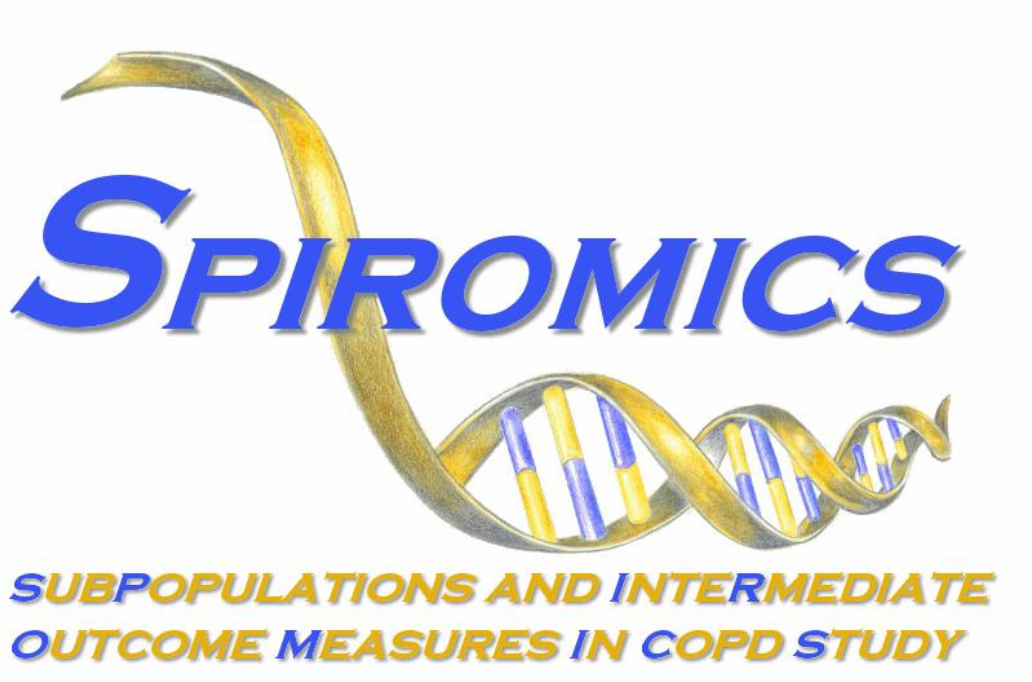


MOP 4

BIOSPECIMEN COLLECTION AND PROCESSING

January 26, 2012

SPIROMICS BIOSPECIMEN COLLECTION AND PROCESSING MOP

1. BIOSPECIMEN COLLECTION AND PROCESSING: Blood and Urine

Subpopulations and intermediate outcome measures in COPD study (SPIROMICS) supports the prospective collection and analysis of phenotypic, biomarker, genetic, genomic, and clinical data from subjects with COPD for the purpose of identifying subpopulations and intermediate outcome measures. It is funded by the National Heart, Lung, and Blood Institute and is coordinated by the University of North Carolina at Chapel Hill.

Research subjects for SPIROMICS will be enrolled, phenotyped, and followed at six SPIROMICS Clinical Centers and associated sub-sites (in Winston-Salem, NC; Ann Arbor, MI; San Francisco, CA; Los Angeles, CA; New York City, NY; and Salt Lake City, UT). Molecular fingerprinting and extensive subject phenotyping will be performed to identify disease subpopulations and to identify and validate surrogate markers of disease severity that will be useful as intermediate outcome measures for future clinical trials. Secondary aims are to clarify the natural history of COPD, to develop bioinformatic resources that will enable the utilization and sharing of data in studies of COPD and related diseases, and to create a collection of clinical, biomarker, radiographic, and genetic data that can be used by external investigators for other studies of COPD.

Aliquots of serum, plasma, urine, sputum, and cell lysate for DNA will be prepared at the field centers and will be stored at the GIC Central Repository. The Central Repository is located at the University of North Carolina at Chapel Hill, Chapel Hill, NC. The GIC coordinating center at the University of North Carolina at Chapel Hill will set-up, operate, and maintain a repository /biobank of biospecimens collected from SPIROMICS participants. The purpose of this particular repository/biobank is to store samples for future research. For SPIROMICS, samples of blood, urine, and sputum will be collected, processed, and stored. A complete list of the aliquots to be prepared is located in Appendix 1.

Investigators will in the future conduct a wide variety of analyses on the specimens banked in the GIC SPIROMICS repository. Some of the analyses will include genetic and genomic analyses to look for relationships between genes, between genes and non-genetic factors (e.g., environment, behaviors), and between genes and other diseases. Other kinds of analyses will include studying the concentrations and mixture of proteins, cells, and RNA found in the various biospecimens. The most important step in maintaining a successful repository, and often times the most difficult to standardize, is the collection and clinical center processing of the blood samples. If the blood, urine, or sputum samples are not collected or processed correctly, laboratory results may not accurately reflect true differences between study participants, even if the laboratory tests are performed accurately. Standardized and quality sample collection and processing is critical to maintaining baseline reproducibility. For this or any study to succeed, biospecimens must be collected, processed, and stored in standardized, safe, and appropriate manner at all clinical sites, as well as in the repository. Thus, it is important that all staff be well-trained, certified, and fully compliant with the protocol for drawing and processing the specimens.

2. PREPARATION for BIOSPECIMEN COLLECTION

Since participation in this study is voluntary, and since it asks a lot of each participant, all SPIROMICS staff should make a concerted effort to make the entire visit as easy and comfortable as possible As always, the blood draw technicians should be calm and confident, particularly when the participant is uneasy or nervous. One way to accomplish this is to be thoroughly knowledgeable about all aspects of the procedures to be conducted.

In summary, the SPIROMICS study will collect approximately 58 - 76 mL of blood in nine tubes from each participant. The blood draw technicians should reinforce the idea that the volume of blood being collected is only about 4 – 5 tablespoons, even if it looks like a lot more, and they should add that this is only about a 10^{th} of the amount of blood that is drawn when a pint of blood is donated.

2.1. Staff Certification Requirements

Blood drawing and processing will be performed by a trained phlebotomist/blood draw technician that has been certified on the SPIROMICS MOP. The technicians will complete a training course taught by either SPIROMICS staff or SPIROMICS-trained-clinical lab certified staff. Each technician must complete the training and pass a written exam before becoming SPIROMICS certified. Recertification should take place annually and will be authorized by clinical center supervisory personnel. Based on individual job duties, biospecimen certification may be necessary in one or all of the following areas.

2.1.1 Blood drawing and urine collection
2.1.2 Blood & urine lab processing and preparation of aliquots
2.1.3 Packaging and shipping of samples

2.2. Blood and Urine Collection Trays and Tubes

One day prior to a scheduled participant visit, the clinical center processing technician will prepare one tray to hold the urine and blood collection supplies and the blood collection tubes. In addition, 7 racks will be prepared to hold intermediate products and final aliquots as outlined in section 2.2.4. Prior to the visit, all urine cups, blood vacutainer tubes, processing tubes and final product aliquot tubes should be tagged with labels that have been provided by the GIC with the appropriate code numbers for the participant. Label sheets will be provided by the GIC and will include the following:

- 8 labels with Laboratory ID (Lab ID); 7 blood tubes for processing and 1 urine specimen cup)

- 14 labels per each blood tube processed with only the Lab ID, visit, specimen type, and aliquot number. These are for the aliquots for the serum or plasma from blood tube #s 1-5, and 8

- [Not currently funded, therefore not included in label sets at this time] 1 label with laboratory ID for tube #6 (if collected) and its processed products to be processed and sent for immunophenotyping.

- 4mL EDTA tube for clinical lab processing will be labeled according to local lab requirements

- 20 Labels for urine specimen aliquots with Lab ID, visit, specimen type, and aliquot number

- 12 Labels for sputum specimens with Lab ID, visit, specimen type, aliquot number as well as two labels for sputum slide and two labels for sputum storage boxes. For aliquot numbering scheme refer to sputum MOP 5.

- 3 labels with Lab ID, visit, and specimen type for cell lysate 50-ml conical centrifuge tube, for rack holding these tubes prior to and during mailing, and 1 label for sheet of paper that will be included with each bagged rack of tubes.

- 4 labels with Lab ID, visit, and box numbers for the top and bottoms of the two aliquot -80ºC storage boxes.

- 2 labels with Lab ID for the top and bottom of the PAXgene RNA blood tube box. These are labeled PXGR – Stor. Sites will need to add the appropriate box number.

- 20 generic labels with Lab ID and visit number to be used at discretion of the technician for items such as blood sample redraw, dropped aliquot tube, etc.

- Labels for blood collection and tube processing racks are not included. Sites are free to choose to use generic labels for this purpose.

2.2.1 Blood and Urine Collection Tray

Prior to the visit the technician organizes and prepares the blood collection tray. The blood collection tray should be made of materials that can be easily cleaned. The tray should have an appropriate amount of space to hold the following supplies:

- urine specimen collection cup
- Urine one-step pregnancy test kit (e.g., QuPid)
- test tube rack that holds at least 9 blood collection tubes (exact tubes to be used are described in the next section)
- 12-inch blood collection set for use with all tubes but mandatory for the P100 plasma collection tubes (this kit includes the needle all except the last three items ion this list)
- sterile, disposable 21 gauge butterfly needles
- plastic vacutainer tube guides
- vacutainer Luer adapters
- sterile alcohol swabs
- gauze sponges
- tourniquet
- bandages ("Band Aids")

Finally, smelling salts, ice packs, and washcloths should be readily available in the blood collection area for participants who become faint during the blood collection.

2.2.2 Blood Collection Tubes

Technicians must be familiar with: the arrangement of blood collection tubes, the order in which the tubes are to be filled, the type of anticoagulant/preservative, and the possible sources of error in handling each tube. These tubes are organized in the test tube rack in the following sequence:

Tubes #1 and #2 are **8.5 mL red stoppered serum tubes (RT)**. Although these tubes do not contain anticoagulant, they do have a clot activator and therefore require mixing immediately following collection. The serum from each of these tubes will be aliquoted into 14 aliquots as described below. It will be used for analysis of known biomarkers or hypothesis driven biomarkers, where it has been previously determined, or reasonably speculated, that serum will serve as the best source for analyte measurement.

Tube #3 is a 10 mL yellow-stoppered tube containing 1.5ml of ACD anticoagulant. This tube should be filled completely in order to standardize the blood to liquid anticoagulant ratio. A full tube is equivalent to collection of 8.5mL of blood. This tube will be used for specifically-defined proteomic analyses of plasma, such as quantification of fibrinogen.

Tubes #4 and 5 are three, 10 mL lavender-stoppered tubes containing a sprayed on K_2EDTA anticoagulant. The plasma from both tubes will be used for hypothesis-driven, specifically-defined, proteomic analyses. Cells from one of the tubes will be processed in two steps to a cell lysis, which will then be used by the GIC repository for DNA extraction. In addition, tube #6 may be used for detailed cellular immunophenotyping analysis (note tube #6 may not be collected).

Tube #7 is a 3 or 4 mL lavender-stoppered tube containing a sprayed on K_2EDTA anticoagulant. This tube will be sent directly to the clinical lab at each site for Complete Blood Counts (CBCs) with differentials.

Tubes #8 is an 8.5ml P100 red-stoppered plasma collection tube with a mechanical separator and sprayed on K_2EDTA anti-coagulant and proprietary additives.
Plasma from this tube will be used for large-scale discovery proteomic analyses.

Tube #9 is a 2.5 mL red-stoppered Paxgene RNA tube containing anticoagulant and RNA stabilizers. The Paxgene tube is the size of a 10 mL collection tube, but because of the liquid stabilizers, only 2.5 mL of blood is collected. This tube must be filled completely in order to standardize the blood to liquid anticoagulant ratio. Partially filled tubes will result in erroneous test results. RNA will be isolated from the lymphocytes and used for gene expression studies. Because there is a large volume of liquid in this tube, be sure to hold the tube below the participant's arm during collection. There is a risk, although extremely small, that the liquid in the tube could flow into the participant's vein if the tube is not held below the arm during collection.

2.2.3 Blood Collection Tubes: Labeling and Set-Up

Blood collection tubes should be set up in advance of the participant visit. Determine participant ID from site coordinator and obtain biospecimen labels with the laboratory ID on them from GIC- provided label rolls.

1. Place a generic Laboratory ID label (or write in the laboratory ID) on the participant Lab ID tracking sheet. This label will serve as a reminder of the participant ID/laboratory ID link. See example of this sheet in Appendix 9.

2. Apply pre-numbered barcoded SPIROMICS participant ID labels (from GIC provided label roll section 2.2 obtained from site coordinator) to each blood collection tube. **Handle only one participant's tubes at a time so the chance of mislabeling is minimized.**

3. Arrange the blood collection tubes in the test tube rack in the same order in which they are to be collected. The nine tubes are to be collected in the following order:
Tube #1: 8.5 mL red stoppered RT tube (Serum)
Tube #2: 8.5 mL red stoppered RT tube (Serum)
Tube #3: 8.5 mL yellow stoppered tube (ACD)
Tube #4: 10 mL lavender stoppered tube (EDTA)
Tube #5: 10 mL lavender stoppered tube (EDTA)
Tube #6: 10 mL lavender stoppered tube (EDTA) (may not be collected)
Tube #7: 4 mL lavender stoppered tube (EDTA)
Tube #8: 8.5 mL red stoppered P100 plasma collection tube
Tube #9: 2.5 mL red stoppered Paxgene tube

4. Note, some participants may be asked to donate one to two additional tubes of blood for quality control purposes. For these additional tubes, use labels from the provided QA/QC/phantom additional ID label sheet. The duplicate sample will be assigned a different Lab ID number, called a Phantom ID. The Phantom ID will be linked to the participant using the Phantom ID Linking form. Please see MOP 7 Quality Control and Quality Assurance for additional details on selecting participants for additional tube collection and for completing the Phantom ID form.

2.2.4 Processing Tubes, Sample Aliquot Rack, and Storage Container Setup

The blood draw/biospecimen technician needs to prepare the following test tube racks (described in detail below) prior to participant arrival. The test tube racks should have the following capacities:

- one test-tube rack capable of holding five 15mL conical centrifuge tubes/vacutainer blood tubes,
- one test-tube rack capable of holding a 50mL conical centrifuge tube or square topped qube,
- one Styrofoam disposable rack capable of holding 25, 50ml conical centrifuge tubes or Qubes for storing room temperature cell lysate prior to shipment and
- five microcentrifuge test-tube racks (suggested rack Fisher catalog #NC9917235-colored flipper racks with tops) capable of holding at least twenty-eight 1.5ml Sarstedt screw cap microcentrifuge freezer vials. Each rack will be used for a different type of aliquot or

intermediate container (urine). It is strongly suggested that each rack holding the aliquot tubes should be a different color or should be labeled with a different colored tape that matches if possible the top of the vacutainer tube from which the aliquots will be coming.

Label racks with the laboratory ID as well as with the aliquot type as shown below in the rack diagrams.

- **Rack 1** will hold 28 vials for the 14 aliquots of serum from each of blood collection tubes #1 & #2 (8.5 mL red stoppered RT tube). Suggested rack or tape color = red.
- **Rack 2** will hold 14 vials for the aliquots of plasma from blood collection tube #3 (8.5 mL yellow stoppered ACD tube). Suggested rack or tape color = yellow
- **Rack 3** will hold 28 vials for the aliquots of plasma from the blood collection tubes #4 and #5 10 mL lavender stoppered EDTA tube. Suggested rack or tape color = purple.
- **Rack 4** will hold one intermediate 15mL tubes for transfer of urine from the participant collection vial into a tube containing preservative. This 15ml tube should be prepared ahead to contain 20mg of preservative (ascorbic acid in crystalline from). Rack 4 will also hold blood collection tube #5 after plasma is removed as well as blood collection tube #6 if collected to be transported O/N to Michigan for immunophenotyping, blood collection tube # 7 for transfer to the on site clinical lab for CBC analysis, and blood collection tube #9 which will be frozen for batch shipment to the UNC-GIC.
- **Rack 5** will hold 20 vials for the aliquots from the urine specimen cup and the intermediate urine tube containing the ascorbic acid preservative. Suggested rack or tape color = white.
- **Rack 6** will hold 14 vials for the aliquots of plasma from blood collection tube #8, 8.5 mL red stoppered P100 plasma collection tube. Suggested rack or tape color = blue, green, or orange.
- **Rack 7** will contain one 50mL conical centrifuge tube for the transfer of the remaining material after plasma removal from tube #5 (EDTA blood). The contents of this tube will be further processed into a red blood cell cleared cell lysate that will be further processed to DNA at the UNC-GIC repository.
- **Box 1 of 2** will contain aliquots from aliquoting racks 1-3 (see section 5.7 for map).
- **Box 2 of 2** will contain aliquots from aliquoting racks 5-6, and sputum aliquots collected in the sputum lab (see section 5.7 for map).
- **Rack 8** Disposable rack capable of holding 25 50ml centrifuge tubes or Qubes, to be used to store and ship room temperature cell lysates. Carefully open the outer bag that wraps the racks of 50ml conical tubes such that the outer bag remains in place over the rack and can then be resealed prior to shipment. This rack will be used to place lysates in as they are prepared. Note initially you will be using qubes that will arrive from the BSP un-racked, please place these in a disposable rack (if possible) for storage and shipment.

2.2.5 Organization and Labeling

Label the screw-top sample aliquot tubes with the laboratory ID number corresponding to this aliquot and arrange in the sample aliquot racks in the following order. After labeling and placing the appropriate tubes in each rack, put on rack cover (if appropriate) and place two rubber bands around cover and place the 5 racks containing the 1.5ml microcentrifuge tubes at 4°C to pre-chill for sample aliquoting.

NOTE: All extra labels are returned to the GIC with the monthly/quarterly frozen specimen shipments

Rack 1 – Label as serum aliquots from Red/Gray stoppered RT tubes #1 & #2

Suggested rack setup and tape color = red

Tube 1 Serum aliquot 1 150 ul	Tube 1 Serum aliquot 2 150 ul	Tube 1 Serum aliquot 3 150 ul	Tube 1 Serum aliquot 4 150 ul	Tube 1 Serum aliquot 5 150 ul	Tube 1 Serum aliquot 6 150 ul	Tube 1 Serum aliquot 7 150 ul	Tube 1 Serum aliquot 8 150 ul	Tube 1 Serum aliquot 9 150 ul	Tube 1 Serum aliquot 10 150 ul		
Tube 1 Serum aliquot 11 250-500 ul	Tube 1 Serum aliquot 12 250-500 ul	Tube 1 Serum aliquot 13 250-500 ul	Tube 1 Serum aliquot 14 250-500 ul								
Tube 2 Serum aliquot 1 150 ul	Tube 2 Serum aliquot 2 150 ul	Tube 2 Serum aliquot 3 150 ul	Tube 2 Serum aliquot 4 150 ul	Tube 2 Serum aliquot 5 150 ul	Tube 2 Serum aliquot 6 150 ul	Tube 2 Serum aliquot 7 150 ul	Tube 2 Serum aliquot 8 150 ul	Tube 2 Serum aliquot 9 150 ul	Tube 2 Serum aliquot 10 150 ul		
Tube 2 Serum aliquot 11 250-500 ul	Tube 2 Serum aliquot 12 250-500 ul	Tube 2 Serum aliquot 13 250-500 ul	Tube 2 Serum aliquot 14 250-500 ul								

Rack 2 – Label as plasma aliquots from 8.5 mL yellow stoppered ACD tube #3

Suggested rack setup and tape color = yellow

Tube 3 Plasma aliquot 1 150 ul	Tube 3 Plasma aliquot 2 150 ul	Tube 3 Plasma aliquot 3 150 ul	Tube 3 Plasma aliquot 4 150 ul	Tube 3 Plasma aliquot 5 150 ul	Tube 3 Plasma aliquot 6 150 ul	Tube 3 Plasma aliquot 7 150 ul	Tube 3 Plasma aliquot 8 150 ul	Tube 3 Plasma aliquot 9 150 ul	Tube 3 Plasma aliquot 10 150 ul		
Tube 3 Plasma aliquot 11 250-500 ul	Tube 3 Plasma aliquot 12 250-500 ul	Tube 3 Plasma aliquot 13 250-500 ul	Tube 3 Plasma aliquot 14 250-500 ul								

Rack 3 – Label as plasma aliquots from 10 mL lavender stoppered EDTA tubes #4/#5

Suggested rack setup and tape color = lavender/purple

Tube 4 Plasma aliquot 1 150 ul	Tube 4 Plasma aliquot 2 150 ul	Tube 4 Plasma aliquot 3 150 ul	Tube 4 Plasma aliquot 4 150 ul	Tube 4 Plasma aliquot 5 150 ul	Tube 4 Plasma aliquot 6 150 ul	Tube 4 Plasma aliquot 7 150 ul	Tube 4 Plasma aliquot 8 150 ul	Tube 4 Plasma aliquot 9 150 ul	Tube 4 Plasma aliquot 10 150 ul		
Tube 4 Plasma aliquot 11 250-500 ul	Tube 4 Plasma aliquot 12 250-500 ul	Tube 4 Plasma aliquot 13 250-500 ul	Tube 4 Plasma aliquot 14 250-500 ul								
Tube 5 Plasma aliquot 1 150 ul	Tube 5 Plasma aliquot 2 150 ul	Tube 5 Plasma aliquot 3 150 ul	Tube 5 Plasma aliquot 4 150 ul	Tube 5 Plasma aliquot 5 150 ul	Tube 5 Plasma aliquot 6 150 ul	Tube 5 Plasma aliquot 7 150 ul	Tube 5 Plasma aliquot 8 150 ul	Tube 5 Plasma aliquot 9 150 ul	Tube 5 Plasma aliquot 10 150 ul		
Tube 5 Plasma aliquot 11 250-500 ul	Tube 5 Plasma aliquot 12 250-500 ul	Tube 5 Plasma aliquot 13 250-500 ul	Tube 5 Plasma aliquot 14 250-500 ul								

Rack 4 – Label as intermediate Urine tubes and Blood tubes that require additional manipulations or alternative shipments. Placement in rack can be adapted to particular rack layout available at clinical sites

15 ml Conical centrifuge tube for 10 mL preserved urine		Blood tube #5 Lavender EDTA after plasma is removed		Blood tube # 6 Lavender EDTA if collected to be sent to Michigan	Blood tube #7 (4 mL) lavender EDTA to be sent to clinical lab		Blood tube# 9 PAXgene RNA tube

Rack 5 – Label as urine aliquots – 1ml aliquots

Suggested rack setup and tape color = white.

Urine not preserved aliquot 1 1ml	Urine not preserved aliquot 2 1ml	Urine not preserved aliquot 33 1ml	Urine not preserved aliquot 4 1ml	Urine not preserved aliquot 5 1ml	Urine not preserved aliquot 6 1ml	Urine not preserved aliquot 7 1ml	Urine not preserved aliquot 8 1ml	Urine not preserved aliquot 9 1ml	Urine not preserved aliquot 10 1ml		
Urine preserved aliquot 1 1ml	Urine preserved aliquot 2 1ml	Urine preserved aliquot 3 1ml	Urine preserved aliquot 4 1ml	Urine preserved aliquot 5 1ml	Urine preserved aliquot 6 1ml	Urine preserved aliquot 7 1ml	Urine preserved aliquot 8 1ml	Urine preserved aliquot 9 1ml	Urine preserved aliquot 10 1ml		

Rack 6 – Label as plasma aliquots from 8.5 mL red stoppered P100 plasma collection tube #8

Suggested rack setup and tape color = blue, green, or orange.

Tube 8 P-100 Plasma aliquot 1 150 ul	Tube 8 P-100 Plasma aliquot 2 150 ul	Tube 8 P-100 Plasma aliquot 3 150 ul	Tube 8 P-100 Plasma aliquot 4 150 ul	Tube 8 P-100 Plasma aliquot 5 150 ul	Tube 8 P-100 Plasma aliquot 6 150 ul	Tube 8 P-100 Plasma aliquot 7 150 ul	Tube 8 P-100 Plasma aliquot 8 150 ul	Tube 8 P-100 Plasma aliquot 9 150 ul	Tube 8 P-100 Plasma aliquot 10 150 ul		
Tube 8 P-100 Plasma aliquot 11 250-500 ul	Tube 8 P-100 Plasma aliquot 12 250-500 ul	Tube 8 P-100 Plasma aliquot 13 250-500 ul	Tube 8 P-100 Plasma aliquot 14 250-500 ul								

Rack 7 – Label as cell lysate

50mL conical centrifuge tube for cell lysate prepared from blood tube #5's cells left after plasma removal		

Rack 8 – Rack for cell lysate storage

This rack should be a disposable rack capable of holding 25, 50ml centrifuge tubes (these tubes are purchased in these racks) or square-topped Qubes, to be used to store and ship cell lysates in prior to room temperature shipment. When using the normal 50ml centrifuge tubes, carefully open the outer bag that wraps the racks of 50ml conical tubes such that the outer bag remains in place over the rack, and can then be resealed prior to shipment. This rack will be used to place

lysates in as they are prepared. Remove tubes as needed, prepare cell lysate, and then place tube back in rack. Place one of the laboratory cell lysate ID labels on the front of the rack, as well as on a sheet of paper that will be sent along with the rack. Note initially you will be using qubes that will arrive from the BSP un-racked, please place these in a disposable rack (if possible) for storage and shipment.

2.2.7 Preparation for Specimen Collection

In the morning, prior to drawing blood from the participants:

1. Check to make sure the blood collection tray is properly equipped. Every item on the checklist (section 2.2.1) must be ready before proceeding (see Appendix 8 for checklist).
2. Check that the urine collection vial and that each Vacutainer tube is properly labeled with the correct SPIROMICS participant ID label.
3. Check that the sample aliquot racks are properly labeled and equipped. Every item on the aliquoting/processing checklist must be ready and in its proper position using the Rack outlines above.
4. Check that each aliquot storage container (two 2-inch 9x9 boxes) is labeled with the correct SPIROMICS participant ID/box labels (on both top and bottom of the box).
5. Perform and record temperature check on centrifuge (21°C ± 2°C). If centrifuges do not meet these specifications call for repair and find an alternative centrifuge to use.
6. Perform and record temperature check on refrigerator temperature (4°C ± 2°C). If refrigerator does not meet these specifications call for repair and find an alternative refrigerator to use.
7. Perform and record temperature check on freezer temperature (-20°C ± 5°C). If freezer does not meet these specifications call for repair and find an alternative freezer to use.
8. Perform and record temperature check on ultracold freezer temperature (-75°C ± 5°C). If freezer does not meet these specifications call for repair and find an alternative freezer to use.
9. Record room temperature.

2.2.8 At Participant Arrival

1. Open the DMS (Data Management System). Confirm that this participant has been appropriately consented and that the consent form data have been entered. **Confirm that the SPIROMICS participant ID on the Biospecimen Collection Form label matches the label on the collection tubes and aliquot containers** (see Appendix 2 for a sample Biospecimen Collection Form).

2. Check that duplicate Quality Control tubes are prepared and labeled, if needed. Please see MOP 7 – Quality Control and Quality Assurance for more details.

3. Note on the Biospecimen Collection Form whether the participant has fasted before the blood draw. Not fasting does not disqualify a participant from having their blood drawn or their urine collected.

2.3. Urine Collection

Prior to blood draw have the participant collect a clean catch urine specimen of at least 20 milliliters, (see Appendix 7 for clean catch urine instructions) into an appropriately labeled specimen cup provided by the biospecimen technician. A portion of the labeling on this specimen cup should have an area for writing down the time of the void. Some clinics may have clean catch collection instructions in the restroom. However; SPIROMICS instructions should be printed and given to each participant, as clean catch procedures may vary across institutions. It is important to make sure the participant understands the procedure. The participant should be instructed to void in the cup and fill it at least half full if possible and then place the lid securely on the cup and follow the clinic specific instructions for recording the time of collection and transporting the specimen to the biospecimen processing technician. The biospecimen processing technician should check to make sure the top is secured, record the time of the collection on the cup, and place the specimen in an appropriate refrigerator until processing.

If the participant cannot void at this time, or if at least 20 mL was not collected they may try again after the blood draw. Every effort should be made to void at least 20mLs at the initial void. If a second void is made this should be added to the Biospecimen Collection form.

3. VENIPUNCTURE PROCEDURE

3.1 Venipuncture Safety Precautions

All specimens should be treated as potentially infectious for laboratory workers. Blood borne pathogens such as hepatitis B and human immunodeficiency virus (HIV) can be transmitted following contact of a tainted blood sample through "broken skin" or intact mucous membrane (mouth, eyes, or nose) or as a result of an inadvertent needle stick. Examples of "broken skin" include open cuts, nicks and abrasions, dermatitis, and acne. OSHA rules mandate that technicians always wear disposable protective gloves when collecting and processing specimens.

Gloves worn during venipuncture must be new and intact (e.g., you cannot tear off the tip of the glove in order to locate the venipuncture site). If the phlebotomist accidentally sustains a stick with a contaminated needle, clean the wound thoroughly with disinfectant soap and water, notify a supervisor, consult the SPIROMICS physician, and follow any local regulations regarding reporting needle sticks or visiting employee health. Never take lab coats worn during the collection and processing of samples outside of the laboratory area except for laundering. Before leaving the laboratory, the technician will remove the lab coat and disposable gloves and wash hands with a disinfectant soap. Use OSHA-approved cleaning solution to clean up any spills of blood, plasma, serum, or urine.

Use this solution to clean all laboratory work surfaces at the completion of work activities. OSHA regulations require that all needles and sharp instruments be discarded into puncture resistant containers. Do not attempt to bend, break, or recap any needle before discarding it. Appropriately discard the butterfly set following each specimen collection.

3.1. Phlebotomy Room

The blood drawing takes place in an area that offers privacy to the study participant. This phlebotomy area should be equipped with all of the necessary blood drawing supplies. A separate work area is equipped with all of the supplies that are used in the blood processing. The biosafety cabinet (or protective shields), centrifuge, refrigerator, and freezers should be nearby.

3.2. Participant Preparation

Phlebotomist should either confirm in the DMS that the participant has completed the informed consent or should check with the study coordinator, to ensure the participant has been appropriately informed of the reason for venipuncture and the associated risks. The consent form also explains the number of tubes and amount of blood that will be drawn

If at all possible, the participant should be in the seated position for a minimum of five minutes prior to the venipuncture. This allows the participant to relax before the procedure. Perform venipuncture with a 21-gauge butterfly needle and 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small thin-walled needle that minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. Give the participant time to relax after the blood collection.

If the participant is nervous or excited, the technician can briefly describe the procedure and purpose, e.g., "I am going draw 4-5 tablespoons of blood. This blood will be used in a range of blood tests including CBC, platelets, and chemistries. We hope to be able to use the results of these tests to better understand the health issues of patients with COPD which may lead to the development of better treatments in the future."

Do not under any circumstances force the participant to have blood drawn. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. If the participant is very anxious, he/she may lie down during the blood collection. A reclining individual will undergo an extravascular water shift, resulting in a dilutional effect on lipid values. If this option is taken, note it on the Biospecimen Collection form by placing an "X" in the appropriate boxes (Appendix 3, Item 14).

NOTE: If a participant in unable to have blood drawn at all, discuss the participant's viability as a subject with the site Principal Investigator. This should be done as soon as the coordinator has concerns in order to avoid unnecessary testing (e.g., it may be preferable to address this before continuing with the visit). If necessary blood can be drawn at a subsequent visit as long as the visit is completed within the study visit window. If the coordinator and/or the Principal Investigator feel it is unlikely the participant will be able to provide blood specimens, the study visit should be stopped. Participants must contribute at least one tube of blood to be enrolled in the study.

If a participant is unwilling to have biospecimens drawn or is unwilling to allow those specimens to be used for research purposes, the study visit should be stopped.

3.3. Venipuncture

Note: Complete the Biospecimen Collection form in the DMS or on a hard copy printout as blood is drawn and urine is collected. If data is collected on a hard copy, transfer this data into the DMS on the same day it is collected.

The participant should be asked if they are on any anticoagulants (blood thinners) or if they have a bleeding disorder. If so, the phlebotomist should apply pressure at the site for 5 minutes after the needle is removed from the vein.

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow). Use a tourniquet to increase venous filling. This makes the veins more prominent and easier to enter. The preferred arm to draw from is the left arm. Use the right arm only if blood collection is not possible from the left arm. This does not mean you must stick the left arm. Only do so if an adequate vein is apparent. Additional reasons to not use the left arm:

*If the participant has had lymph node dissection or radiation therapy to the left axilla (armpit) for both males and females

*If the participant has tubes, rashes, dressings, casts, open sores hematomas, wounds, an arteriovenous (AV) shunt, or any other IV access device

PRECAUTIONS WHEN USING A TOURNIQUET: The tourniquet should be on the arm for the shortest time possible. Never leave the tourniquet on for longer than two minutes. To do so may result in hemoconcentration or a variation in blood test values. If a tourniquet must be applied for preliminary vein selection, and it remains on the arm for longer than two minutes, it should be released and reapplied after a wait of two minutes.

Instruct the participant that he/she should not clench their fist prior to the venipuncture. Doing so could cause fluctuations in the results in several of the analytes being measured. If the participant has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.

A. Apply tourniquet.

1. Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.

2. Tuck the end of the tourniquet under the last round.

3. If a Velcro tourniquet is used, adhere the ends to each other.

B. Identify vein:

1. 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. 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.

C. Assemble the butterfly-Vacutainer set.

1. Attach the Luer adapter to the Vacutainer holder.

2. Attach the Luer end of the butterfly needle set to the Luer adapter.

D. Cleanse the venipuncture site.

1. Remove alcohol prep from its sterile package.

2. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.

3. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the patient when the venipuncture is performed.

4. 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.

E. Perform venipuncture.

1. 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.

2. With the needle bevel upward, enter the vein in a smooth continuous motion.

3. Once blood appears in the butterfly tubing, place tube #1 (8.5 mL red RT serum) into the Vacutainer holder. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.

4. Make sure the participant's arm is in a flat or downward position while maintaining the 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.

5. Remove the tourniquet after tube #3 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. If the tourniquet is loosened or reapplied, note this on the Biospecimen Collection form.

6. Keep a constant, slight forward pressure (in the direction of the adapter) on the end of the 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.

7. Fill each Vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a Vacutainer tube fills only partially, remove the tube and attach another without removing needle from vein.

8. When the blood flow into the collection tube ceases, remove the tube from the holder. The shutoff valve covers the point, stopping blood flow until the next tube is inserted (if necessary). Gently invert tubes #1 & #2 5x, tubes 3-8, 8x and tube #9 12 times, immediately following removal of the tube from the adapter while the next tube is filling. (See section 7.3.5 for mixing instructions.)

9. When collecting the P100 tube #8 and the PAXgene tube #9, be sure to keep the tube upright and below the participant's arm. When collecting the P100 tube observe the following instructions to insure to minimize premature separation of the mechanical separator from the stopper.

- Push tube onto non-patient end in one swift action.
- Hold tube on non-patient end during drawing. If mechanical separator moves in the P100 tube during blood collection, discard the tube and redraw.
- Please note that extreme care should be taken when drawing this tube so that this does not occur because of the high cost of these tubes.

10. 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.

11. If the blood flow stops before collecting all of the tubes, repeat the venipuncture on the participant beginning with the first unfilled tube**. Because of the ratio of anticoagulant to blood, tubes #3 and #8 must be completely filled in order not to have a dilution of the plasma**. As always, the tourniquet must never be on for longer than two minutes.

F. Overcoming Problems

If a blood sample is not forthcoming, the following manipulations may be helpful.

1. If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.

2. If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm. The same technician should not attempt a venipuncture more than twice (once in each arm). If a third attempt is necessary, a different phlebotomist should attempt the venipuncture.

3. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.

If a complete set of 9 tubes cannot be drawn with three venipunctures (using a 2nd phlebotomist for the 3rd venipuncture), the coordinator should assess whether the participant should be rescheduled for a second screening visit. Coordinators should check with his or her site PI or supervisor. If a second visit is schedule, the study coordinator should remind the participant to fast starting at midnight, and encourage the participant to drink plenty of water during the fast, especially during the morning hours prior to the blood draw. If a complete set of blood tubes was not taken at the initial visit, during the follow-up visit the blood draw should resume with the first tube missed. If it is clear that a participant will not be able to provide a full set of blood tubes, coordinators should begin with Tube 1 (RT) and collect as many blood tubes as possible, dropping the duplicate RT and EDTA tubes as needed.

G. Bandaging the arm.

1. Under normal conditions:
    a. Determine if the participant has allergies to tape/adhesives. If so, use gauze pad, dressing only
    b. Slip the gauze pad down over the site, continuing mild pressure.
    c. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
    d. If the participant continues to bleed:
    1. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
2. Wrap a gauze bandage tightly around the arm over the pad.
3. Tell the participant to leave the bandage on for at least 15 minutes.

H. PRECAUTIONS - When a Participant Feels/Looks Faint Following the Blood Drawing:

1. Have the person remain in the chair. If necessary, have him/her lie on the floor with legs elevated. Use of a transfer belt may be indicated in this situation.

2. Take an ampule of smelling salts, crush it, and wave it under the person's nose for a few seconds.

3. Provide the person with a basin if he/she feels nauseated.

4. Have the person stay seated until the color returns and he/she feels better.

5. Have someone stay with the person to prevent them from falling and injuring themselves if he/she should faint.

6. Place a cold wet cloth on the back of the person's neck or on their forehead.

7. Once the episode has passed, some fruit juice may be given to the participant in order to counteract any possible hypoglycemia due to their pre-clinic visit fast.

8. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member for further direction.

3.4 Blood and Urine Processing Safety Precautions

Universal and Occupational Safety and Health (OSHA) and all **institutional-specific (such as bloodborne pathogen training)** precautions and training requirements should be followed when processing blood.

- Gloves **must** be worn **at all times** when handling specimens.
- Care should be taken to minimize aerosols when opening and handling the blood tubes. For instance, holding a piece of gauze or similar product (i.e., BloodBloc Biohazard wipes) over the stopper while slowly removing it from the tube is a practice that should be followed. In addition, locking centrifuge bucket covers should be used.
- The ideal situation for the processing of whole blood would be to perform all the manipulations in a class II biological safety cabinet (lamina flow hood), while wearing a lab coat and gloves. Alternatively, to minimize infection risks the technician can wear a lab coat and a mask in combination with an eye protection device, such as goggles or glasses with solid side shields or a chin-length face shield, or as an another alternative a desk-mounted or under-shelf mounted clear plastic shield could be used.
- Place all used Vacutainer tubes and blood-contaminated products in biohazard bags for proper institutional dictated disposal.
- Clean up any spills with an appropriate disinfectant (10% bleach, 70-85% ethanol, or appropriate commercial product such as Decon or DuPont RelyOn).
- **Do not perform any pipetting by mouth.**
- If a blood borne pathogen contamination occurs from a skin prick allow wound to bleed freely for a minute and then wash with copious amounts of soap and water. If contamination occurs with a mucous membrane (eyes, nose, mouth), rinse surface with copious amounts of water. Eyes should be irrigated with the emergency eye wash station for at least 15 minutes. Finally, follow all institution reporting and follow-up requirements (i.e., a visit to your employee health services may be warranted) if a blood borne pathogen exposure occurs.
- For more detailed safety information see (http://www.osha.gov/SLTC/biologicalagents/index.html).

3.5. Blood Tube Mixing and Storage During Venipuncture

All tubes must be mixed. For tubes containing anticoagulants this is required to optimize coagulation, however even tubes #1 and #2 must be mixed since they contain a clot activator that needs to be mixed with the blood. Gently invert tubes #1 & #2 5x, tubes 3-8, 8x and tube #9, 12 times. Eight inversions should take 6 to 8 seconds. Inversions should be performed on each tube while the next tube is being drawn.

1. **Tubes #1 & #2** (8.5 mL red stoppered RT tubes). Mix 5 times by complete inversion immediately after the sample is drawn. Let sit vertical in a test tube rack at room temperature for a **minimum** of 30 minutes and a **maximum** of 2 hours, prior to centrifugation and removal and aliquoting of serum. Protect tubes from light by placing a box over the rack until centrifugation. Note time this tube was drawn on the Biospecimen Collection Form.

2. **Tube #3** (8.5 mL yellow stoppered ACD plasma tube). Invert 8 times immediately after the sample is drawn. Centrifugation and aliquoting of plasma should occur within 2 hours of draw. These tubes should be kept covered at room temperature (RTemp) prior to centrifugation. Note time this tube was drawn on the Biospecimen Collection Form.

3. **Tube #4 and #**5 (10 mL lavender stoppered EDTA plasma tubes). Invert 8 times immediately after the sample is drawn. Centrifugation and aliquoting of plasma should occur within 2 hours of draw. These tubes should be kept covered at RTemp prior to centrifugation. Note time this tube was drawn on the Biospecimen Collection Form.

4. **Tube #6** (10 mL lavender stoppered tube EDTA plasma tube). ***(this substudy is not yet funded)*** Invert 8 times prior to preparation for on site staining protocol prior to overnight mailing to Michigan for immunophenotyping analysis. This tube will only be drawn in a subset of subjects. Note time this tube was drawn on the Biospecimen Collection Form.

5. **Tube #7** (4 mL lavender stoppered tube EDTA plasma tube). Invert 8 times immediately after the sample is drawn and prior to transport to clinical center lab for CBC counts with differentials. Note time this tube was drawn on the Biospecimen Collection Form. Follow site-specific clinical laboratory procedures for labeling this tube. Sample can be held at room temperature up to four hours, after which it should be kept at **4°C**. Samples should be transported to the clinical lab as quickly as possible.

6. **Tube #8** (8.5 mL red stoppered P100 plasma collection tube). **Immediately** invert 8 times after the sample is drawn. Centrifugation and aliquoting of plasma should occur within 2 hours of draw. These tubes should be kept covered at RTemp prior to centrifugation. Note time this tube was drawn on the Biospecimen Collection Form.

7. **Tube #9** (2.5 mL red stoppered PAXgene RNA stabilized EDTA plasma tube). Immediately after blood is drawn, gently invert 12 times and place in a rack at room temperature for at least 4 hr. Note time this tube was drawn on the Biospecimen Collection Form. **After 4 hours, freeze this sample as described in section 5.6.**

4. SNACK

Allow time before the participant proceeds to the next SPIROMICS procedure for the participant to obtain a snack. Coordinators should be certain to ask the participant whether he or she has any dietary restrictions while scheduling the study visit. Please see MOPs 2 and 5 (PFTs and Sputum Induction) for notes on the amount of time between eating and these procedures.

5. BLOOD PROCESSING

5.1 Operating the Centrifuge

Refer to Centrifuge Operating Manual for specific operating and balancing instructions. Always use the Relative Centrifugal Force (RCF) or x g values, in this MOP not Revolutions Per Minute (RPM) when centrifuging blood samples. If your centrifuge does not display RCF values, consult the manual for a conversion chart or use the following formula:

> RCF = 1.12r (RPM/1000) where r = radius in millimeters (Measure radius from center of rotor pin to middle of swinging bucket or center of fixed angle rotor) and RPM = revolutions per minute.

If a clinical center's centrifuge is not capable of creating a certain RCF, increase the centrifugation time until the rcf-minutes total the equivalent amount it would have spun at the higher RCF value. If, for example, the maximum force is 2000 RCF rather than 3000 RCF then increase the time from 30 to 45 minutes. To balance the centrifuge, place tubes of the same size and with equal volume of blood (or blank tube with water) as determined visually in opposite positions in the bucket/holders.

5.1.1 Stage One: Immediate Processing

Note: Process blood from only one participant at a time.
Immediately, or as soon as possible after completion of venipuncture, begin centrifugation of tubes for plasma and serum extraction in the following order. All centrifugations will be conducted at RTemp. Record on the Biospecimen Collection Form the time at which these tubes began to spin. **NOTE: For all of the following centrifugations, if a swinging bucket rotor is used, bucket covers must be used when centrifuging whole blood.**

1. Centrifuge tubes #3, #4, and #5 at RTemp, at 1100-1300 RCF (relative centrifugal force) for 10 minutes in a swinging bucket rotor or 15 minutes in a fixed angle, balancing centrifuge as appropriate. Remove plasma from these tubes as described in Section 5.2.1 (this can be done while tubes #1 & 2 are spinning).

2. After a minimum of 30 minutes upright at RTemp (to allow the blood to clot), load tubes #1 and #2 into the centrifuge, and centrifuge these tubes at RTemp at 1100-1300 RCF for 10 minutes in a swinging bucket rotor or 15 minutes in a fixed angle. Alternatively, if tubes have already incubated at RTemp for 30 minutes, they can be spun along with tubes #3, #4, and #5.

3. [*Collection and Processing for this step is currently unfunded*] Place tube #6 in refrigerator until daily shipment on ice to the Michigan for immunophenotyping.

4. Place tube #7 in appropriate storage per clinical lab requirements at each site in preparation for CBC analysis.

5. After removal of tubes #1 and #2 from the centrifuge, place tube #8 in the centrifuge and spin at 2500 RCF at RTemp for 15-20 minutes. Alternatively if centrifuge does not reach 2500 RCF,

spin for 30 minutes at 1100-1600 RCF. If a fixed angle centrifuged is used, a 45 degree angle rotor is needed. **Use the appropriate sized tube holders and cushions to hold 16 x100 mm tubes with hemogard closures**. If this instruction is not followed, P100 tube may shatter upon centrifugation, or they may sink to the bottom of the tube holder, which will require the use of forceps to remove the tube from the holder, or the hemogard closure may become dislodged. If you are unsure what is needed for your particular centrifuge, contact your local Becton-Dickinson representative for assistance.

6. Leave tube #9 in an upright position at room temperature for at least 4 hours.

5.2. Stage Two: Processing of Blood Tubes

Note: Process blood from only one participant at a time. In addition, open and process only one type of blood tube at a time.

Stage two begins immediately or as soon as possible or after each centrifugation. As described above, working in a biosafety cabinet is recommended, but at the very least, face protection, gloves and lab coat must be used for all blood processing. All other rules regarding the safe blood specimen handling as described above (Section 3.4) must also be observed.

5.2.1. Removal of plasma from tubes #3, #4, and #5.

- Remove tubes from centrifuge carefully so as not to disturb cell layers, and place in test-tube rack in direct proximity to samples aliquoting racks 2 and 3.
- **Confirm that the label on the blood tube corresponds to the labels on the aliquoting tubes, both for participant ID and specimen type (i.e., yellow top plasma tube#3)**
- Loosen and remove lids from aliquot tubes in racks 2 and 3 as each type of blood tube is processed.
- Slowly, so as not to disturb the cell layers or cause aerosols, remove stopper or hemogard using a piece of gauze.
- When removing the plasma after centrifugation do not disturb the white blood cells layer, also called the buffy coat, which forms as a thin layer between the upper plasma layer and the lower layer of packed red blood cells. Stop collecting the plasma within ½ to ¾ of an inch above the surface of the buffy coat. If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube using the initial processing conditions. Indicate in Item 14 of the Biospecimen Collection Form that the tube was re-centrifuged.
- If lipids (oily/fatty layer) are floating on top of plasma do not collect this layer, place tip of pipette below this surface before aspirating off plasma. Indicate the presence of lipids on top of the plasma on the Biospecimen Collection Form.
- Carefully remove plasma using a pipette aid (“set to slow”) and a 2mL sterile disposable serological pipette (an automatic pipetter can also be used) and directly transfer 150ul into aliquots 1-14 (note on the biospecimen form if less than 150ul is placed in any of these 14 aliquots).
    - No aliquots should receive less than 150uL. If the coordinator is unable to fill all 14 aliquots with 150uL, then any left over plasma should be added to the last

aliquot with 150uL of plasma already. The total number of aliquots and the volume of the final aliquot should be noted on the BIO form.

- If there is more plasma left in the pipette after all 14 aliquots are filled with 150uL, then the remaining plasma should be aliquoted into the 14th aliquot, and the volume should be noted on the BIO form.

- Place screw caps on aliquoting tubes and dispose of blood tube #3 in biohazard waste as specified by individual institution. **Keep blood tubes #4 and #5 until one is successfully processed into cell lysate as described in section 5.2.3.**
- Place aliquots into box 1 of 2 for this participant in slots 31-74 as described in section 5.7. Keep this box on dry ice until all aliquots are placed in it and then transfer to a -80ºC for temporary storage prior to batch shipment to the GIC repository. Alternatively, after each blood tube is processed, place aliquot racks 2 & 3 temporarily at -80°C until other aliquots are processed. After completion of all processing, aliquots for each individual participant will be consolidated into two 2" 9x9 sectioned freezer boxes (again see section 5.7).

5.2.2. Removal of serum from tubes #1, and #2.

- Remove tubes from centrifuge and place in test-tube rack in direct proximity to sample aliquoting rack 1.
- **Confirm that the label on the blood tube corresponds to the labels on the aliquoting tubes, both for participant ID and specimen type (i.e., RT tube serum)**
- Loosen vial screw caps and remove (if working in a biosafety cabinet) lids from aliquot tubes in rack1 as each blood tube is processed.
- Slowly, so as not to cause aerosols, remove stopper using a piece of gauze. Stop collecting the serum within 1/4 of an inch above the surface of the tube's floating polymer gel barrier. **Do not** re-centrifuge this tube. As Becton-Dickinson explains, "A potential for inaccurate test results is possible. Analytes from cellular leakage/exchange, accentuated by clot retraction, will then be centrifuged into the serum."
- Carefully remove serum using a pipette aid set to slow and a 2mL sterile disposable serological pipette (an automatic pipetter can also be used) and directly transfer appropriate 150ul aliquots 1-14 (note on the biospecimen form if less than 150ul is placed in any of these 14 aliquots).
  - No aliquots should receive less than 150uL. If the coordinator is unable to fill all 14 aliquots with 150uL, then any left over serum should be added to the last aliquot with 150uL of serum already. The total number of aliquots and the volume of the final aliquot should be noted on the BIO form.
  - If there is more serum left in the pipette after all 14 aliquots are filled with 150uL, then the remaining serum should be aliquoted into the 14th aliquot, and the volume should be noted on the BIO form.
- Place screw caps on aliquoting tubes and dispose of blood tube in biohazard waste as specified by individual institution.
- Place aliquots into box 1 of 2 for this participant in slots 1-29 as described in section 5.7. Keep this box on dry ice until all aliquots are placed in it and then transfer to a -80ºC for temporary storage prior to shipment to the UNC-GIC repository.

Alternatively, after each blood tube is processed, place aliquot rack temporarily at -80°C until other aliquots is processed. After completion of all processing, aliquots for each individual participant will be consolidated into two 2" 9x9 sectioned freezer boxes (again see section 5.7).

5.2.3. Processing blood tube #5 (or #4 if needed) to red blood-cell-depleted cell lysate

- Transfer contents of blood tube#5, which has had its plasma removed, to the labeled 50-ml conical centrifuge tube.
- Add 35mLs (if original blood volume was less than 5 mLs, use tube #4 or combine both tubes to no more than 10mLs total) of Qiagen Puregene Red Blood Cell Lysis buffer to tube. Mix by inversion and incubate for 6.5 minutes (set timer) at room temperature.
- Immediately after room temperature incubation, centrifuge at RTemp at 3,000 RCF for 5 minutes.
- After centrifugation, pour off supernatant into biohazardous liquid waste container (see waste components in appendix 3), leaving behind the visible white blood cell pellet and about 200ul of residual fluid.
- Close tube and vortex tube vigorously for 10 seconds.
- Add 10mLs of Qiagen Puregene Cell Lysis Solution to pellet, close tube, and vortex again for 10 seconds.
- Wrap the top of tube with parafilm and then with packing tape. Store samples at room temperature in 50ml tube racks until quarterly (or more frequent as needed) batch shipments to the GIC.
- Place one of the laboratory cell lysate ID labels on the front of the rack, as well as on a sheet of paper that will serve as this particular rack's inventory. This sheet of paper will eventually be sent to the BSP along with this particular rack

5.2.4. Removal of plasma from tube #8

- Remove tube from centrifuge and place in test-tube rack in direct proximity to sample aliquoting rack 6.
- **Confirm that the label on the blood tube corresponds to the labels on the aliquoting tubes, both for participant ID and specimen type (i.e., P100 plasma)**
- Loosen vial screw caps and remove (if working in a biosafety cabinet) lids from aliquot tubes in rack 6 as each blood tube is processed.
- Slowly, so as not to cause aerosols, remove stopper using a piece of gauze. Stop collecting the serum within 1/4 of an inch above the surface of the tube's floating mechanical separator.
- Carefully remove plasma using a pipette aid set to slow and a 2mL sterile disposable serological pipette (an automatic pipetter can also be used) and directly transfer appropriate 150ul aliquots 1-14 (note on the biospecimen form if less than 150ul is placed in any of these 14 aliquots).
    - No aliquots should receive less than 150uL. If the coordinator is unable to fill all 14 aliquots with 150uL, then any left over plasma should be added to the last aliquot with 150uL of plasma already. The total number of aliquots and the volume of the final aliquot should be noted on the BIO form.

  - If there is more plasma left in the pipette after all 14 aliquots are filled with 150uL, then the remaining plasma should be aliquoted into the 14th aliquot, and the volume should be noted on the BIO form.
- Place screw caps on aliquoting tubes and dispose of blood tube in biohazard waste as specified by individual institution.
- Place aliquots into box 2 of 2 for this participant in slots 23-36 as described in section 5.7. Keep this box on dry ice until all aliquots are placed in it and then transfer to a -80ºC for temporary storage prior to shipment to the UNC-GIC repository. Alternatively, after each blood tube is processed place aliquot rack temporarily at -80°C until other aliquots are processed. After completion of processing, aliquots for each individual participant will be consolidated into two 2" 9x9 sectioned freezer boxes.

5.2.2 Processing blood tube #6 for immunophenotyping analysis *(this substudy is currently unfunded)*

If this blood tube is collected follow staining and shipping protocol as provided in the Immunophenotyping MOP.

5.3 Urine Processing

- Remove specimen cup from refrigerator and place in direct proximity to rack containing the intermediate processing tubes and the aliquoting rack 5. **Confirm that the participant ID on the specimen cup corresponds to the participant ID on the intermediate urine processing tubes and the urine aliquot vials.**
- Invert urine specimen cup 8 times.
- Loosen 15 mL centrifuge tube cap and remove (if working in a biosafety cabinet).
- Loosen aliquoting vial screw caps and remove (if working in a biosafety cabinet) lids from aliquot tubes in racks 2 and 3 as each blood tube is processed.
- Using a 10mL plastic serological pipette remove 10 mLs of urine and transfer 1mL of urine into each of the 10 **non-preserved aliquoting vials**.
- Using a 10mL plastic pipette remove 10 mLs of urine and transfer to the 15ml centrifuge tube containing the ascorbic acid preservative.
- Invert this tube until preservative is dissolved.
- Using a 10mL plastic pipette remove 10 mLs of urine and transfer 1mL of urine into each of the 10 **preserved** aliquoting vials.
- Place aliquots into box 2 of 2 for this participant in slots 1-21 as described in section 5.7. Keep this box on dry ice until all aliquots are placed in it and then transfer to a -80ºC for temporary storage prior to shipment to the UNC-GIC repository. Alternatively, place aliquot rack temporarily at -80°C until other aliquots are processed. At that time aliquots for each individual participant will be consolidated into two 2" 9x9 sectioned freezer boxes.
- Dispose of excess urine and specimen cup in biohazard waste according to institutional regulations.
- Note any comments about urine collection and processing in section 19 on the Biospecimen Collection Form (i.e., if two voids were necessary, if there was blood in the urine, etc., if 20 mls total was not collected indicate volume that was collected)

5.4 Transfer of Tube #6 to Michigan *(this substudy is currently unfunded)*

If this blood tube is collected follow staining and shipping protocol as provided in the Immunophenotyping MOP.

5.5 Transfer of Tube # 7 to individual site clinical lab
Transfer this tube to your clinical lab per each institution's policy for CBC and differentials.

5.6 Final Processing of Tube #9
Place tubes from the individual patients horizontally directly into a -80°C freezer into the current (non-sectioned) 2" x 2" box. Keep adding tubes to this box as patients are seen until the box is filled (24 tubes should fit) or until your next shipment to the BSP at the GIC. Label these boxes starting with 1. Start with a new box number (i.e., 2, 3, etc) each time you either fill a box or ship a box to the UNC-BSP.

In addition, place a participant ID label on the top of the box for each participant's tube placed in the box. Each month this box will be shipped on dry ice along with the frozen aliquot tubes to the UNC-GIC repository.

5.7 Freezer boxing
Aliquots should be places in the 2" x 2", 9x9 sectioned freezer boxes in the order outlined below. During the transfer from the racks to the boxes, the boxes should be kept on dry ice. These freezer boxes should be pre-labeled with the laboratory ID and the designation box 1 of 2 (1/2) or box 2 of 2 (2/2) on both the top and bottom of each box. The order shown below reflects placing the aliquots into the boxes based on the original rack order, skipping one space after each blood/urine tube's worth of aliquots. Frozen aliquots will be shipped monthly to the GIC Repository. Box should be labeled with a marker on the inside in the upper left corner, to orient the box for sample #1.

Box 1

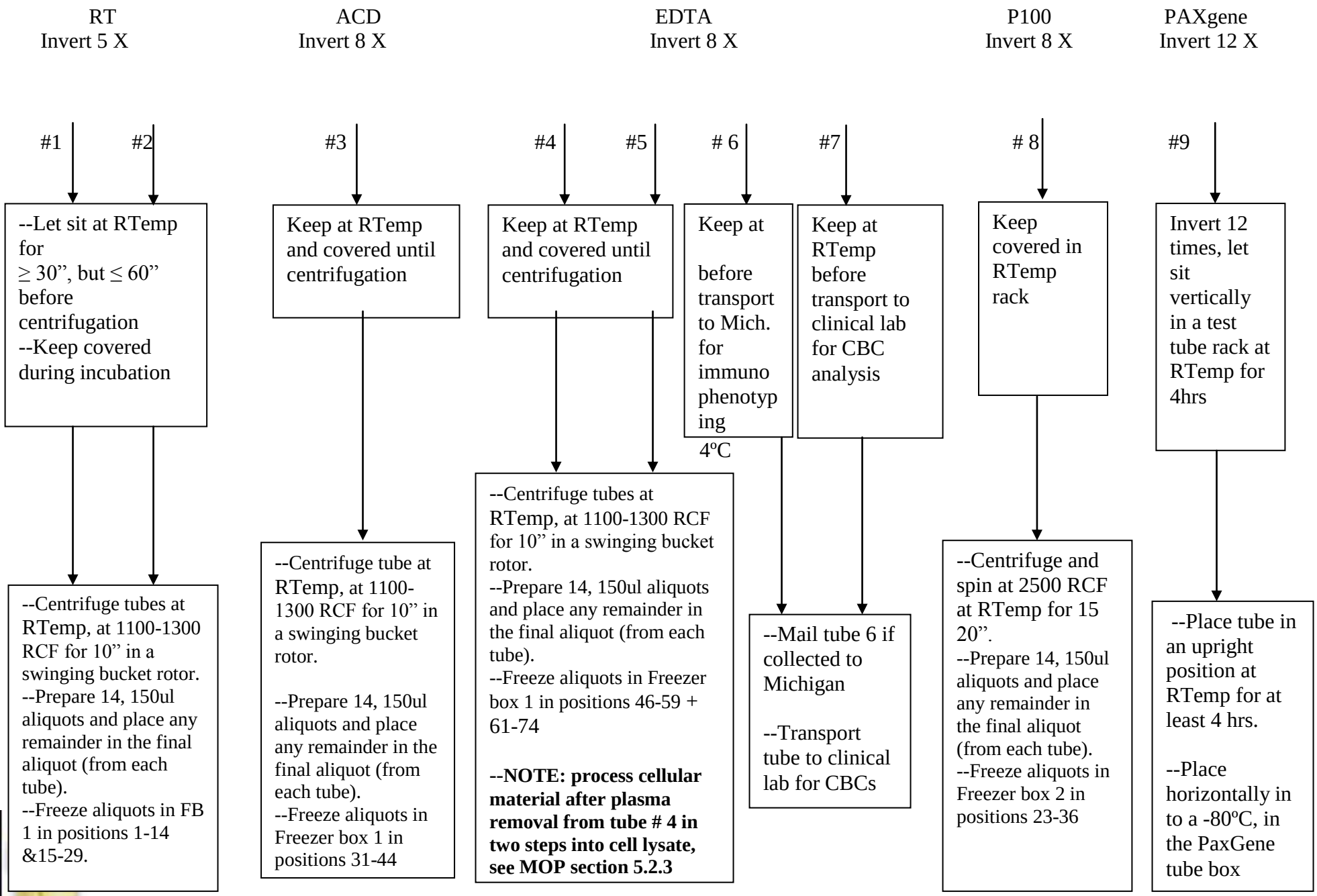
● = box orientation label Tube 1 red RT Serum aliquot 1	Tube 1 red RT Serum aliquot 2	Tube 1 red RT Serum aliquot 3	Tube 1 red RT Serum aliquot 4	Tube 1 red RT Serum aliquot 5	Tube 1 red RT Serum aliquot 6	Tube 1 red RT Serum aliquot 7	Tube 1 red RT Serum aliquot 8	Tube 1 red RT Serum aliquot 9
Tube 1 red RT Serum aliquot 10	Tube 1 red RT Serum aliquot 11	Tube 1 red RT Serum aliquot 12	Tube 1 red RT Serum aliquot 13	Tube 1 red RT Serum aliquot 14		Tube 2 red RT Serum aliquot 1	Tube 2 red RT Serum aliquot 2	Tube 2 red RT Serum aliquot 3
Tube 2 red RT Serum aliquot 4	Tube 2 red RT Serum aliquot 5	Tube 2 red RT Serum aliquot 6	Tube 2 red RT Serum aliquot 7	Tube 2 red RT Serum aliquot 8	Tube 2 red RT Serum aliquot 9	Tube 2 red RT Serum aliquot 10	Tube 2 red RT Serum aliquot 11	Tube 2 red RT Serum aliquot 12
Tube 2 red RT Serum aliquot 13	Tube 2 red RT Serum aliquot 14		Tube 3 Yellow ACD plasma aliquot 1	Tube 3 Yellow ACD plasma aliquot 2	Tube 3 Yellow ACD plasma aliquot 3	Tube 3 Yellow ACD plasma aliquot 4	Tube 3 Yellow ACD plasma aliquot 5	Tube 3 Yellow ACD plasma aliquot 6
Tube 3 Yellow ACD plasma aliquot 7	Tube 3 Yellow ACD plasma aliquot 8	Tube 3 Yellow ACD plasma aliquot 9	Tube 3 Yellow ACD plasma aliquot 10	Tube 3 Yellow ACD plasma aliquot 11	Tube 3 Yellow ACD plasma aliquot 12	Tube 3 Yellow ACD plasma aliquot 13	Tube 3 Yellow ACD plasma aliquot 14	
Tube 4 EDTA Plasma aliquot 1	Tube 4 EDTA Plasma aliquot 2	Tube 4 EDTA Plasma aliquot 3	Tube 4 EDTA Plasma aliquot 4	Tube 4 EDTA Plasma aliquot 5	Tube 4 EDTA Plasma aliquot 6	Tube 4 EDTA Plasma aliquot 7	Tube 4 EDTA Plasma aliquot 8	Tube 4 EDTA Plasma aliquot 9
Tube 4 EDTA Plasma aliquot 10	Tube 4 EDTA Plasma aliquot 11	Tube 4 EDTA Plasma aliquot 12	Tube 4 EDTA Plasma aliquot 13	Tube 4 EDTA Plasma aliquot 14		Tube 5 EDTA Plasma aliquot 1	Tube 5 EDTA Plasma aliquot 2	Tube 5 EDTA Plasma aliquot 3
Tube 5 EDTA Plasma aliquot 4	Tube 5 EDTA Plasma aliquot 5	Tube 5 EDTA Plasma aliquot 6	Tube 5 EDTA Plasma aliquot 7	Tube 5 EDTA Plasma aliquot 8	Tube 5 EDTA Plasma aliquot 9	Tube 5 EDTA Plasma aliquot 10	Tube 5 EDTA Plasma aliquot 11	Tube 5 EDTA Plasma aliquot 12
Tube 5 EDTA Plasma aliquot 13	Tube 5 EDTA Plasma aliquot 14							

Box 2

● = box orientation label Urine un-preserved aliquot 1	Urine un-preserved aliquot 2	Urine un-preserved aliquot 3	Urine un-preserved aliquot 4	Urine un-preserved aliquot 5	Urine un-preserved aliquot 6	Urine un-preserved aliquot 7	Urine un-preserved aliquot 8	Urine un-preserved aliquot 9
Urine un-preserved aliquot 10		Urine preserved aliquot 1	Urine preserved aliquot 2	Urine preserved aliquot 3	Urine preserved aliquot 4	Urine preserved aliquot 5	Urine preserved aliquot 6	Urine preserved aliquot 7
Urine preserved aliquot 8	Urine preserved aliquot 9	Urine preserved aliquot 10		Tube 8 P-100 Plasma aliquot 1	Tube 8 P-100 Plasma aliquot 2	Tube 8 P-100 Plasma aliquot 3	Tube 8 P-100 Plasma aliquot 4	Tube 8 P-100 Plasma aliquot 5
Tube 8 P-100 Plasma aliquot 6	Tube 8 P-100 Plasma aliquot 7	Tube 8 P-100 Plasma aliquot 8	Tube 8 P-100 Plasma aliquot 9	Tube 8 P-100 Plasma aliquot 10	Tube 8 P-100 Plasma aliquot 11	Tube 8 P-100 Plasma aliquot 12	Tube 8 P-100 Plasma aliquot 13	Tube 8 P-100 Plasma aliquot 14
Reserved for sputum specimen	Reserved for sputum specimen	Reserved for sputum specimen	Reserved for sputum specimen	Reserved for sputum specimen	Reserved for sputum specimen	Reserved for sputum specimen	Reserved for sputum specimen	Reserved for sputum specimen
Reserved for sputum specimen	Reserved for sputum specimen							

6.0. Overview of Specimen Collection

Blood Tube Processing Flow Chart



7. PACKAGING AND SHIPPING

7.1. Storage, Packaging, and Shipping (For Monthly Frozen Specimens)

Ship frozen specimens on Mondays- Wednesdays only. Email the GIC Repository at all of the emails listed below at least 48 hours in advance to insure they are ready for your shipment. In this email please include as attachments all excel shipping manifest forms (located on secure SPIROMICS website under forms) as appropriate (see appendix 6). Determine the number of boxes to be shipped that month and gather up the appropriate number of shipping containers needed for shipment. Each shipping container consists of a Styrofoam container surrounded by a cardboard shipping box. Approximately 16 two-inch boxes should fit per carton along with 5-8lbs of dry ice. Therefore, if 15 patients are seen per month, 2 shipping cartons will be needed. These cartons can be recycled (the GIC-repository can send back empty cartons via ground transportation). **Note: during the packing process the aliquot and Paxgene blood tube boxes should be kept on dry ice at all times.**

7.1.1 Packaging Frozen Specimens for Monthly Shipment

- Package samples on the afternoon of shipment, if done earlier maintain package at -80ºC until it is shipped.
- Include the excel spreadsheet manifest prepared 48 hours in advance (see above). Place a manifest into each of the shipping containers, and check off each item as it is packed.
- Put dry ice in a tray or other temporary container for transport of the frozen aliquot and PAXgene blood tube boxes to shipping preparation area.
- Place a rubber band around each of the frozen boxes.
- Remove Styrofoam container from cardboard box before beginning packaging.
- Place some dry ice in the Styrofoam inner container (enough to cover well the bottom of the shipping container), and begin layering boxes and dry ice into the container. Make sure all air pockets contain dry ice. Repeat for additional shipping containers as needed.
- The amount of dry ice in the shipping container should total approximately 5 pounds.
- Put one piece of strapping tape across Styrofoam box top.
- Place the GIC repository address and the paper shipping manifest (with the address on top into a plastic folder or ziplock plastic bag and tape to top of Styrofoam container.
- Put top on Styrofoam container.
- Put Styrofoam box back into card board box.
- Seal the cardboard box containing the Styrofoam box inside securely with strapping tape.
- Affix a Biological Substance Category B (UN3373) label and a Dry Ice label (UN 1845) to the outside of the cardboard box. Please note these labels are not provided by the GIC.
- Affix the completed (see below for specifics) FedEx airbill to the following address to the outside of the box.
    - Record the site address and telephone number in section 1. Record the GIC's FedEx account number on the appropriate line (contact the GIC for this information)
    - Record the GIC internal billing reference in Section 2 (contact the GIC for this information)

- Section 3 should be the GIC repository address (below):
  BioSpecimen Processing Facility
  Rm. 3213 Michael Hooker Research Center
  135 Dauer Drive
  Campus Box 7406
  The University of North Carolina at Chapel Hill
  Chapel Hill, NC 27599-7406
- Section 4--**Check priority overnight**
- Section 5 (packaging) = check **other**
- Section 6 –check **Yes** shippers declaration not required and check **dry ice** and write on the estimated number of pounds
- Section 7 – Check **Third Party**
- Use your institution's FedEx shipping procedures or call 1-800-GO-FEDEX for pickup.

– Send an e-mail message containing the tracking number and date of shipment to all of the addresses below.
Heena Mehta (hmehta@med.unc.edu), Hednrik Dejong (hdejong@email.unc.edu), Amy Perou (amyperou@med.unc.edu), Patricia Basta (patricia_basta@unc.edu), and Betsy Carretta (betsy.carretta@unc.edu)

7.2 Storage, Packaging, and Shipping (For Quarterly Room Temperature Specimens-Cell Lysates)

Email the GIC Repository (BSP) 48 hours in advance at the emails listed below to insure they are ready for your shipment. In this email please include as attachments all Excel shipping manifest forms (located on secure SPIROMICS website under forms) as appropriate (see appendix 6). Determine the number of 50mL Styrofoam tube racks to be shipped that quarter and gather up the appropriate number of shipping containers needed for shipment. Each shipping container consists of a sturdy cardboard shipping box, lined plastic (a garbage bag could be used) These cartons can be recycled (the GIC-repository can send back empty cartons via ground transportation).

7.2.1 Packaging Room Temperature Cell Lysates for Quarterly (monthly shipments can be made if room temperature storage area of the clinical center reaches capacity) Shipment

- Include the excel spreadsheet manifest prepared 48 hours in advance (see above). Place a manifest into each of the shipping containers.
- Top of each lysate tube should be wrapped with parafilm and then with packing tape.
- Line a sturdy cardboard box with a plastic liner
- Prepare the rack of cells lysates by placing the label sheet in the bag with the lysate tubes. This is the sheet which you have been adding labels to each time you place a lysate tube in the rack. You should have the styrofoam rack (with labels on its side), the lysate tubes, the label sheet, and several sheets of absorbent pads all inside the bag the tubes originally arrived in. If you do not have a disposable Styrofoam rack, tubes can be placed in gallon zip locked bags in layers separated by absorbent pads. Do not forget to include the label sheet in the bag. Place the rack into a bag. The original bag

that came with the racked tubes can be used, or alternatively two gallon ziplocked bags can be placed over each end and sealed in the middle.

- Arrange cell lysates in the bagged disposable 50ml test-tubes racks in carton.
- Place packing material generously around bagged test tube racks.
- Place the GIC repository address and the paper shipping manifest (with the address on top into a plastic folder and place on top of tubes.
- Seal carton with strapping tape.
- Affix an "Exempt Human Specimens" label to the outside of the cardboard box. Please note these labels are not provided by the GIC.
- Affix the completed (see below for specifics) FedEx airbill to the following address to the outside of the box.
    - Record the site address and telephone number in section 1. Record the GIC's FedEx account number on the appropriate line (contact the GIC for this information)
    - Record the GIC internal billing reference in Section 2 (contact the GIC for this information)
    - Section 3 should be the GIC repository address (below):
      BioSpecimen Processing Facility
      Rm. 3213 Michael Hooker Research Center
      135 Dauer Drive
      Campus Box 7406
      The University of North Carolina at Chapel Hill
      Chapel Hill, NC 27599-7406
    - Section 4--**Check priority overnight**
    - Section 5 (packaging) = check **other**
    - Section 6 –check **No**
    - Section 7 - Check **Third Party**

- Use your institution's FedEx shipping procedures or call 1-800-GO-FEDEX for pickup.
- Send an e-mail message containing the tracking number and date of shipment to all of the addresses below.
  Heena Mehta (hmehta@med.unc.edu), Hednrik Dejong (hdejong@email.unc.edu), Amy Perou (amyperou@med.unc.edu), Patricia Basta (patricia_basta@unc.edu) , and Betsy Carretta (betsy.carretta@unc.edu)

7.3 Storage, Packaging, and Shipping (For Monthly 4°C Temperature Sputum Specimen for Mucin Analysis)-Note quarterly shipments are possible if not many subjects were recruited during the month.

Email the GIC Repository at least 48 hours in advance at the emails listed below to insure they are ready for your shipment. In this email please include as attachments all excel shipping manifest forms as appropriate (see appendix 6). Determine the number of 2" refrigerated boxes to be shipped that month and gather up the appropriate number of shipping containers (most likely one) needed for shipment. Each shipping container consists of a sturdy cardboard shipping box, with an internal Styrofoam container. Mote use smallest box possible. These

cartons can be recycled (the GIC-repository can send back empty cartons via ground transportation).

7.3.1 Packaging Samples for 4°C Shipping

- Include the excel spreadsheet manifest prepared 48 hours in advance (see above).
- Place appropriate number of frozen gel packs on bottom of Styrofoam container to line the bottom.
- Place at least a half inch of padding on top of frozen gel pack before placing the 2-inch box(es) containing the sputum specimens destined for mucin analysis.
- Place an additional half inch of padding on top of box and place another frozen gel pack on top of box.
- Place packing material generously around gel packs and box(es).
- Place the GIC repository address and the paper shipping manifest (with the address on top) into a plastic folder and place on top of tubes.
- Seal carton with strapping tape.
- Affix an "Exempt Human Specimens" label to the outside of the cardboard box. Please note these labels are not provided by the GIC.
- Affix the completed (see below for specifics) FedEx airbill to the following address to the outside of the box.
    - Record the site address and telephone number in section 1. Record the GIC's FedEx account number on the appropriate line (contact the GIC for this information)
    - Record the GIC internal billing reference in Section 2 (contact the GIC for this information)
    - Section 3 should be the GIC repository address (below):
      BioSpecimen Processing Facility
      Rm. 3213 Michael Hooker Research Center
      135 Dauer Drive
      Campus Box 7406
      The University of North Carolina at Chapel Hill
      Chapel Hill, NC 27599-7406
    - Section 4--**Check priority overnight**
    - Section 5 (packaging) = check **other**
    - Section 6 – check **No**
    - Section 7 - Check **Third Party**
- Use your institution's FedEx shipping procedures or call 1-800-GO-FEDEX for pickup.
- Send an e-mail message containing the tracking number and date of shipment to all of the addresses below.
  Heena Mehta (hmehta@med.unc.edu), Hednrik Dejong (hdejong@email.unc.edu), Amy Perou (amyperou@med.unc.edu), Patricia Basta (patricia_basta@unc.edu), and Betsy Carretta (betsy.carretta@unc.edu)

8. General Quality Control

General Good laboratory Practice Checklist

1) All staff collecting, processing, and shipping samples must be certified on the SPIROMICS MOP. In addition, they should comply with all additional Institutional and Government regulations for working with human blood specimens.
2) For collecting and processing samples follow procedures outlined in this MOP.
3) Process all blood specimens within 2 hours of collection, except the PaxGene which much remain at room temperature for 4 hours prior to freezing..
4) Maintain a daily log of freezer, refrigerator, and room temperatures.
5) Pipettes should be calibrated and certified every six months.
6) Centrifuges speeds should be certified yearly with a tachometer by a certified technician.
7) All biological safety cabinets should be certified yearly, by a licensed technician.

8.1. Quality Control Duplicate Blood Samples

Please refer to QA/QC MOP.

9. TRAINING PROCEDURES

This study will not provide general phlebotomy training, which must be provided by the clinical centers. This MOP will serve as the official training guide for all other aspects of biospecimen collection and processing. A central training will be performed at UNC, and at this time, all sites will need to have present representatives that become certified on the procedures outlined in this MOP. Once certified, these individuals can train and certify new clinical site staff as necessary. Annual re-certification will be required of all current staff.

Appendices

Appendix 1---Aliquots to be collected

1.) **Serum from Tube #1** (8.5 mL red stoppered RT tube--Serum)
   a. 13 150ul aliquots
   b. 1 aliquot with remaining volume

2.) **Serum from Tube #2** (8.5 mL red stoppered RT tube (Serum)
   a. 13 150ul aliquots
   b. 1 aliquot with remaining volume

3.) **Plasma from Tube #3** (8.5 mL yellow stoppered tube--ACD)
   a. 13 150ul aliquots
   b. 1 aliquot with remaining volume

4.) **Plasma from Tube #4** (10 mL lavender stoppered tube--EDTA)
   a. 13 150ul aliquots
   b. 1 aliquot with remaining volume

5.) **Plasma from Tube #5** (10 mL lavender stoppered tube--EDTA)
   a. 13 150ul aliquots

b. 1 aliquot with remaining volume
c. Cellular material made into a red cell-depleted cell lysate

6.) Plasma from Tube #8 (8.5 mL red stoppered P100 plasma collection tube)
a. 13 150ul aliquots
b. 1 aliquot with remaining volume

7.) Urine from unpreserved urine specimen cup
a. Ten 1000ul aliquots

8.) Urine from preserved urine tube
a. Ten 1000ul aliquots

Appendix 2. Biospecimen Processing Form

See study website: www.cscc.unc.edu/spir

Secure Site → Study Documents → Forms

Appendix 3. Puregene Biohazardous Waste Components

38% Tris EDTA, Sodium Bicarbonate
24% Human Blood
19% Ammonium Chloride
19% Water

Appendix 4. Equipment and Supplies:

Supplies to be provided by the Central Laboratory:

Blood Urine Collection Supplies

Tube Name Abbreviation	Tube Color	Size	Anticoagulant	tube type	Specification
10ml LT	Lavender	10ml / 16x100mm	K2EDTA (sprayed on) - 15% sol./vol / shelf life= 365 days	plastic	see-thru label and hemoguard closure
4ml LT	Lavender	4 ml /13x75mm	K2EDTA --- 7.2mg / shelf life= 480 days	plastic	paper label and hemoguard closure
10 ml YT	Yellow	8.5ml / 16x100mm	ACD tri-sodium citrate solution-A (ACD Solution A of trisodium citrate, 22.0g/L; citric acid, 8.0 g/L; and dextrose 24.5 g/L, 1.5 mL, 1.5 mL ACD solution A) / shelf life 720 days	glass	paper label and conventional closure
RT	Red	10 ml / 16x100mm	RT tube with silica activator / shelf life=365 days	plastic	paper label and conventional closure
P100*	Red	8.5ml / 16x100mm	K2EDTA --- 7.2mg / protein stabilizers / store at 2 -8^{o}C shelf life= 365 day		
Paxgene Tube	reddish / orange	8.5ml / 16x100mm / only holds 2.5 ml blood	preservatives and RNA stabilizers --- suggest 21 G needle / 12 inch tubing / shelf life=	plastic (treat like glass once frozen)	paper label
60 ml Cup		60ml / sterile	Urine collection		

*Phlebotomy supplies included in P100 kit

1) BD Vacutainer® One Use Tube Holders, Single Use Only, Translucent

2) BD Vacutainer® Safety-Lok™ Blood Collection Set, 21 G Butterfly Needle, 12" Tubing, Sterile, Use Once and Discard

3) BD™ Alcohol Swab: Isopropyl Alcohol - 70%

Blood and Urine Processing Supplies

Product Class	Product	Description
Tubes / containers	1.7ml screw cap	1.7ml Sarstedt screw cap tube / conical / Oring cap / Sterile / assembled
	15 ml conical tube	flat top / costar brand / conical btm / poly prop / sterile/ racked / 17x119mm / 12,000xG
	50 ml conical tube	flat top / costar brand / conical btm / poly prop / sterile / racked / 28x115mm / 15,500xG
	2" boxes	2" fiberboard box / Revco 5954
	9x9 dividers	9x 9 divider / revco # 6212
	Lg Styrofoam Shipping containers	
	Cardboard Shipping box	Thermosafe: Come as unit : foam container assembled in corrugated carton
	Bar code labels	Labels for blood collection tubes, specimen aliquots, and freezer boxes will be provided.
	Labels - FedEx	Fedex labels/specimen shipping provided by GIC
chemicals	Ascorbic Acid	to be used with the Treated Urine Samples
	Red Blood Cell Lysis Solution	use to process Blood in the first step
	Cell Lysis Solution	used to process blood in the second step

Equipment purchased and maintained by Field Centers:

Table-top centrifuge with swinging buckets, refrigerated, and capable of producing 3,000 x *g*

Freezer capable of maintaining -70° C with a minimum of 5 cu ft storage

Refrigerator 4° C for storing urine containers and 4 mL EDTA tubes prior to shipping.

Appendix 5 Partial Biospecimen Collection Procedure
Participant Sample Set

If a complete set of blood tubes was not taken at the initial visit, during the follow-up visit the blood draw should resume with the first tube missed. A follow-up visit to complete the blood draws should be scheduled within a month of the first visit.

Appendix 6 Excel Shipping forms

Please see study website: www.cscc.unc.edu/spir

Secure Site → Study Documents → Forms

Appendix 7 Clean Catch Urine Instructions—Male and Female

INSTRUCTIONS FOR FEMALES

1. Wash hands with soap and warm water.
2. Place clean paper towel on counter.
3. Open the urine cup and place the lid and cup face up on the clean paper towel being careful not to touch the rim or inside of the lid or cup (to prevent contaminants from entering the clean field).
4. Spread the labia (folds of skin) apart with one hand and wipe with the towelette provided. Wipe from front to back.
5. Continue holding the labia apart. As you start to urinate, allow a small amount of urine to fall into the toilet bowl. (This clears the urethra of contaminants) Do not touch the inside of the cup.
6. After the urine stream is well established, urinate into the cup. Once an adequate amount of urine fills the cup (the cup only needs to be half-full), remove the cup from the urine stream.
7. Pass the remaining urine into the toilet.
8. Screw the lid on the cup tightly (do not touch the inside of the cup or lid).
9. Wash your hands with soap and warm water.
10. Record the time of the urine collection on the label on the cup, with the pen provided.
11. Place the cup in the biospecimen bag provided***
12. Give the cup to the Study Coordinator/technician

*** Alternately "leave specimen in bathroom and notify the technician that the specimen is waiting" or "place specimen in wall cabinets"

INSTRUCTIONS FOR MALES

2. Wash hands with soap and warm water.

2. Place clean paper towel on counter.

3. Open the urine cup and place the lid and cup face up on the clean paper towel being careful not to touch the rim or inside of the lid or cup (to prevent contaminants from entering the clean field).

13. If uncircumcised, retract foreskin.

14. Wipe the end of penis with towelette provided. As you start to urinate, allow a small amount of urine to fall into the toilet bowl. (This clears the urethra of contaminants) Do not touch the inside of the cup.

15. After the urine stream is well established, urinate into the cup. Once an adequate amount of urine fills the cup (the cup only needs to be half-full), remove the cup from the urine stream.

16. Pass the remaining urine into the toilet.

17. Screw the lid on the cup tightly (do not touch the inside of the cup or lid).

18. Wash your hands with soap and warm water.

19. Record the time of the urine collection on the label on the cup, with the pen provided.

20. Place the cup in the biospecimen bag provided

21. Give the cup to the Study Coordinator/technician

*** Alternately "leave specimen in bathroom and notify the technician that the specimen is waiting" or "place specimen in wall cabinets"

Appendix 8 Biospecimen Collection Preparation List

SPIROMICS BIOSPECIMEN COLLECTION PREPARATION CHECKLIST

Urine Collection:

□ urine specimen collection cup, towelette

Blood Collection Tray

□ test tube rack that holds at least 9 blood collection tubes

□ 12-inch blood collection set for use with all tubes

****mandatory for P100 plasma collection tubes**

- □ sterile, disposable 21 gauge butterfly needles
- □ plastic vacutainer tube guides
- □ vacutainer Luer adapters
- □ sterile alcohol swabs
- □ gauze sponges
- □ tourniquet
- □ bandages (“band aids”)
- □ GIC provided labels (for 9 blood tubes, 1 urine cup)

Appendix 9 Participant Lab ID tracking sheet

Participant ID:		
Visit	**Date**	**Lab ID**