

1.0 STANDARD OPERATING PROCEDURE (SOP): BLOOD COLLECTION AND PROCESSING

1.1 General Guidelines and Supplies

All biospecimen samples will be collected from participants who have agreed to participate in the study and have signed the Informed Consent Form.

Universal precautions, Occupational Safety and Health Administration guidelines (OSHA), and institutional requirements will be followed in the collection and handling of biospecimens in this study. Universal precautions are a method of infection control in which all human tissue, blood, and body fluids are treated as if infectious and appropriate personal protective equipment (e.g., gloves, yellow gown, eye protection, etc) is worn.

All materials (e.g., tubes, needles, pipettes, etc) must be properly disposed of in biohazard containers, in accordance with institutional requirements. All equipment used in the collection, storage, and shipping must be labeled as biohazard.

All biospecimens must be aliquoted to an appropriate test volume prior to freezing to avoid freeze-thaw cycles. Freezers should have a back-up generator or other emergency system.

Collection and processing supplies are provided by Boston University. To request supplies, please submit an Inquiry to Coord Ctr in VSIMS.

1.2 Blood Kit and Associated Supplies

Contents of Blood Kit (created by site using supplies provided by Boston University)

- a. Small, biohazard plastic bags that contain:
 - I. Two Vacutainer® tubes (no additive, silicone coated): e.g. BD Vacutainer Plus plastic serum tube, 10 mL, red top (Fisher #02-685-112)
 - II. Two Vacutainer® tubes containing anticoagulant (e.g. BD Vacutainer plastic K2 EDTA (spray-dried) tube, 10 mL, lavender top (Fisher #02-657-32)
 - III. Two PAXgene Tubes (Fisher #23-021-01)
 - IV. Kit ID Labels for labeling Vacutainer® tubes (provided by DMCC)
- b. two 15 mL-conical tube with for second spin for both serum and plasma
- c. ten 0.1 mL volume small serum (red) aliquot tubes 1.5 mL size
- d. ten 0.5 mL volume serum (yellow) aliquot tubes 1.5 mL size
- e. ten 0.1 mL volume small plasma (green) aliquot tubes 1.5 mL size
- f. ten 0.5 mL volume plasma (blue) aliquot tubes 1.5 mL size
- g. serological pipettes with 0.1 mL and 0.5 mL markings (sterile)
- h. three Specimen Worksheets - One labeled "Red Top Tubes (serum)", one labeled "Purple Top Tubes (plasma)" and one labeled "PAXgene" (provided by DMCC)
- i. barcode labels for aliquots (provided by DMCC)

Supplies needed, but not provided in the Blood Kit

- a. Blood drawing supplies (needles, Vacutainer® adapter, tourniquet, bandages)
 - b. Ice or ice packs
 - c. Extra red and purple Vacutainer® tubes and extra PAXgene tubes
- All Vacutainer® tubes and transfer vials get labeled by the site staff using the Kit Label provided, which corresponds to the Specimen Worksheets.
 - All aliquot tubes should be labeled as the aliquots are created or ahead of time. Only aliquot the serum and plasma from tubes with the same Kit # that the aliquot tubes are from.
 - NOTE: The aliquot tube labels do not contain any reference to the Kit ID. The specimen aliquot tubes cannot ever be labeled with a Kit ID.
 - When processing specimens for multiple participants:
 - Pay particularly close attention not to mix up kit components. If necessary- once the aliquot tubes are in a rack, the label from the aliquot tube bag can be removed and temporarily attached to the rack, to help

- keep things organized.
- It is imperative that ALL Vacutainer® tubes be labeled *immediately* after collection and prior to any processing.
- Never “borrow” labeled kit components from one kit to use in another.
- It is acceptable to label a Kit Bag with a participant ID label to keep things organized.

1.3 Blood Sample Collection Procedure

1. Do not collect blood specimens unless consent has been signed
2. Obtain a new, unused Specimen Kit
3. Put on gloves and prepare needle (if used, 21 gauge recommended) and red, purple top Vacutainer® tubes and PAXgene tubes
4. Using a Vacutainer® adapter collect the blood in the following order:
 - a. 2 red top Vacutainer® tubes.
 - b. 2 purple top Vacutainer® tubes. These must be filled to line on label for proper EDTA: blood ratios, invert gently 10 times to mix (careful not to shake too hard or buffy coat cells could lyse)
 - c. 2 PAXgene tubes (2.5 mL of blood per tube) for RNA
5. After collection, place a Kit ID label on each of the tubes
6. On the Red Top Tubes (Serum), Purple Top Tubes (Plasma) and PAXgene Worksheets:
 - a. Label the worksheet with a Kit ID Label and a Participant ID Label. This is critical for associating a Kit to a single participant.
 - b. Enter the date and time of the draw.
 - c. Mark the fasting status.
 - d. Also check the appropriate boxes labeled “1st/2nd Red Top Vacutainer® Tube Collected” and 1st/2nd Purple Top Vacutainer® Tube collected and PAXgene Tube collected to indicate if the full draw was completed.

1.4 Notes About The Blood Samples

1. Wear gloves at all times when handling aliquot tubes
2. Fill the aliquot tubes in numerical order according to the SR or PL numbers on the barcode labels
3. **PLEASE NOTE THAT RED AND PURPLE TOP VACUTAINER® TUBES ARE HANDLED AND PROCESSED DIFFERENTLY**
4. Additional processing supplies needed and supplied, but not contained in the Blood Kit
 - a. Refrigerated centrifuge with brake off feature and non-refrigerated centrifuge with brake off feature
 - b. Racks for holding the small tubes
 - c. Styrofoam holder for 15 mL conical tubes and blood collection tubes
 - d. Disposable, sterile packaged pipets for RNA later
 - e. 9x9 slotted freezer boxes
 - f. Site specific numbering schema for freezer boxes (provided by DMCC)
 - g. Sharpie® or other marker to label freezer boxes

1.5 Red Top Vacutainer® Tubes:

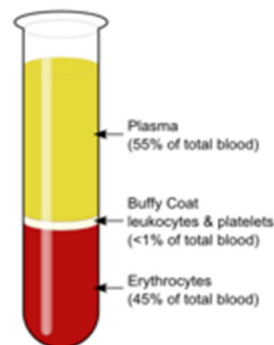
1. For serum collection, draw whole blood into vacutainer tube(s) containing no anticoagulant. Serum samples should be processed and stored within two hours of blood collection.
2. Incubate in an upright position at room temperature for 30-45 min (no longer than 60 min) to allow clotting.
3. Put on ice or upright at 4°C until centrifuged (do not exceed 2 hours).
4. Centrifuge for 15 minutes at manufacturer’s recommended speed (1300g) in a benchtop centrifuge (NOT refrigerated). Do not use brake to stop centrifuge.
5. Carefully aspirate off the serum at room temperature and pool into a centrifuge tube, taking care not to

disturb the cell layer or transfer any cells. Use a clean pipette for each tube. After centrifugation, use a disposable transfer pipet to draw the serum (top layer) off into 15mL polypropylene collection tubes. Centrifuge tubes at 1500g for 10 minutes to pellet any contaminating mononuclear and other cells.

6. Transfer serum into a total of 20 cryovials: 10 cryovials (100µl/vial – red 1.5 mL) and remaining serum into 10 cryovials (500µl/vial – yellow 1.5 mL). At the end of the procedure check that all aliquot vial caps are secure.
7. Store vials upright in a labeled specimen box in a -80°C freezer. All specimens should remain at -80°C prior to distribution.
8. Document the data point information on the Worksheets as follows:
 - Date and time of blood collection
 - Fasting status
 - Number and volume of aliquots prepared
 - Date and time serum placed into -80°C freezer
 - Any freeze-thaw that occurs with a sample for any reason
 - Additional information as specified in the Worksheet

1.6 Purple (lavender) Vacutainer® Tubes:

1. Draw blood into two (2) vacutainer tubes containing ~1.8 mg K2EDTA per mL blood (may vary depending on manufacturer). Be sure to draw the full volume to ensure the correct blood-to anticoagulant ratio.
2. After collection in vacutainer, gently mix the blood by inverting the tube 10 times to mix blood and anticoagulant. Store vacutainer tubes on ice or upright at 4°C until centrifugation. Plasma samples should be processed and stored within two hours of blood collection.
3. Centrifuge samples immediately. for a minimum of 10 minutes at 1300g) in a refrigerated +4°C centrifuge. Please refer to speeds and times recommended by manufacturer. Do not use brake to stop centrifuge.
4. Post centrifugation, the specimen will contain three layers: (from top to bottom of tube) plasma, leukocytes (buffy coat), and erythrocytes (red blood cells, RBCs).



5. Carefully collect the plasma layer at room temperature and pool in a centrifuge tube. Take care not to disrupt the leukocytes (buffy coat) layer or transfer any cells.
6. Transfer the plasma into 15 mL conical tube and spin at 1500g for 5 minutes to remove all potentially remaining cells in a refrigerated +4°C centrifuge.
7. Inspect plasma for turbidity. Turbid samples should be centrifuged and collected again to remove remaining insoluble matter.
8. Carefully collect the plasma layer with an appropriate transfer pipette without disturbing the leukocyte (buffy coat) layer.

Please note: The Lung Team Project #2 is not storing buffy coat

9. Pipet the plasma into appropriate sized aliquots, attempting not to disturb any of the cell pellet. Transfer plasma into a total of 20 cryovials: 10 cryovials (100µl/vial – green 1.5 mL) and remaining plasma into 10 cryovials (500µl /vial – blue 1.5 mL). Label all samples appropriately. At the end of the procedure, check that all aliquot vial caps are secure.
10. Store vials upright in a labeled specimen box in an -80°C freezer. All specimens should remain at -80°C prior to distribution.
11. Document the data point information appropriately on the Worksheets.
 - Date and time of blood collection
 - Fasting status
 - Number and volume of aliquots prepared
 - Date and time placed into -80°C freezer
 - Any freeze-thaw that occurs with a sample for any reason
 - Additional information as specified in the Worksheets

1.7 PAXgene™ Tubes:

PAXgene™ Blood RNA Tube should be drawn last in the phlebotomy procedure, so the interior volume of the blood collection set used during phlebotomy can be primed.

Storage Conditions for materials:

PAXgene RNA spin columns, PAXgene Shredder spin columns, proteinase K, and Buffers BR1 to BR5 can be stored dry at room temperature (15–25°C).

The RNase-Free DNase Set, which contains DNase I, Buffer RDD, and RNase-free water (tube), is shipped at ambient temperature. Store all components of the RNase-Free DNase Set immediately upon receipt at 2–8°C.

Ensure that the PAXgene™ Blood RNA Tube is at 18°C to 25°C prior to use and properly labeled with Kit #.

1. Using a blood collection set and a holder, collect blood into the PAXgene™ Blood RNA Tube using your institution's recommended procedure for standard venipuncture technique.
2. The following techniques shall be used to prevent possible backflow:
 - Place donor's arm in a downward position.
 - Hold tube in a vertical position, below the donor's arm during blood collection.
 - Release tourniquet as soon as blood starts to flow into tube.
 - Make sure tube additives do not touch stopper or end of the needle during venipuncture.
3. Allow at least 10 seconds for a complete blood draw to take place. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The PAXgene™ Blood RNA Tube has a vacuum designed to draw 2.5mL of blood into the tube.
4. Immediately after blood collection, gently invert the PAXgene™ Blood RNA Tube 8 – 10 times.
5. Store the PAXgene™ Blood RNA Tube upright at room temperature (18°C to 25°C) for a minimum of 2 hours but no more than 4 hrs.
6. Stand the PAXgene™ Blood RNA Tube upright in a wire rack. Do not freeze tubes upright in a styrofoam tray as this may cause the tubes to crack. Store tubes in a -80°C freezer.
7. Document the data point information appropriately on the Worksheet
 - Date and time of blood collection
 - Fasting status
 - Number and volume of aliquots prepared
 - Date and time placed into -80°C freezer
 - Any freeze-thaw that occurs with a sample for any reason
 - Additional information as specified in the Worksheet

Lung Team Project #2 Specimen Flow Sheet

1 SERUM



2 x 10mL red top Vacutainer® tubes

- Clot 30-45 min (no more than 60min) then on ice or 4°C until centrifuged (no longer than 2 hours)
- Centrifuge (15 min at 1300g, with brake off, in non-refrigerated centrifuge)

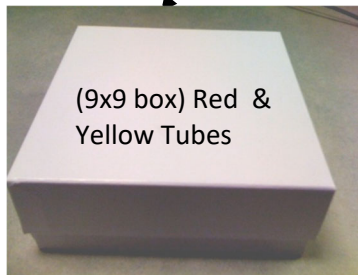
→ Centrifuge 15 mL tube with combined serum for 10 more min at 1500g



10 x 0.1 mL aliquot vials (red 1.5 mL) fill with 100µL of serum



10 x 0.5 mL aliquot vials (yellow 1.5 mL) fill with 500 µL of serum



Store at -80°C

PAXgene



Wire Rack

2 PLASMA



2 x 7mL purple EDTA Vacutainer® tubes

- Invert gently 10 times
- on ice or 4°C until centrifuged (no longer than 2 hours)
- Centrifuge (10 min at 1300g with the brake off, in refrigerated centrifuge)

→ Centrifuge 15 mL tube with plasma for 5 more min at 1500g

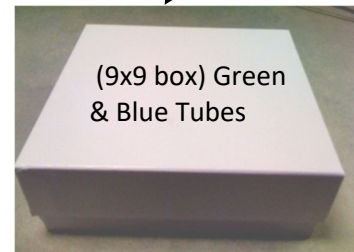


10 x 0.1 mL aliquot vials (green 1.5 mL) fill with 100µL of plasma



10 x 0.5 mL aliquot vials (blue 1.5 mL) fill with 500 µL of plasma

Store at -80°C



LTP2 RED TOP TUBES (SERUM)

Kit Label Here		Participant ID Label Here
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Staff ID: _____

Date Collected: ____ - ____ - ____

Time Collected: ____:____ AM PM

Participant Fasting? Yes No

Time at RT to Clot (30-45 min - no more than 60 min) _____ Minutes

Tubes Placed at 4°C (0-2 hours) Yes No If yes: ____:____ AM PM (Exact Time)

Time 1st Centrifuge Started (Benchtop, Non-refrigerated, no brake: 15 min at 1300g/1000-2000RCF) ____:____ AM PM (Exact Time)

Time 2nd Centrifuge Started (10 min at 1500 RCF) ____:____ AM PM (Exact Time)

Time Placed in Freezer ____:____ AM PM (Exact Time)

Freezer Temperature -80°C or colder Yes No










1st Red Top Tube Collected

2nd Red Top Tube Collected

if collected

	Volume	Freezer #	9x9 Box #	Row	Column	Hemolyzed/Lipemic/Icteric	SOP followed?
<input type="checkbox"/> 3541516622 SR01	100ul					H, L, I	Y / N
<input type="checkbox"/> 3541516704 SR02	100ul					H, L, I	Y / N
<input type="checkbox"/> 354151688 4SR03	100ul					H, L, I	Y / N
<input type="checkbox"/> 354151696 6SR04	100ul					H, L, I	Y / N
<input type="checkbox"/> 3541517048 SR05	100ul					H, L, I	Y / N
<input type="checkbox"/> 354151712 8SR06	100ul					H, L, I	Y / N
<input type="checkbox"/> 354151720 9SR07	100ul					H, L, I	Y / N
<input type="checkbox"/> 3541517389 SR08	100ul					H, L, I	Y / N
<input type="checkbox"/> 3541517460 SR09	100ul					H, L, I	Y / N
<input type="checkbox"/> 3541517537 SR10	100ul					H, L, I	Y / N
<input type="checkbox"/> 3541517613 SR11	500ul					H, L, I	Y / N

LTP2 RED TOP TUBES (SERUM)

<input type="checkbox"/>	3541517795 SR12 	500ul					H, L, I	Y / N
<input type="checkbox"/>	354151787 6SR13 	500ul					H, L, I	Y / N
<input type="checkbox"/>	354151795 6SR14 	500ul					H, L, I	Y / N
<input type="checkbox"/>	354151803 6SR15 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541518116 SR16 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541518294 SR17 	500ul					H, L, I	Y / N
<input type="checkbox"/>	354151837 6SR18 	500ul					H, L, I	Y / N
<input type="checkbox"/>	354151845 6SR19 	500ul					H, L, I	Y / N
<input type="checkbox"/>	354151860 6SR20 	500ul					H, L, I	Y / N

If all tubes were not created give reason: _____

LTP2 PURPLE TOP TUBES (PLASMA)

Kit Label Here		Participant ID Label Here
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Staff ID: _____

Date Collected: _____ - _____ - _____

Time Collected: _____:_____ AM PM

Participant Fasting? Yes No

Tubes inverted 10 times? Yes No

Tubes Placed at 4°C (0-2 hours) Yes No If yes: _____:_____ AM PM (Exact Time)

Time 1st Centrifuge Started (Benchtop, refrigerated, no brake: 10 min at 1300g/1000-2000RCF) _____:_____ AM PM (Exact Time)

Time 2nd Centrifuge Started (5 min at 1500 RCF) _____:_____ AM PM (Exact Time)

Time Placed in Freezer - Plasma _____:_____ AM PM (Exact Time)











Freezer Temperature -80°C or colder Yes No

1st Purple Top Tube Collected 2nd Purple Top Tube Collected

if collected Volume Freezer # 9x9 Box # Row Column Hemolyzed/Lipemic/Icteric SOP followed?

<input type="checkbox"/>	3541516626 PL01	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541516706 PL02	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541516886 PL03	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541516966 PL04	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541517046 PL05	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541517125 PL06	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541517205 PL07	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541517384 PL08	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541517465 PL09	100ul					H, L, I	Y / N
<input type="checkbox"/>	3541517536 PL10	100ul					H, L, I	Y / N

LTP2 PURPLE TOP TUBES (PLASMA)

<input type="checkbox"/>	3541517617 PL11 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541517793 PL12 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541517875 PL13 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541517956 PL14 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541518036 PL15 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541518116 PL16 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541518296 PL17 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541518378 PL18 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3545184589 PL19 	500ul					H, L, I	Y / N
<input type="checkbox"/>	3541518605 PL20 	500ul					H, L, I	Y / N

If all tubes were not created give reason: _____

LTP2 PAXgene TUBES

Kit Label Here		Participant ID Label Here
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Staff ID:

Date Collected: - -

Time Collected: : AM PM

Participant Fasting? Yes No



Inverted 8-10 times? Yes No

Hours stored at room temperature (minimum of 2 hrs, no more than 4 hrs)

Time Placed in Freezer : AM PM (Exact Time)

Freezer Temperature -80°C or colder Yes No

if collected

<input type="checkbox"/>	Volume	Freezer #	Rack#	Slot#	SOP followed?
<input type="checkbox"/> 3541516626 PG01 	n/a				Y / N
<input type="checkbox"/> 3541516706 PG02 	n/a				Y / N

SAMPLE