

33. BIOREPOSITORY CORE BLOOD COLLECTION OVERVIEW AND BLOOD DRAWING PROCEDURES

33.1 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 34 describes processing and shipping of blood specimens.

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

33.2 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, e-mail pvd-biorepos@bio.ri.ccf.org.

33.3 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).

During the study, the following blood will be collected:

- o Venous blood
- o Systemic artery blood (optional)
- o Pulmonary capillary blood (Wedge)
- o Pulmonary artery blood (Mix)

Table 33.1 provides the blood volumes needed for the OMICS studies.

(see table on next page)

Table 33.1 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 (PAXgene®)		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

33.4 Personnel Blood Collection Training Requirements

The Biorepository and the DCC will conduct a webinar 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 prior to analyses. Each PVDOMICS center will designate a study staff member who will be responsible for training the appropriate personnel.

33.5 Supplies for Blood Collection and Shipment

The Biorepository provides sample collection/shipping kits. Five 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 center recruitment. If kits are not received for any reason, or extra supplies are needed, centers should enter an Emergency Supplies Request Form 340 in the database.

Table 33.2 PVDOMICS Blood Collection 1 (C1) Kit

Item Number	Description	Quantity
Fisher: 02-683-97 BD: 367986	5 ml Serum Separator Gold top Vacutainer™ blood tube (gel barrier)	2
Fisher: 02-689-5 BD: 367871	4 ml NA-Heparin Green top Vacutainer™ blood tube	1
Fisher: 02-687-107 BD: 367862	4 ml EDTA Plasma Lavender top Vacutainer™ blood tube	1
Fisher: 02-688-26 BD: 369714	4.5 ml NA-Citrate Blue top Vacutainer™ blood tube	1
Fisher: 02-657-32 BD: 366643	10 ml EDTA Plasma Lavender top Vacutainer™ blood tube	1

Table 33.3 PVDOMICS Blood Collection 2 (C2) Kit: RHC with or without iCPET at Rest

Item Number	Description	Quantity
Fisher: 23-02101 BD: 762165	2.5 ml <i>PAXgene</i> ® Red top Vacutainer™ blood tube	4
Fisher: 02-657-32 BD: 366643	10 ml EDTA Plasma Lavender top Vacutainer™ blood tube	2
Fisher: 02-683-97 BD: 367986	5 ml Serum Separator Gold top Vacutainer™ blood tube (gel barrier)	1
Fisher: 02-687-107 BD: 367862	4 ml EDTA Plasma Lavender top Vacutainer™ blood tube	3

**Table 33.4 PVDOMICS Blood Collection 2 (C2) Kit:
RHC with iCPET during Rest, Peak and Post**

Item Number	Description	Quantity
Fisher: 23-02101 BD: 762165	2.5 ml <i>PAXgene</i> ® Red top Vacutainer™ blood tube	8
Fisher: 02-657-32 BD: 366643	10 ml EDTA Plasma Lavender top Vacutainer™ blood tube	2
Fisher: 02-683-97 BD: 367986	5 ml Serum Separator Gold top Vacutainer™ blood tube (gel barrier)	1
Fisher: 02-687-107 BD: 367862	4 ml EDTA Plasma Lavender top Vacutainer™ blood tube	7

Standard phlebotomy kits that include items such as: alcohol wipes or swabs, adhesive tape, gloves, tourniquets and 21 gauge needles are not part of the collection kit and must be purchased using local center funds.

The peripheral venous blood collection needs to occur with a 21 gauge needle.

In addition, the following materials are needed for Collection 2 which occurs during RHC and are not provided by DCC:

- o BD Vacutainer Blood transfer device BD 364880
- o BD 10 ml Syringe Luer-Lok™ tip: BD 309604
- o BD 30 ml Syringe Luer-Lok™ tip: BD 309650

33.6 Preparation for Each PVDOMICS Blood Collection

Blood will be collected 2 times during the study. The first blood draw (C1) will generally occur at the first study visit. The second blood draw (C2) takes place during RHC with or without iCPET visit. It is strongly recommended to avoid scheduling Collection 1 draw on a Friday. Additional instructions for the timing of blood Collection 1 and Collection 2 are provided in Chapter 33 Addendum 1.

Several days prior to each collection, the study coordinator prepares the collection kit for the participant and the data collection forms. The coordinator needs to check expiration dates on the Vacutainer™ blood tubes included in the kit, and the participant ID and collection number. If the Vacutainer™ blood tubes included in the kit have expired or any other issues are discovered, the coordinator should enter an Emergency Supplies Request Form 340 in the study database.

The coordinator contacts the participant the day prior to the collection to remind them to avoid all food and liquids except for small sips of water for pills, for 8-10 hours prior to blood collection. Every effort should be made to schedule the biospecimen collections in the morning to avoid potential diurnal effects of measures.

Specific instructions for blood collection for healthy control subjects are included in Chapter 33, Addendum 2.

33.7 Blood Draw Collection 1 (C1) Procedure

- o All participants will need to **avoid all food and liquids** except water for 8-10 hours prior to blood collection. Record the number of hours the participant fasted on Form 300.
- o Blood collection needs to occur after vital signs and prior to physical exam.
- o Before beginning the blood collection, participants need to be resting in a chair for 15 min. This time should be in a quiet area with minimal talking and the participant should not be doing questionnaires or other study components.
- o Complete Form 300 to document the blood draw.

Procedure

1. Apply tourniquet.
 - o Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site. Tuck the end of the tourniquet under the last round. If a Velcro tourniquet is used adhere the ends to each other.
2. Identify vein.
 - o Palpate and trace the path of veins several times with the index finger. Unlike veins, arteries pulsate, are more elastic, and have a thick wall. Thrombosed veins lack resilience, feel cord-like, and roll easily – these should be avoided. If superficial veins are not readily apparent, lowering the extremity over the arm of the chair will allow the veins to fill to capacity. Identify the best available vein.

3. Assemble the butterfly-Vacutainer™ set, if butterfly is used. If a straight phlebotomy needle is used, confirm that it is a 21g and then attach it to the Vacutainer™ holder with the Luer adapter.
 - o Attach the Luer adapter to the Vacutainer™ holder.
 - o Attach the Luer end of the butterfly needle (21 gauge needle) set to the Luer adapter.
4. Cleanse the venipuncture site.
 - o Remove alcohol prep from its sterile package.
 - o Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.
 - o Allow the area to dry to prevent possible hemolysis or contamination of the specimen and a burning sensation to the participant when the venipuncture is performed.
5. If venipuncture becomes difficult, the vein may need to be touched again with a gloved hand. If this happens, cleanse the site again with alcohol and allow the skin to dry.
6. Perform venipuncture.
 - o Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches (2.5 or 5.0 cm) below the venipuncture site.
 - o With the 21 gauge needle bevel upward, enter the vein in a smooth continuous motion.
 - o Once blood appears in the butterfly tubing, place the first Vacutainer™ blood tube into the Vacutainer™ holder (Table 33.5). Grasp the flange of the needle holder and push the Vacutainer™ blood tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.
 - o Make sure the participant's arm is in a flat or downward position while maintaining the Vacutainer™ blood tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support. **DO NOT HAVE THE PARTICIPANT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN.**
 - o Remove the tourniquet after Vacutainer™ blood tube #1 fills. Once the draw has started, do not change the position of a tube until it is withdrawn from the needle. The tourniquet may be reapplied if blood flow is slow without it. If the color of the arm turns red or blue, the tourniquet is applied too tightly. Loosen it and continue.
 - o Keep a constant, slight forward pressure (in the direction of the adapter) on the end of the Vacutainer™ blood tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.
 - o Fill remaining Vacutainer™ blood tubes in the order specified in Table 33.5.
 - o Fill all Vacutainer™ blood collection tubes as completely as possible, i.e., until the vacuum is exhausted and blood flow ceases. If a Vacutainer™ tube fills only partially, remove the Vacutainer™ blood tube and attach another without removing needle from vein.
 - o When the blood flow into the Vacutainer™ blood tube ceases, remove the Vacutainer™ blood tube from the holder. The shutoff valve covers the point, stopping blood flow until the next Vacutainer™ blood tube is inserted (if necessary).

- o To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into needle box. **DO NOT ATTEMPT TO RECAP NEEDLES.** Have the participant hold the gauze pad firmly for one to two minutes to prevent bruising.
- o If the blood flow stops before collecting all of the Vacutainer™ blood tubes, repeat the venipuncture on the participant beginning with the first unfilled Vacutainer™ blood tube. As always, the tourniquet must never be on for longer than two minutes.

Order of Peripheral Venous Blood Collection 1 at Visit 1

Clinical Blood Collection: This blood is collected and analyzed at each clinical center. Vacutainer™ blood tubes are not provided by the DCC. **Do not send** to Biorepository. For details about clinical blood collection refer to Chapter 30 and Chapter 31.

Center CBC w Diff Results	<u>Note:</u> Lab draw must be no more than 1 month before the Form 100 date.
Center Complete Metabolic Panel (CMP) Results	<u>Note:</u> Lab draw <u>must be no more than one month before</u> the Form 100 date.
Center Antinuclear Antibody (ANA) Results	<u>Note:</u> Lab draw must be no more than 45 days before the Form 100 date.
Center HIV Antigen/Antibody Screen Result	If there is a historical positive HIV test, no need to repeat screen. If HIV screen was completed <u>within the past year</u> , no need to repeat screen.
Center Hepatitis Screen Result	If there is a historical positive hepatitis test, no need to repeat screen. If hepatitis screen was completed <u>within the past year</u> , no need to repeat screen.
Center Prothrombin Time (PT) Results	<u>Note:</u> Lab draw <u>must be on or after</u> the Form 100 date.

- The following clinical bloods done locally: CBC, CMP, PT/INR, and pregnancy test (if applicable) must be completed and **resulted before** right heart catheterization.
- OMICS Collection 1 blood draw must be completed before CPET, CT with contrast, RHC and V/Q scan.
- All clinical and OMICS blood draws need to be done either before the V/Q scan or at least 3 days following V/Q.
- Pregnancy testing (either blood or urine) needs to be completed and resulted prior to completion of any other testing at each study visit.

OMICS Blood Collection: Vacutainer™ blood tubes are provided by the DCC.

1. The order of blood draw is shown in Table 33.5
2. Immediately after drawing the samples, gently invert the Vacutainer™ blood collection tubes 10 times to mix the clot activator or anticoagulant with the blood.
3. Place all Vacutainer™ blood tubes on wet ice **except** the Green top (4ml) Plasma “Genomics” and Lavender top (10ml) “Cell Biomix” which will remain at room temperature and can be placed into a biohazard bag for transport.
4. Transport all Vacutainer™ blood tubes to the laboratory. All blood needs to be transported to the lab **within 1 hour of collection.**

Table 33.5 Order of OMICS Blood Draw Collection 1

Order	Color	Additive	Volume	Core	Transport to Lab
1	Gold	SST	5 ml	CC-PRL	Wet ice
2	Gold	SST	5 ml	CC-PRL	Wet ice
3	Green	Na-Heparin	4 ml	Genomic	Room temperature
4	Lavender	EDTA	4 ml	OMICS	Wet ice
5	Blue	Na-Citrate	4.5 ml	OMICS	Wet ice
6	Lavender	EDTA	10 ml	Cell Biomix	Room temperature

*** If you are unable to obtain all the blood in one venipuncture, please try another time on the same visit. **YOU WILL NEED TO HAVE THE PARTICIPANT REST FOR 15 MINUTES PRIOR TO BLOOD COLLECTION AGAIN** - If you are still unable to obtain all the blood after two attempts, then you will have to have the participant come in for another visit to obtain the blood. If you are unable to obtain the blood at visit one, you may attempt to obtain the clinical and OMICS blood at visit 2 only if you will not exceed your institution’s policy for maximum amount of blood drawn at one visit. If you were unable to draw due to volume limit constraints, the OMICS blood collection can then occur at Visit 3. Remember that the participant will need to **avoid all food and liquids** except water for 8-10 hours prior to each blood collection and needs to be resting 15 minutes prior each blood collection.

33.8 Blood Draw Collection 2 (C2) Procedure

- o All participants will need to **avoid all food and liquids** except water for 8-10 hours prior to blood collection. Record the number of hours the participant fasted on Form 304 (Form 307 if Invasive CPET center).
- o Before beginning the blood collection, participants need to be resting in a chair for 15 min. This time should be in a quiet area with minimal talking and the participant should not be doing questionnaires or other study components.
- o Non-invasive CPET Clinical centers **21, 22, 31, and 61** complete Blood Draw Form 304.
Invasive CPET Clinical centers **11, 41, and 51** complete Blood Form 307 for **all participants**.

During the RHC procedure with or without iCPET, venous blood, pulmonary capillary blood (Wedge), and pulmonary artery blood (Mix) will be collected. In some cases, systemic artery blood will also be collected.

The order of blood collection is as follows:

1. Venous blood
2. Systemic artery blood (optional)
3. Pulmonary capillary bed blood (Wedge)
4. Pulmonary artery blood (Mix)

Blood Collection During RHC with/without iCPET

Venous Blood Collection 2

1. After the introducer has been placed but before any other procedures or measurements are performed, draw the venous blood sample from the side port of the sheath. Remember participant needs to be resting for 15 min prior to this blood draw.
2. Draw back 3mL of blood in a sterile syringe and waste the sample.
3. Then, using a second 30 ml sterile syringe with a Luer-Lok™ tip, draw 27.5 ml of blood from the side port.
4. Transfer the blood in the syringe into the appropriate Vacutainer™ blood collection tubes using the Vacutainer™ blood transfer device system.
5. Order of *Venous Blood Collection 2* is shown in Table 33.6.
6. Immediately after filling the Vacutainer™ blood tubes, gently invert the Vacutainer™ blood tubes 10 times to mix the clot activator or anticoagulant with the blood.
7. Place all Vacutainer™ blood tubes on wet ice **except** the Red *PAXgene*® tube.
8. All blood needs to be transported to the lab within 1 hour of collection.
9. A study team member should be designated to transport blood samples to the lab.

Table 33.6 Order of Blood Draw Collection 2: Venous Blood Draw

Order	Blood Type	Color	Additive	Volume	Core	Transport to Lab
1	Venous	Red <i>PAXgene</i> ®	RNA Preservative solution	2.5 ml	Genomic	Room temperature
2	Venous	Lavender	EDTA	10 ml	OMICS	Wet ice
3	Venous	Lavender	EDTA	10 ml	OMICS	Wet ice
4	Venous	Gold	SST	5 ml	Bank	Wet ice

NO VENOUS BLOOD WILL BE DRAWN DURING THE CHALLENGES (INCLUDING PEAK OR POST EXERCISE)

Systemic Artery Blood Collection (optional)

Collection of systemic artery blood (radial or femoral) is optional and is preferred to collect the blood after the venous blood draw. This blood collection is not required. If arterial blood collection for arterial blood gases (ABG) occurs at the time of spirometry, there is no need to repeat ABG collection during RHC. If collecting at the time of spirometry, remember to collect the OMICS systemic artery blood **before** spirometry starts.

1. Draw back 3 ml of blood and waste the sample.
2. Draw at least 1 ml of blood into a sterile heparinized syringe for arterial blood gas analysis per protocol at local center.
3. Use a second 10 ml non-heparinized sterile syringe with a Luer-Lok™ tip, draw back 6.5 mL of blood from the arterial line for OMICS analysis.
4. Transfer the arterial blood from this second syringe into the appropriate Vacutainer™ blood collection tubes using the Vacutainer™ blood transfer device system.
5. Order of *Systemic Artery Blood Collection 2* is shown in Table 33.7.
6. Immediately after filling the Vacutainer™ blood tube, gently invert the Vacutainer™ blood tube 10 times to mix the clot activator or anticoagulant with the blood.
7. Place all Vacutainer™ blood tube on wet ice **except** the Red PAXgene® tube.
8. All blood needs to be transported to the lab within 1 hour of collection.

Table 33.7 Order of Blood Draw Collection 2: Systemic Artery Blood

Order	Blood Type	Color	Additive	Volume	Core	Transport to Lab
1	Systemic artery	Lavender	EDTA	4 ml	OMICS	Wet ice
2	Systemic artery	Red PAXgene®	RNA Preservative solution	2.5 ml	Genomic	Room temperature

Pulmonary Capillary Blood (Wedge) and Pulmonary Artery Blood (Mix) Collection

Pulmonary capillary wedge and pulmonary artery mixed blood must be drawn after all hemodynamic measurements except thermodilution cardiac output and prior to any of the challenges.

1. During the right heart catheterization, there will be two different blood draws:
 - a. Pulmonary capillary (Wedge) blood, drawn while the catheter is in the wedge position, after the pulmonary capillary wedge pressure measurements are complete.
 - b. Pulmonary artery (Mix) blood, drawn while the catheter is in the pulmonary artery position, after the pulmonary artery pressure measurements are complete.
2. Blood will be drawn from the distal port in both cases.
3. Pulmonary capillary (Wedge) blood.

- a. After all pulmonary capillary wedge pressure measurements are completed, and while the tip of the catheter is still in the capillary wedge position with the balloon inflated, draw back 3 mL of blood using a sterile syringe and waste the sample. Then, using a second 10 ml sterile syringe with a Luer-Lok™ tip, draw back 6.5mL of blood per your institution’s guidelines for drawing from a Swan-Ganz catheter.
 - b. Transfer the blood in the syringe into the appropriate Vacutainer™ blood collection tubes using the Vacutainer™ blood transfer device system.
4. Pulmonary artery (Mix) blood.
 - a. Similarly, after all pulmonary artery pressures are completed, and while the tip of the catheter is still in the pulmonary artery position, draw back 3 mL of blood using a sterile syringe and waste the sample. Then, using a second 10 ml sterile syringe with a Luer-Lok™ tip, draw back 6.5 mL of blood per your institution’s guidelines for drawing from a Swan Ganz catheter.
 - b. Transfer the blood in the syringe into the appropriate Vacutainer™ blood collection tubes using the Vacutainer™ blood transfer device system.
5. Order of blood draw during Collection 2 is shown in Table 33.8.
6. Immediately after filling the Vacutainer™ blood tube, gently invert the Vacutainer™ blood tubes 10 times to mix the clot activator or anticoagulant with the blood.
7. Place all Vacutainer™ blood tubes on wet ice **except** the Red *PAXgene*® tube.
8. All blood needs to be transported to the lab within 1 hour of collection.

Table 33.8 Order of Blood Draw Collection 2: Procedure for Pulmonary Artery Blood (Mix) and Pulmonary Capillary Blood (Wedge)

Order	Blood type	Color	Additive	Volume	Core	Transport to lab
1	Pulmonary Capillary “Rest Wedge”	Lavender	EDTA	4 ml	OMICS	Wet ice
2	Pulmonary Capillary “Rest Wedge”	Red <i>PAXgene</i> ®	RNA Preservative solution	2.5 ml	Genomics	Room Temperature
3	Pulmonary Artery “Rest Mix”	Lavender	EDTA	4 ml	OMICS	Wet ice
4	Pulmonary Artery “Rest Mix”	Red <i>PAXgene</i> ®	RNA Preservative solution	2.5 ml	Genomics	Room Temperature

If OMICS blood is collected during PEAK and POST exercise, the blood draw should be done following the procedure for the collection during REST outlined above. The recommended order of the blood draw is specified in Table 33.9.

Table 33.9 Recommended Order of Collection 2 Blood Draw During Right Heart Catheterization

Blood type	Order	Color	Additive	Volume	Cat # Fisher or BD	Core	Transport to lab
Venous	1	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomic	Room temperature
Venous	2	Lavender	EDTA	10ml	Fisher:02-657-32 BD #366643	OMICS	Wet ice
Venous	3	Lavender	EDTA	10ml	Fisher:02-657-32 BD #366643	OMICS	Wet ice
Venous	4	Gold	SST	5ml	Fisher:02-683-97 BD:367986	BANK	Wet ice
Systemic Artery “Rest”	5*	Lavender	EDTA	4ml	Fisher:02-687-107 BD:367862	OMICS	Wet ice
Systemic Artery “Rest	6*	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomics	Room temperature
Pulmonary Capillary “Rest Wedge”	7	Lavender	EDTA	4ml	Fisher:02-687-107 BD:367862	OMICS	Wet ice
Pulmonary Capillary “Rest Wedge”	8	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomics	Room Temperature
Pulmonary Artery “Rest Mix”	9	Lavender	EDTA	4ml	Fisher:02-687-107 BD:367862	OMICS	Wet ice
Pulmonary Artery “Rest Mix”	10	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomics	Room Temperature
Pulmonary Artery “Peak Mix”	11**	Lavender	EDTA	4ml	Fisher:02-687-107 BD:367862	OMICS	Wet ice
Pulmonary Artery “Peak Mix”	12**	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomics	Room Temperature
Systemic Artery “Peak	13**	Lavender	EDTA	4ml	Fisher:02-687-107 BD:367862	OMICS	Wet ice
Systemic Artery “Peak”	14**	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomics	Room Temperature
Pulmonary Artery “Post Mix”	15**	Lavender	EDTA	4ml	Fisher:02-687-107 BD:367862	OMICS	Wet ice
Pulmonary Artery “Post Mix”	16**	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomics	Room Temperature
Systemic Artery “Post”	17**	Lavender	EDTA	4ml	Fisher:02-687-107 BD:367862	OMICS	Wet ice
Systemic Artery “Post”	18**	Red	RNA Preservative solution	2.5ml	Fisher: 23-02101 BD:762165	Genomics	Room Temperature

Notes: Blood tubes 1-4 and 7-10 will be collected on all participants.

* Some participants will have blood tubes 5 and 6 collected before the resting right heart catheterization, at the same time as the initial venous collection.

** Some participants will have blood tubes 11-18 collected during the iCPET.

Addendum 1: Timing of OMICS and CLINICAL Blood Collection 1 and Collection 2

The timing of Collection 1 blood draw needs to be BEFORE Collection 2 blood draw. Blood draw is performed for clinical/safety reasons, and for OMICS analyses. Collection 1 results can be skewed by the RHC and CPET procedures, and should not be done after these procedures.

If both OMICS Collection 1 and 2 are done on the same day, the following should be done:

1. This needs to be approved by the local IRB as this may exceed local policies regarding quantity of blood draw at one visit for a research study.
2. The clinical results need to be reviewed prior to the RHC procedure by the PI or doctor performing procedure.
3. Participant rests for vitals (before blood draw)
4. Participant rests for each blood draw for 15 minutes and is fasting

Remember:

- No testing intervention (e.g., RHC, CT, MRI, VQ, etc.) before collection of blood
- Participant needs to be resting for 15 min and must be fasting prior to blood draw

Addendum 2: Biorepository Blood Collection: Healthy Controls

Healthy participants who enroll in the PVDOMICS study will be asked to provide Clinical and OMICS blood and urine. All procedures need to be performed as described at Biorepository Core blood collection overview and blood drawing procedures (Chapter 33). Participant identification numbers for healthy control subjects will have “9” as third digit, i.e. a healthy control from center 51 would have an ID “519001”. The blood collection kits for healthy control participants will be marked with an orange sticker.



Clinical Blood Collection: This blood is collected and analyzed at the clinical center. Vacutainer™ blood tubes are not provided by the DCC. **Do not send** to Biorepository. For details about clinical blood collection refer to Chapter 30 and Chapter 31.

Center CBC w Diff Results	<u>Note:</u> Lab draw must be no more than 1 month before the Form 100 date.
Center Complete Metabolic Panel (CMP) Results	<u>Note:</u> Lab draw <u>must be no more than one month before the</u> Form 100 date.
Center Antinuclear Antibody (ANA) Results	<u>Note:</u> Lab draw must be no more than 45 days before the Form 100 date.
Center HIV Antigen/Antibody Screen Result	If there is a historical positive HIV test, no need to repeat screen. If HIV screen was completed <u>within the past year</u> , no need to repeat screen.
Center Hepatitis Screen Result	If there is a historical positive hepatitis test, no need to repeat screen. If hepatitis screen was completed <u>within the past year</u> , no need to repeat screen.
Center Prothrombin Time (PT) Results	<u>Note:</u> Lab draw <u>must be on or after</u> the Form 100 date.

- Pregnancy testing (either blood or urine) needs to be completed and resulted prior to completion of any other testing at each study visit.

OMICS Blood Collection: Only Resting Venous Blood will be collected at Collection 1 (C1) and Collection 2 (C2).

If approved by IRB, the clinical blood collection and OMICS blood collection 1 and 2 can occur at one visit as described in Addendum 1. The blood volumes and the order of the blood draws are described in Tables 33.1, 33.5 and 33.6. The tables are listed below again for convenience:

This subset of Table 33.1 provides the blood volumes needed for the OMICS studies

Vacutainer™ Blood Tubes	Collection 1 (C1)	Collection 2 (C2)
Gold top (SST)	2 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
Red top (<i>PAXGene</i> ®)		1 x 2.5 ml
Blue top (Na-Citrate)	1 x 4.5 ml	
TOTAL Blood Collected	32.5 ml	27.5 ml

Table 33.5 Order of OMICS Blood Draw Collection 1

Order	Color	Additive	Volume	Core	Transport to Lab
1	Gold	SST	5 ml	CC-PRL	Wet ice
2	Gold	SST	5 ml	CC-PRL	Wet ice
3	Green	Na-Heparin	4 ml	Genomic	Room temperature
4	Lavender	EDTA	4 ml	OMICS	Wet ice
5	Blue	Na-Citrate	4.5 ml	OMICS	Wet ice
6	Lavender	EDTA	10 ml	Cell Biomix	Room temperature

Table 33.6 Order of Blood Draw Collection 2: Venous Blood Draw

Order	Blood Type	Color	Additive	Volume	Core	Transport to Lab
1	Venous	Red <i>PAXgene</i> ®	RNA Preservative solution	2.5 ml	Genomic	Room temperature
2	Venous	Lavender	EDTA	10 ml	OMICS	Wet ice
3	Venous	Lavender	EDTA	10 ml	OMICS	Wet ice
4	Venous	Gold	SST	5 ml	Bank	Wet ice

Processing of samples for healthy controls is the same as the processing of samples for pulmonary hypertension participants and is described in Chapter 34.