2

Cryovial label	Color Cryovial Screw cap	Number of cryovials	Storage box
Serum Rest Venous	Yellow	10	SERUM/BC/URINE
Plasma Rest Venous	Lavender	40	PLASMA
Plasma Rest Mix	Lavender	11	PLASMA
Plasma Rest Wedge	Lavender	11	PLASMA
Plasma Rest Syst Art	Lavender	11	PLASMA
Buffy coat Rest Venous	Clear	1	SERUM/BC/URINE
Plasma Peak Mix	Lavender	11	PLASMA
Plasma Peak Syst Art	Lavender	11	PLASMA
Plasma Post Mix	Lavender	11	PLASMA
Plasma Post Syst Art	Lavender	11	PLASMA
<i>PAXgene</i> ® Tubes	Red	- 4 tubes for non-exercise sites - 8 tubes for exercise sites	<i>PAXgene</i>

SHIPPING of Collection 2 Cryovials and *PAXgene*® to Biorepository

All samples from Collection 1 and Collection 2 stored at the centers should be shipped to the Biorepository on Monday – Wednesday as long as the following day is not 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 34.10 for details.

34.10 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 34.10 and MOP Chapter 35.11 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 the following day is not 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 PVDOMICS Biological Sample Tracking (BST) system online and scan the barcodes of all cryovials in Form 336 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 and participant information (participant ID number, collection number, specimen collection date). Please note that 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 be 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 303 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 Medical 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.