1.0 PURPOSE

1.1 The purpose of this standard operating procedure (SOP) is to provide instructions to BBRB-subcontracted biospecimen source sites (BSSs) for pre-anesthesia blood collection and processing. This SOP is to be followed by the BBRB-subcontracted BSSs. Pre-anesthesia blood will be collected for the preparation of plasma, RNA, and DNA from all study donors. ***Pre-anesthesia blood must be collected within 14 calendar days before the surgical event from which the submitted tissue biospecimens are derived.***

2.0 SCOPE

2.1 This procedure encompasses all BBRB activities required to properly collect and process blood at all BSSs for Phase II of the BPV program. This procedure is to be followed by all personnel performing the collection and processing of blood biospecimens.

3.0 RESPONSIBILITY

3.1 It is the responsibility of the Principal Investigator (PI) at each BSS to ensure that the phlebotomy and blood-processing lab personnel have been trained in accordance with this SOP, that the training is documented, and that this procedure is followed.

3.2 It is the responsibility of the phlebotomy and blood processing lab personnel to ensure that each of them has read, understands, and follows the SOP when processing blood samples.

3.3 It is the responsibility of the project staff designated by the PI or BSS to ensure that all the required case report forms (CRFs) in the BBRB Comprehensive Data Resource (CDR) are completed.

3.4 Any deviation or change from this SOP known before a collection should be approved by the BSS technical project manager (TPM) and ***well documented by the site***.

3.5 Any deviation or change that is unexpected or identified during or after a collection should be well documented by the site. Such deviations should be submitted to the BSS TPM along with a corrective action description for the documentation and comment.

3.6 It is the responsibility of the comprehensive biospecimen resource (CBR) to provide blood collection supplies (in the overpack) and kits for case shipment and to receive and store all biospecimens from the BSSs.

4.0 DEFINITIONS

4.1 Definitions

Case ID: An eight-character identification (e.g., BPV-XXXXX) obtained from the kits and assigned to the donor by the BSS at the time of specimen procurement

Specimen ID: Identifies each blood biospecimen from a study subject and is used on

all tubes, including cryovials, Vacutainers®, and PAXgene™ tubes

g-force: A unit of inertial force on a body that is subjected to rapid acceleration or gravity, equal to 32 feet per second at sea level; g = 1.12r (rpm/1000)2, where g = g-force, r = radius of rotor in mm, and rpm = revolutions per minute

4.2 Acronyms

BPV	Biospecimen Pre-Analytical Variables
BSS	Biospecimen Source Site
CBR	Comprehensive Biospecimen Resource
CDR	Comprehensive Data Resource
CRF	Case Report Form
EDTA	Ethylene Diamine Tetraacetic Acid
PPE	Personal Protective Equipment
SOP	Standard Operating Procedure
TPM	Technical Project Manager

5.0 ENVIRONMENTAL HEALTH AND SAFETY

5.1 Universal Precautions (CDC-1987) shall be used for all phases of blood collection and handling.

5.2 Comply with institutional policies regarding blood-borne pathogens and the use of appropriate PPE at all times.

5.3 Dispose of all contaminated supplies in the appropriate biohazard and sharps containers.

5.4 Handle all chemicals appropriately according to Material Safety Data Sheets.

Note: The contents of the PAXgene™ Blood RNA Tubes are not compatible with disinfecting reagents containing bleach.

6.0 MATERIALS AND EQUIPMENT

6.1 **Materials Required**

6.1.1 The CBR will provide collection kits and kit materials. Refer to **BPV Kit Receipt, Supplies, and Shipping Procedure, OP-0014** for specific instructions.

6.1.2 It is a requirement to use CBR-issued kits and kit materials for collection.

6.1.3 The BSS will be responsible for any additional materials or equipment to be utilized during a case collection that is not provided by the CBR.

6.2 **Materials to Be Provided by BSS**

Description	Quantity per Blood Draw	Vendor	Catalog Number
Rubber (non-latex) band for tourniquet	1	may vary	n/a
Antiseptic wipes	1	may vary	n/a
18- or 22-gauge needle	1	may vary	n/a
Needle holder (Luer-Lok Access/Blood Collection Device)	1	BD	364902
3-mL vacuum tube for capturing 3-mL discard blood	1	may vary	n/a
Dry ice	Variable	may vary	n/a
1000-µL sterile filtered pipet tips	Variable	may vary	n/a
Cryovial storage box (1-mL–2-mL cryovials)	Variable	Simport	T314-281B
Sterile conical tube	Variable	may vary	n/a
Liquid nitrogen (LN2)	Variable	may vary	n/a

6.3 **Materials Provided by CBR**

Overpack

Blood Bag (Red Kit)

Blood Red Kit (LN2 Vapor Shipper) plasma aliquots components:

- 1 yellow 2-inch storage box containing Vacutainer® tubes for storage of frozen PAXgene™ Vacutainers®

White Kit (dry ice kit) PAXgene™ blood components:

- 1 prelabeled PAXgene™ Blood DNA Tube
- 1 prelabeled PAXgene™ Blood RNA Tube
- 1 prelabeled 10-mL K2 EDTA tube
- 1 label for conical centrifuge tube (conical tube not provided)

Silver Kit (dry ice kit) plasma aliquots and whole-cell pellet aliquots components:

- 1 white 2-inch storage box with 81-count grid containing cryovials
- 21 prelabeled 1.2 mL cryovials
- Extra blood collection tube labels
- 1 plasma randomization key
- 21 extra pre-printed cryovial ID labels with attached secondary randomization key
- Extra grid (81-slot) for 2-inch box of cryovials

6.4 Equipment Required

1. Centrifuge capable of 1,500–2,000 x g
2. P1000 micro pipette
3. Blood tube rack (open/wire)
4. Cryovial rack
5. Dry ice freezing tray
6. Tabletop LN2 Dewar

7.0 PROCEDURE

7.1 Data Entry into the Required CRFs in BBRB's CDR database:

7.1.1 The **PR-0005-F1_BPV Blood Collection and Processing Form** must be completed in the CDR within 4 days of blood draw.

7.2 Pre-Blood Collection Preparation:

7.2.1 General

7.2.1.1 Pre-anesthesia blood must be collected within 14 calendar days before the surgery up until the time of induction of anesthesia at surgery, with the day of surgery as day 0. It is ideal that all three blood tubes shall be collected during the same blood draw. However, if minimum blood requirement is not met on the day of first blood draw, blood can still be collected at a later date, as long as it is collected within 14 calendar days before the time of induction of anesthesia at surgery.

7.2.2 Blood Collection Supplies

7.2.2.1 Before blood collection, each BSS will receive blood sample collection kits containing supplies for a single subject's blood draw from the BBRB CBR. These blood collection kits will be preassembled and should be quality controlled by the BSS before use to ensure accuracy and completeness of the blood sample collection kit (correct items, IDs, and labels).

7.2.2.2 Check the expiration date of blood collection tubes prior to use. Do not use expired tubes. Notify both the CBR and the BSS TPM if any kits contain expired tubes. The BSS can utilize additional clean or sterile supplies or materials as needed to ensure a successful collection.

7.2.2.3 Use appropriate PPE and universal precautions during blood collection and processing.

7.2.2.4 Before blood collection, confirm accuracy of consent status and assigned BPV Case ID.

7.2.3 Biospecimen Labeling

7.2.3.1 Each biospecimen will be identified by using a unique specimen ID. The complete specimen ID is composed of two elements—a Case ID (e.g., BPV-XXXXX) and a sequence number (e.g., 01)—that together form the final alphanumeric Specimen ID (e.g., BPV-XXXXX-01).

7.2.3.2 The blood collection staff or the BPV collection team member present at the blood collection is responsible for recording their initials and date on the blood tube or on the corresponding paper form and in each field in the

PR-0005-F1_BPV Blood Collection and Processing Form. This includes but is not limited to recording the date and time of blood draw, the name of the individual who performed the blood draw, and the time and date of receipt in the processing lab.

7.3 Blood Collection

7.3.1 Sample Collection Tubes

7.3.1.1 Each blood draw will collect a maximum of three filled blood collection tubes (Table 1). The maximum volume for the blood collection will be approximately 21 milliliters.

7.3.1.1.1 The EDTA Vacutainer® is required for participation in the study.

NOTE: *As the EDTA tubes are not graduated or marked for volume, the creation of volume reference tubes is recommended. Pipette 1.0 milliliter of water into a spare EDTA tube, and mark the tube with a permanent marker to indicate the level of fluid within the tube. Repeat adding 1.0 milliliter of water and marking the EDTA tube all the way up to 10 milliliters. The markings may then be used as a reference for assessing the amount of blood collected following a draw from a donor.*

7.3.1.1.2 After collecting the required EDTA tube of blood, the DNA and RNA PAXgene™ tubes should be collected.

NOTE: *As the PAXgene™ Blood RNA and DNA Tubes are not graduated or marked for volume, the creation of a volume reference tube is recommended. Pipette 1.0 milliliter of water into a spare PAXgene™ Blood RNA Tube and 4.0 milliliters of water into a spare PAXgene™ Blood DNA Tube. Use a permanent marker to indicate the level of the fluid within the tubes. That marking may then be used as a reference for assessing the amount of blood collected following a draw from a donor.*

7.3.1.1.3 The blood derivatives, aliquot size, number of resulting aliquots, and size of the final cryovial (container) to be used are shown in Table 1.

7.3.1.1.4 Prepare one 3-milliliter vial for the collection of initial discard blood. All other tubes will come prelabeled. This will include one 10-milliliter EDTA Vacutainer®, one 8.5-milliliter PAXgene™ Blood DNA Tube, and one 2.5-milliliter

PAXgene™ Blood RNA Tube per draw. Organize all tubes for convenient access during the blood collection procedure. The technician collecting the blood should initial and date the blood tube at the time of the blood draw.

7.3.2 **Table 1: Summary of Blood Collection Tubes, Cryovial Sizes, Aliquot Amounts, and Numbers**

BLOOD COLLECTION

Processed at	Collection Priority	Tube Type	Processed For	Tube Size	Number of Aliquots (total volume, mL)
BSS	**1**	BD Vacutainer® EDTA Tube*	Plasma and whole cell pellet	10 mL	A minimum of 12 0.25-mL aliquots of plasma in 1.2-mL cryovials. The remaining plasma should be aliquoted in 0.25-mL aliquots. A minimum of 3 1.0-mL aliquots of whole cell pellet in 1.2-mL cryovials. The remaining whole-cell pellet will be aliquoted in 1.0-mL aliquots.
CBR	**2**	PAXgene™ Blood DNA Tube	DNA	8.5 mL	Tubes will be frozen and shipped to the CBR for processing.
CBR	**3**	PAXgene™ Blood RNA Tube	RNA	2.5 mL	Tubes will be frozen and shipped to the CBR for processing.

*= EDTA tube is required for every case.

7.3.3 **Participant Position**

7.3.3.1 The participant should be immobile for at least 5 minutes before the blood draw. This may include sitting or reclining.

7.3.3.2 The arm should be positioned in a straight line from the shoulder to the wrist. The arm should not be bent at the elbow.

7.3.4 **Source of the Venous Blood**

7.3.4.1 Collect blood from the median, cubital, basilic, or cephalic veins.

7.3.4.2 In the event that blood will be collected from a port, it is essential that the initial blood collected from the port be discarded in a separate discard tube (3 milliliters) and that after blood collection; the port is properly flushed and maintained according to appropriate clinical standards. If participant blood is collected (before blood for this study), then this will serve as the flush and discard blood.

7.3.5 **Blood Drawing**

7.3.5.1 Apply a tourniquet 2 inches above the antecubital fossa or above or below the elbow (as deemed appropriate by phlebotomist) with enough pressure to provide adequate vein visibility.

7.3.5.2 The elapsed time for the use of the tourniquet should be less than 1 minute. In the event that additional time is required, the tourniquet must be removed in a fashion that restores both the circulation and normal skin color.

7.3.5.3 Clean the blood collection site of the forearm with an antiseptic wipe. Allow the antiseptic to dry.

7.3.5.4 Anchor the vein by placing the thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving.

7.3.5.5 By using the dominant hand, insert the 18- or 22-gauge needle (connected to the tube holder or blood collection device) into the participant's vein, insert the first blood tube into the blood collection device, and allow it to fill with blood.

7.3.5.6 NOTE: *Do not allow the preservative of the PAXgene™ tubes to flow back while collecting. Since PAXgene™ blood collection tubes contain a chemical additive, it is important to avoid possible backflow from the tube, with the possibility of adverse donor reactions. To guard against backflow, observe the following precautions:*

7.3.5.6.1 Place the donor's arm in a downward position.

7.3.5.6.2 Hold the tube in a vertical position, below the donor's arm, during blood collection.

7.3.5.6.3 Release the tourniquet as soon as blood starts to flow into the tube.

7.3.5.6.4 Make sure that tube additives do not touch the stopper or the end of the needle during venipuncture.

7.3.5.7 Remove the tourniquet as soon as it is reasonable to do so, either after the

blood begins to flow into the first tube, after all blood collection tubes have been filled, or between the first and last tubes, exercising discretion regarding the continued likelihood of blood flow from that participant. In all cases, be sure that the tourniquet has been removed before removing the needle from the participant's arm.

7.3.5.8 Carefully remove the needle from the participant's arm when all tubes have been filled. Apply pressure on the blood collection site with sterile gauze and apply a bandage.

7.3.5.9 Discard all remaining blood collection supplies, PPE, and needles in biohazardous waste and sharps containers, respectively, as per institutional safety and waste disposal policies.

7.3.5.10 Please collect blood in each blood tube following the priority outlined in Table 1 (the EDTA 10-milliliter tube is required and should be collected first). If possible, collect additional blood in the PAXgene™ tube for DNA and the PAXgene™ tube for RNA.

7.3.6 **Inversion of EDTA Tube**

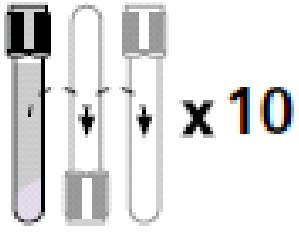
7.3.6.1 Immediately after allowing EDTA tube to completely fill, slowly and gently invert the tube 8–10 times. (Do not invert or shake the tube vigorously.)

Note: **Sample processing must begin within 30 minutes of EDTA tube blood collection. Freezing of prepared aliquots and collection of whole-cell pellet must be completed within 90 minutes of collection.**

7.3.7 **Inversion of PAXgene™ Blood DNA and RNA Tubes**

7.3.7.1 Blood draw and collection in PAXgene™ Blood DNA and RNA Tubes should be carried out per the Pre-Analytix protocol provided in the overpack from the CBR.

7.3.7.2 Immediately after blood collection, gently invert the PAXgene™ Blood DNA and RNA Tubes 8–10 times, as shown in the picture below.



(Source: *Picture taken from online manufacturer's manual: PAXgene_Blood_RNA_Tube_Product_Circular v02*)

7.3.8 **Minimum Blood Collection Requirements**

7.3.8.1 Mandatory: The volume of blood to be collected in the EDTA tube is 10 milliliters, so at least 12 plasma aliquots (0.25 milliliters plasma per aliquot) and three whole-cell pellet aliquots (1.0 milliliters whole cell pellet per aliquot) are collected.

7.3.8.1.1 If 12 plasma aliquots and three whole-cell pellet aliquots are not collected and you have the ability to collect blood again, repeat the collection as a second blood draw.

7.3.8.1.2 If 12 plasma aliquots and three whole-cell pellet aliquots are not collected after a second attempt to collect blood was made or there is not an opportunity to do a second blood draw, please contact your TPM immediately for approval to continue with the tissue collection.

7.3.8.1.3 If the participant's blood has not been processed in advance of surgery and plasma aliquots are not yet available, a minimum of 8 milliliters of blood must have been collected in the EDTA tube to proceed with the tissue collection. If less than 8 milliliters of blood is collected, please contact your TPM immediately for approval to continue with the tissue collection.

7.3.8.2 Optional: The minimum volume of blood to be collected in the PAXgene™ Blood DNA tube is 4.0 milliliters. The minimum volume of blood to be collected in the PAXgene™ Blood RNA Tube is 1.0 milliliter. Do not ship DNA or RNA PAXgene™ tubes if the minimum volumes are not collected.

NOTE: *An extra EDTA tube will be provided to use as backup in case of any issues related to a defect in the original tube supplied in the kit or in the event that a second blood draw is needed. These extra tubes will be provided unlabeled as a batch. An extra label to use for these tubes will be included in each of the overpack kits. If there is an issue with drawing the required amount of blood in the original kit EDTA tube, or if a second blood draw occurs, please use one of these extra tubes (be sure to identify the tubes by using the extra labels provided in the overpack kit). On the* ***PR-0005-F1_BPV Blood Collection and Processing Form*** *in the CDR, "add" this tube and scan the barcode ID on the label.*

7.4 Blood Processing

7.4.1 Preparation of Blood Processing Supplies and Cryovials

Before blood processing, prepare necessary supplies. Preparation of processing supplies includes the following:

7.4.1.1 Place the labels provided in the kit on the sterile conical centrifuge tubes (to be provided by the BSS).

7.4.1.2 Have the plasma randomization key out and ready to be used, scan the key ID into the CDR database and/or record on the paper CRF.

7.4.1.3 **First blood draw:**

7.4.1.3.1 Place the cryovials in sequential order (by specimen ID) onto a tube rack. Only the first 12 cryovials will be randomized by the randomization key for plasma aliquots. Any additional tubes collected will be divided evenly between storage temperatures.

7.4.1.3.2 Place the last five sequential (by specimen ID) cryovials onto a tube rack for collection of whole cell pellet aliquots. (It is possible that not all five of these designated cryovials will be used.)

7.4.1.4 **Second blood draw (if needed):**

7.4.1.4.1 Use the extra pre-printed specimen ID labels for cryovials, provided in the overpack, to label 1.2-milliliter cryovials sent in bulk. Place the cryovials in sequential order (by specimen ID) onto a tube rack. Only the first 12 cryovials will be randomized by the randomization key for plasma aliquots. Any additional tubes collected will be divided evenly between storage temperatures.

7.4.1.4.2 Place the last five sequential (by specimen ID) cryovials onto a tube rack for collection of whole cell pellet aliquots. (It is possible that not all five of these designated cryovials will be used.)

7.4.1.5 Have the CDR database and/or paper CRF (**BPV Blood Collection and Processing Form, PR-0005-F1**) ready for data entry.

7.4.2 **Plasma Preparation (Two Centrifugation Steps)**

7.4.2.1 Enter the time that processing began on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or paper CRF.

7.4.2.2 First centrifugation: Within 30 minutes of blood collection, centrifuge the EDTA tube designated for plasma preparation at 1,500 grams for 15 minutes at room temperature (20°C to 25°C or 68°F to 77°F) with centrifuge brake setting "low."

7.4.2.3 After the first centrifugation, assess whether gross hemolysis is present in the plasma sample. Gross hemolysis will be defined as bright pink or red plasma. The presence of gross hemolysis should be noted on the **BPV Blood Collection and Processing Form, PR-0005-F1** in CDR database.

7.4.2.4 Use a P-1000 manual pipet (set to a 500-microliter volume) with attached sterile filtered disposable 1000-microliter pipet tip to transfer the plasma from the EDTA tube to the correspondingly labeled 15-milliliter polypropylene conical tube.

7.4.2.5 When transferring supernatant plasma, be sure not to disrupt any material at the interphase of the spin tube. It is acceptable to leave some plasma in the original tube (approximately 0.5 milliliters) so that transferred plasma is not contaminated with any cellular debris. Document the volume of plasma transferred on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or the corresponding paper form.

7.4.2.6 Second centrifugation: Centrifuge the transferred plasma (in 15-milliliter polypropylene conical tube) a second time at 1,500 grams for 15 minutes at room temperature (20°C to 25°C or 68°F to 77°F) with brake setting "low" to remove any remaining platelets or cellular debris.

7.4.2.7 After the second centrifugation, aliquot the resulting supernatant into 0.25-milliliter aliquots. The original tube of 10 milliliters of whole blood should generate approximately 5.0 milliliters of plasma, or 16 0.25-milliliter aliquots of plasma, leaving 0.5 milliliters of plasma in the EDTA tube and 0.5 milliliters of plasma in the conical tube. Use a P-1000 manual pipet (set to a 250-microliter volume) with an attached sterile, filtered, disposable, 1,000-microliter pipet tip to transfer, in sequential order, 250-microliter aliquots of plasma from the 15-milliliter polypropylene conical tube to the ordered set of 16 labeled 1.2-milliliter cryovials.

7.4.2.7.1 Do not rely on markings on the cryovial. Please aliquot according to the amount set on the P-1000 manual pipette.

7.4.2.7.2 Do not disturb the last 0.5 milliliters of plasma in the bottom of the 15-milliliter polypropylene conical tube.

7.4.2.7.3 Document the volume of plasma transferred on the **PR-0005-F1_BPV Blood Collection and Processing Form** in the CDR database or on the corresponding paper form. Place caps on the 1.2-milliliter cryogenic vials containing plasma. Scan the barcodes of all cryogenic vials into the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database, or document them on the paper form. All plasma aliquots collected shall be used for experimental purposes.

7.4.2.7.4 The 15-milliliter conical tube should be discarded according to institutional policies.

7.4.2.8 Record the time that plasma processing was completed on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the corresponding paper form.

7.4.2.9 Freeze the plasma in the cryovials (via step-down freezing) by temporary placement on dry ice. Record the time that plasma was placed on dry ice, on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the corresponding paper form. Note: The elapsed time from blood collection to freezing should be 90 minutes or less.

If you have not scanned the plasma randomization key ID into the CDR, do so now before dividing the aliquots for storage.

If you do not collect the required 12 plasma aliquots after a second blood collection or cannot attempt a second blood collection, you must have approval from the TPM to continue with the collection. The plasma aliquots should still be distributed according to the randomization key. This may result in an uneven distribution of aliquots to the -80°C or LN2 vapor freezer. Please store as indicated by the randomization key regardless if the division is uneven or if all plasma aliquots are designated for storage in the -80°C or LN2 freezer.

7.4.2.10 Within 1 hour of processing (freezing plasma aliquots on dry ice), transfer the experimental frozen cryogenic vials designated by the randomization key to be stored in LN2 to a pre-chilled cryovial storage box and store them in an LN2 vapor freezer. Record the time at which the plasma was transferred to LN2 vapor storage on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the corresponding

paper form.

7.4.2.11 Within 1 hour of processing, (freezing plasma aliquots on dry ice), transfer the experimental frozen cryogenic vials designated by the randomization key to be stored at -80°C to a pre-chilled cryovial storage box and store in a -80°C freezer. Record the time that plasma was transferred to -80°C storage on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the corresponding paper form.

7.4.2.12 Within 1 hour of processing (freezing plasma aliquots on dry ice), transfer remaining experimental frozen cryogenic vials to pre-chilled cryovial storage boxes. Any plasma aliquots created beyond the required 12 aliquots should be divided evenly between storage at -80°C and in LN2. Record which tube is placed at each temperature for storage. Record the time that the plasma was transferred to either -80°C or LN2 on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the corresponding paper form.

NOTE: *Change pipet tips as needed to ensure the use of a clean pipet tip between plasma samples from a single participant and between different participants.*

7.4.3 **Whole-Cell Pellet Preparation**

7.4.3.1 After all plasma is removed from the EDTA tube, record the volume of whole-cell pellet remaining in the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the corresponding paper form.

7.4.3.2 Use a 5- to 10-milliliter serological pipet to gently aspirate and expel (two to three times) any remaining plasma, buffy coat, and erythrocytes before transferring and aliquoting the contents into the appropriate number of sterile prelabeled cryovials.

7.4.3.3 Aliquot the resulting mixture of whole cell pellet into 1.0-milliliter aliquots. After removing 4.5 milliliters of plasma from the original 10-milliliter tube, there should be approximately 5.5 milliliters left over (plasma, buffy coat, and erythrocytes) that will be mixed and referred to as the whole-cell pellet. The whole-cell pellet should generate approximately five 1-milliliter aliquots from the 5.5-milliliter volume. The final five cryovial specimen IDs in the sequential order of cryovials will be used to collect whole-cell pellet aliquots. Use a P-1000 manual pipet with an attached sterile, filtered, disposable, 1,000-microliter pipet tip to transfer 1,000-microliter aliquots from the EDTA collection tube to the five pre-labeled 1.2-milliliter cryovials

designated for whole-cell pellet collection. Place caps on the 1.2-milliliter cryogenic vials containing the whole-cell pellet. Scan the barcodes of all cryogenic vials into the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database, or document them on the corresponding paper form. Document the volume of whole-cell pellet transferred into each aliquot on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the paper form.

7.4.3.4 Freeze the whole cell pellet aliquots directly in a -80°C freezer. Record the time at which the whole-cell pellet aliquots were placed into the freezer on the **BPV Blood Collection and Processing Form, PR-0005-F1** in the CDR database or on the corresponding paper form.

7.4.4 **PAXgene™ Blood DNA Tube**

7.4.4.1 Blood samples collected in the PAXgene™ Blood DNA Tubes must be stored at -22°C to -18°C in an open wire rack for a minimum of 24 hours and a maximum of 72 hours. Enter the time at which the tube was stored at -22°C to -18°C on the CRF (**BPV Blood Collection and Processing Form, PR-0005-F1**) in the CDR database.

7.4.4.2 After freezing the tubes for at least 24 hours at -22°C to -18°C, transfer the DNA PAXgene blood tubes to -90°C to -70°C for long-term storage. Enter the time at which the tube was stored at -90°C to -70°C on the CRF (**BPV Blood Collection and Processing Form, PR-0005-F1**) in the CDR database.

7.4.4.3 Blood samples in the PAXgene™ Blood DNA Tube should be kept stored at -90°C to -70°C until shipment, per the instructions provided by the CBR (**BPV Kit Receipt, Supplies, and Shipping Procedure, OP-0014**).

7.4.5 **PAXgene™ Blood RNA Tube**

7.4.5.1 Incubate the PAXgene™ Blood RNA Tubes upright at room temperature (18°C to 25°C) for a minimum of 2 hours and a maximum of 12 hours.

7.4.5.2 Blood samples collected in the PAXgene™ Blood RNA Tubes should be first stored at -22°C to -18°C in an open wire rack for a minimum of 24 hours and a maximum of 72 hours and then transferred to -80°C to -70°C.

7.4.5.3 Enter the time that tubes were transferred to -22°C to -18°C and to -90°C to -70°C on the CRF (**BPV Blood Collection and Processing Form, PR-0005-F1**) in the CDR database.

7.4.5.4 Blood samples in the PAXgene™ Blood RNA Tube should be kept stored at -90°C to -70°C until shipment, per the instructions provided in **BPV Work Instruction for the White Kit, OP-0014-W3.**

7.4.6 **Storage and Shipment**

7.4.6.1 Plasma should be stored in an LN2 vapor phase freezer until shipment except for the experimental vials that should be stored at -80°C. PAXgene™ Blood DNA and RNA Tubes are to be stored at -90°C to -70°C before shipment. Biospecimens should never be allowed to thaw.

7.4.6.2 If two blood collections were processed, ship only the blood collection with the required 12 plasma aliquots and three whole-cell pellet aliquots or the aliquots associated with a collection where the TPM approved less than the required plasma and whole cell pellet aliquots. Do not ship both blood collections.

7.4.6.3 Timing of Blood Shipping:

7.4.6.3.1 Cases with blood and tissue collected: Plasma aliquots and whole-cell pellet aliquots should have data entry completed and shipped within 39 to 44 days from the day of blood collection.

7.4.6.3.2 Cases with only blood collected: Plasma and whole-cell pellet aliquots should have data entry completed and shipped within 25 to 30 days from the day of blood collection.

7.4.6.4 Pack and ship all blood biospecimens to the CBR according to the **BPV Kit Receipt, Supplies and Shipping Procedure, OP-0014** and **BPV Work Instruction for the Red Kit (OP-0014-W2)**, **BPV Work Instruction for the White Kit (OP-0014-W3)**, or **BPV Work Instructions for Silver Kit (OP-0014-W4)**, depending on which specimens are to be shipped.

7.4.6.5 Ship frozen PAXgene™ Blood DNA and RNA Tubes and experimental plasma aliquots that were temporarily stored at -80°C on dry ice to the CBR.

7.4.6.6 Ship the remaining plasma aliquots that were temporarily stored in LN2 vapor by using a LN2 Vapor Phase Shipper provided by the CBR.

8.0 REFERENCES

8.1 BD Instructions for Use: Preparing a Quality Sample (http://www.bd.com/vacutainer/products)

8.2 Common Blood Collection and Plasma Processing Protocol: Clinical Proteomics Technologies Assessment for Cancer Biospecimen Working Group (2008)

8.3 https://www.preanalytix.com/products/blood/dna/paxgene-blood-dna-kit/US?cHash=a7c9a10ec586bc528688c5e39530187c

8.4 https://www.preanalytix.com/products/blood/rna-/-mirna/paxgene-blood-rna-tube-ivd/DE?cHash=a8705841c1048aa1d98cb94f51a12164

8.5 https://www.preanalytix.com/products/blood/dna/paxgene-blood-dna-tube/DE?cHash=db172aa62dd2bd6e14093cf5bcecf077

9.0 ATTACHMENTS

9.1 BPV Blood Collection and Processing Form, PR-0005-F1