MESA EXAM 7 Field Center Procedures – Biospecimens Manual of Operations

Laboratory

Most of the procedures, laboratory equipment, and supplies used in Exam 7 for the MESA Classic (Core) collection are similar to those in previous Exams.

I. PURPOSE

MESA is a multicenter, longitudinal epidemiological study of the incidence and progression of subclinical atherosclerotic cardiovascular disease. The Central Blood Analysis Laboratory (CBAL) will have responsibilities for special blood collection and handling protocols as well as training and QC monitoring at the Clinical Centers. The laboratory will also be responsible for performing assays and reporting results.

The blood samples collected and processed by Clinical Center technicians are the foundation for all of these tests. The most important step – and potentially the most variable – is the collection and processing of the blood samples. If the blood sample itself is not correctly drawn and processed, the laboratory results may not be precise or may not be valid. Consistency in this step across the multiple clinical centers is vital to the study.

MESA Exam 7 Core blood collection involves the collection of ~69.5 mLs of blood from the MESA Classic cohort and includes additional blood to be collected specifically for a few of the ancillary studies. Blood for ancillary studies is included in the core blood draw. For details regarding collection and processing for MESA Lung hair and nasal cells and processing of cells for MESA Epigenomics at Northwestern refer to the individual ancillary study protocols within the MESA 7 Manual of Operations.

II. EQUIPMENT & SUPPLIES

The following supplies will be provided in bulk by the CBAL:

Styrofoam Shipping Containers.
Cryovials– 0.5 mL, and 2.0 mL with color-coded caps (blue, red, yellow, and green).
10 mL transfer tubes and caps
Absorbent material
Shipping labels (FedEx, Dry Ice, Category B Biospecimen)

The blood collection area should have the following supplies:

Lab coats and gloves
Phlebotomy chair
Basin (just in case)
Washcloths/Towels
Smelling salts
Lab mats and wipes
10% bleach solution or approved biohazard disinfectant
Plastic cart with wheels for phlebotomy supplies (or plastic tray with compartments)
Butterfly needles (21G) with luer adapter (BD #367281)
Vacutainer barrels
Tourniquets
Alcohol prep pads

Gauze pads (2x2)
Surgical tape – paper tape (easier on participants)
Band-Aids
MESA Blood collection tubes (keep extras on hand):
- MESA Classic (Core + MIND) collection (Standard sites):
  - 5 – 10 mL EDTA tubes (BD# 366643)
  - 1 – 10 mL Serum tubes (BD# 367820)
  - 1 – 5 mL Serum tubes (BD# 367814)
  - 1 – 2 mL EDTA tubes (BD #367841)
  - 1 – 2.5 mL PAXgene RNA tubes (BD# 762165)
- MESA Classic (Core+MIND+Epi) collection (NWU):
  - 4 – 10 mL EDTA tubes (BD# 366643)
  - 1 – 6 mL EDTA tubes (BD# 367863)
  - 1 – 10 mL Serum tubes (BD# 367820)
  - 1 – 2 mL EDTA tubes (BD #367841)
  - 1 – 2.5 mL PAXgene RNA tubes (BD# 762165)
  - 1 – 8 mL CPT (BD# 362753)

Draw tube rocker
Draw tube racks
Ice bucket with crushed ice – filled 10 min before draw
Stopwatches or timers
Scissors
Pens and Sharpie pens
MESA Participant Labels
MESA Phlebotomy / Classic Processing Form
Blood Spill Kit
Biohazardous waste containers
Needle/sharps container
Material for urine collection for MESA Classic – urine specimen collection containers (Covidien Sterile Midstream Urine Collection Systems (FisherScientific Cat# 14-375-143))

III. METHODS

1. Safety Issues and Precautions for Handling Blood Specimens.

In accordance with the OSHA regulations on bloodborne pathogens, the CBAL recommends the following laboratory safety protocol for the field center laboratories:

Use of non-permeable lab coats, nitrile or latex gloves, and face shields when handling any blood in any situation where splashes, spray, spatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonably anticipated.

Use of aerosol containers in all centrifuges.

Follow ‘Universal Precautions’ when handling any blood products.

Immediately place contaminated needles and sharps in a puncture-resistant, leak-proof container.

Never recap or break needles.

Offer Hepatitis B vaccine to all unvaccinated technicians handling/working with blood, blood products, or equipment contaminated with blood. Documentation of vaccination, or technician’s declining to be vaccinated, should be kept on file at the Clinical Center.

2. Biospecimen ID Labels

The Coordinating Center is supplying each field center with sheets of Biospecimen ID number labels to use for labeling draw tubes, pooling tubes, cryovials, freezer boxes, and forms. There will be a total of 105 labels for MESA 7 collection including labels needed for urine collection and processing.

MESA Classic

- MESA Phlebotomy & Processing Forms (3)
- MESA Draw tube labels (7)
- MESA Cryovials labels (84)
- MESA EDTA Pooling tube (1)
- MESA Serum Pooling tube (1)
- MESA Urine Collection container (1)
- MESA Shipping Form (1)
- MESA Freezer boxes (3)
- Extra labels in case needed for replacement draw tubes, etc. (4)

MESA QC (Select Participants)

- MESA QC Cryovial labels – EDTA and urine or serum, hair follicle, hair shaft, and nasal cell
- MESA QC Shipping Form (1)

The Biospecimen ID is a 5 digit ID number that will be assigned to the participant at the time of biospecimen collection. This is a change from previous exams where the MESA ID number was used to identify biospecimens. Each set of labels has the same 5-digit Biospecimen ID number (the first digit identifies the clinic – Wake Forest =3, Columbia =4, John's Hopkins =5, UMinn =6, Northwestern =7, and UCLA =8). The exam year, '7', is represented as the 6th digit in the 8 digit number on the barcode cryovial labels. The last two digits on the cryovial label represents the number assigned to that cryovial. This 2-digit number (01 to 84) uniquely identifies each cryovial within a biospecimen ID which is of key importance in tracking the repository. Note – sample collected in the PAXgene RNA and 2 mL EDTA whole blood (WB) draw tube will be stored in the draw tube, hence why the PAXgene RNA draw tube is assigned the 2-digit repository/cryovial identifier "81" and the 2 mL EDTA WB draw tube is assigned the 2-digit repository/cryovial identifier "80" as indicated on the barcode label for the draw tube. See Appendix for proper orientation of the barcode label on the cryovial and an example set of MESA 7 labels.

Also, there will be special QC ID labels for the blind duplicate samples. The MESA QC labels for the blind duplicate aliquots will have a 5-digit QC ID number with the first digit corresponding to the MESA site and the second digit "9" to identify it as a QC ID, 1-digit for the visit year, and a 2-digit cryovial identifier barcoded on them along with text stating sample type. See the section on blind duplicates for further information on the procedure.

It is essential that all biospecimen samples be correctly labeled throughout the collection and processing stages. Pre-label sets of MESA Classic collection tubes and cryovials prior to the participant's arrival. Biospecimen IDs will be assigned to participants at the time of collection so if, for some reason, a participant does not show for their appointment the labeled tubes and cryovials can be used for the next person that is scheduled. It is not recommended to label phlebotomy and processing forms ahead of time.

3. Forms

The MESA Phlebotomy Form and Processing Form provides the vital link between the Biospecimen ID, Participant ID number, and the samples. The Forms aid in the efficient collection of the samples,

facilitate the monitoring of sample collection, processing and other quality assurance parameters, and provide information critical to the interpretation of results and for future repository use.

The MESA Exam 7 Phlebotomy Form is generated at the time of the participant's visit and lists all exam draw tubes. Tubes are listed in the correct order of collection. The phlebotomist uses the form to complete draw tube set-up and labeling for the participant's blood collection. All questions on the Phlebotomy Form are completed at the time of phlebotomy. The phlebotomy form is also used to link the Biospecimen ID to the MESA participant ID, so care should be taken to double check this match before moving on with specimen collection.

The MESA Exam 7 Processing Forms are used to record the processing (centrifuge) start times of the Core draw tubes and to account for all the MESA 7 aliquots (blood and urine) – cryovials# 1-81. Completed MESA 7 Processing Forms, along with a completed MESA 7 Shipping Form identifying all the participant samples in a shipment, are to be included in the shipments to Vermont (CBAL).

Ancillary studies involving the collection and processing (and storage if applicable) of additional samples, have their own study specific Processing Form (or Collection Form). Refer to the individual ancillary study protocols for details.

It is important that all forms are labeled with the correct participant MESA ID number and all appropriate sections completed legibly. The completed MESA Phlebotomy Forms do not need to be included in the sample shipment as an electronic version will be forwarded to CBAL from the Coordinating Center.

4. Participant Refusal of Phlebotomy or Urine collection

Rarely, a participant will refuse to provide a urine sample or will refuse phlebotomy. Please keep a list of MESA Enrollment ID numbers of any of these participants and identify which test they refused. Properly document this information on the Phlebotomy Form.

5. MESA Classic Urine Collection & Venipuncture

5.1 Initial preparation for specimen collection prior to the arrival of participants is similar to that of previous MESA Exams.

5.2 Urine Collection & Processing
A random, spot urine will be collected on all MESA 7 participants upon morning arrival at the clinic. The urine will be aliquoted and stored as part of the sample repository in Vermont and will be measured for urinary creatinine and microalbumin as samples are received.

Keep urine samples correctly labeled throughout the collection and processing stages. Pre-label collection containers and cryovials prior to the participant's visit, and cross-check the labels with each participant's Biospecimen ID number prior to specimen collection.

5.2.1 Preparation of Participants for Urine Collection

Urine should be collected before venipuncture; preferably as early in the visit as possible. Encourage participants to stay hydrated (water only) even while fasting for the visit. However, do not collect samples after acute fluid load (>24 oz) or after participant exertion. Participants having difficulty producing a urine specimen may be offered a glass of water, and a second (and third) urine specimen may be collected later in the visit to bring the volume up to the required amount.

It is suggested that participants use the Covidien Sterile Midstream Urine Collection Systems for urine collection. Instructions are included in the package.

5.2.2 Urine Collection:

Specimen containers for routine random urine collection should be chemically clean, be able to hold about 100+ mLs, and must have a tight fitting lid to prevent leakage during transport. (Suggestion for standard sterile specimen container: Covidien Sterile Midstream Urine Collection Systems (FisherScientific Cat# 14-375-143))

Instructions for Participants:

The participant's privacy should be assured.

Orient the participant to the supplies and explain the procedure (Covidien Sterile Midstream Urine Collection Systems have clear packaging for ease in describing contents).

Steps to be followed:

1. Wash hands before and after voiding. Open or remove clothing to make voiding and collection easier.
2. Remove the cap from the collection container. Void directly into the container until approximately half full.
3. Carefully seal the cap of the container so that it is tight and leak proof.
4. Bring the urine container to the MESA staff member.

The MESA staff member will record if urine is collected, time of collection, and approximate volume on the Phlebotomy Form.

5.2.3 Urine Processing

MESA 7 - Urine Aliquots

Collection	Sample Type	Number of Aliquot Vials	Color Code	Volume per Aliquot Vial
Urine	Urine (plain)	6 (Cryos# 69-74)	Yellow	1.5 mL urine in 2.0 mL cryovial

Keep urine refrigerated or on ice until processing can be done.

Gently swirl urine immediately prior to aliquoting to ensure sample is thoroughly mixed.

Aliquot 1.5 mL urine into six pre-labeled 2.0 mLs cryovials, Cryovials# 69-74. Keep cryovials on ice. These cryovials will be color-coded with yellow caps.

Do NOT overfill the cryovials. There must be space for the urine to expand when frozen. These should all be 2.0 mL size cryovials.

Double-check urine aliquots have the correct Biospecimen ID labels and caps are securely tightened on filled cryos.

Discard any extra urine.

Check off on the MESA 7 Processing Form the number of urine aliquots made (Cryovial#s 69-74).

Freeze cryovials in an upright position at -80°C or colder, within 10 minutes of aliquoting.

5.2.4 Blind Duplicate Urine Sample:

All participants selected for EDTA QC, will also have a Urine QC cryovial made. The QC ID number will be the same for both the EDTA and urine blind duplicate cryovials, however the two-digit QC cryovial identifier (the last two digits on the barcode label) will be different.

After aliquoting the participant's urine into repository Cryovial#s 69-70, aliquot 1.5 mL of urine into a 2.0 mL cryovial pre-labeled with the participant's QC ID number. Urine blind duplicate cryovials will be color-coded with yellow caps and have "03" as their two-digit QC cryovial identifier.

Resume aliquoting the participant's remaining urine repository Cryovial#s 71-74.

Freeze all aliquots immediately (within 10 minutes of aliquoting) in the upright position at -80°C. (See section **V. Quality Assurance**, Blind Duplicates and Urine Blind Duplicates for further details.)

5.3 Priority of Tubes & Preparation of Phlebotomy Draw-tubes and Aliquot Racks

For the MESA collection, a maximum of approximately 69.5 mL of blood will be collected from each participant into 9 draw tubes. There are no additional tubes collected specifically for quality control purposes.

The order in which the tubes are collected is important and is done as follows:
MESA 7 Draw Tubes: (All sites except Northwestern)

1. 10 mL EDTA purple top
2. 10 mL EDTA purple top
3. 10 mL EDTA purple top
4. 10 mL EDTA purple top
5. 10 mL Serum red top
6. 5 mL Serum red top
7. 2 mL EDTA purple top
8. 10 mL EDTA purple top
9. 2.5 mL PAXgene RNA red top

MESA 7 Draw Tubes Northwestern:

1. 10 mL EDTA purple top
2. 10 mL EDTA purple top
3. 10 mL EDTA purple top
4. 10 mL EDTA purple top
5. 10 mL Serum red top
6. 2 mL EDTA purple top
7. 6 mL EDTA purple top

8. 8 mL Heparin CPT red/green tiger top
9. 2.5 mL PAXgene RNA red top

Note: For MESA 7, draw tubes collected and the priority of tubes has changed from previous MESA Exams.

MESA 7 Draw Tube Collection and Processing

1. 10 mL EDTA Tubes (#1-4 & 8) are purple-topped EDTA tubes (BD# 366643). After filling, these tubes are mixed for ~30 seconds on the tube rocker then placed on wet ice. The draw tubes are centrifuged and the plasma pooled and aliquoted into 54 cryovials (#01-54) color-coded with blue caps. Participants selected for EDTA QC will have 0.5 mL plasma aliquoted, from the same pooled plasma as the repository cryovials, into a 0.5 mL cryovial labeled with the participant's EDTA blind duplicate label. (Participants selected for EDTA QC are also selected for urine QC.)

1. 10 mL & 5 mL Serum Tubes (#5 & #6) are red-topped Serum tubes (BD# 367820 & 367814). These tubes are silicone coated with a clot activator. Immediately after filling, gently invert tube 5 times to ensure proper mixing of the clot activator with the blood. After collection and initial mixing, these tubes remain upright at room temperature for a minimum of 60 minutes, but no longer than 90 minutes, to allow the blood to clot. Serum from the 10 mL and 5 mL centrifuged draw tubes is pooled then aliquoted into 14 cryovials (#55 – 68) color-coded with red caps. Participants selected for serum QC will have 0.5 mL serum aliquoted, from the same pooled serum as the repository cryovials, into a 0.5 mL cryovial labeled with the participant's serum blind duplicate label.

2. 2 mL EDTA Tubes (#7) is a purple-topped EDTA tube (BD# 367841). After filling, these tubes are mixed for ~30 seconds on the tube rocker then placed on wet ice before placing upright in a -80°C freezer. Do NOT centrifuge the EDTA whole blood tube.

3. PAXgene RNA Tube (#9) is a red-topped PAXgene Blood RNA tube (BD# 762165) containing 6.9 mL of proprietary RNA stabilization additive. Even though these tubes are tall, similar to a 10 mL tube, the sample collection is only 2.5 mL. Prior to collection, ensure the PAXgene RNA tube is at room temperature and correctly labeled with the provided MESA barcode draw tube label. After filling, these tubes are gently inverted 8-10 times, and can be kept at room temperature until placing upright in a -80°C freezer. Do NOT centrifuge the PAXgene RNA tubes.

MESA 7 Draw Tube Collection and Processing @ NWU

1. 10 mL EDTA Tubes (#1-4) and 6 mL EDTA Tube (#7) are purple-topped EDTA tubes (BD# 366643 and 367863). After filling, these tubes are mixed for ~30 seconds on the tube rocker then placed on wet ice. The draw tubes are centrifuged and the plasma pooled and aliquoted into cryovials (#01-46) color-coded with blue caps. Participants selected for EDTA QC will have 0.5 mL plasma aliquoted, from the same pooled plasma as the repository cryovials, into a 0.5 mL cryovial labeled with the participant's EDTA blind duplicate label. (Participants selected for EDTA QC are also selected for urine QC.)

2. 10 mL Serum Tube (#5) is a red-topped Serum tubes (BD# 367820). These tubes are silicone coated with a clot activator. Immediately after filling, gently invert tube 5 times to ensure proper mixing of the clot activator with the blood. After collection and initial mixing, these tubes remain upright at room temperature for a minimum of 60 minutes, but no longer than 90 minutes, to allow the blood to clot. Serum from the 10 mL centrifuged draw tube is aliquoted into 9 cryovials (#55 – 63) color-coded with red caps. Participants selected for serum QC will have 0.5 mL serum aliquoted, from the same pooled serum as the repository cryovials, into a 0.5 mL cryovial labeled with the participant's serum blind duplicate label. (Participants selected for serum QC are also selected for hair and nasal brushing QC samples.)

3. 2 mL EDTA Tubes (#6) is a purple-topped EDTA tube (BD# 367841). After filling, this tube is mixed for ~30 seconds on the tube rocker then placed on wet ice before placing upright in a -80°C freezer. Do NOT centrifuge the EDTA whole blood tube.

4. 8 mL Heparin Cell Prep Tube (#8) is red/green tiger topped CPT (BD# 362753). Ensure the tubes are at room temperature before filling. After filling, gently invert 8-10 times to ensure proper mixing. Place upright at room temperature until hand off to MESA Epi technician.

5. PAXgene RNA Tube (#9) is a red-topped PAXgene Blood RNA tube (BD# 762165) containing 6.9 mL of proprietary RNA stabilization additive. Even though these tubes are tall, similar to a 10 mL tube, the sample collection is only 2.5 mL. Prior to collection, ensure the PAXgene RNA tube is at room temperature and correctly labeled with the provided MESA barcode draw tube label. After filling, these tubes are gently inverted 8-10 times, and can be kept at room temperature until placing upright in a -80°C freezer. Do NOT centrifuge the PAXgene RNA tubes.

Summary of Blood Mixing During Venipuncture

Each tube should be treated as follows:

Serum tubes	Gently invert 5 times to mix; place in rack at room temperature for 60 minutes, but no longer than 90 minutes.
EDTA tubes	Place on mixer for ~30 seconds, then place in rack on wet ice until centrifuging.
EDTA WB tube	Place on mixer for ~30 seconds, then place in rack on wet ice until freezing upright in -80°C freezer.
PAXgene RNA	Gently invert 8-10 times to mix; Keep at room temperature until freezing upright in -80°C freezer.
Heparin CPT (NWU)	Gently invert 8-10 times to mix; keep at room temperature until hand off to MESA Epi technician

5.4 Preparation of Phlebotomy Room – is similar to that of previous MESA Classic Exams.

The blood draw is done in an isolated room, or participants are separated by room dividers.

Setup of Draw Tube and Aliquot Racks

Correct labeling and accurate tracking of collected specimens is vital, and correct draw tube order is important (see section 5.3 for specifics). It is recommended to set up pre-labeled draw tubes for the MESA 7 collection in a blood collection tube rack prior to the participant's arrival. Setting up aliquot racks with the corresponding pre-labeled cryovials prior to processing the samples is strongly recommended. Depending on how many participant sample sets are expected to be processed simultaneously, it may work well to have all cryovials for one participant – and only that participant's cryovials – in one cryovial rack. Alternately, a separate aliquot rack for the serum cryovials (red capped cryovials), maybe helpful as the serum is generally centrifuged at a different time from the EDTA tubes. Processing technicians are to use their discretion as to which set-up ensures accuracy and is most efficient for their situation.

Preparation for Specimen Collection

Preparation for specimen collection is done in the following manner. Early morning, prior to arrival of any participants:

1. Make sure venipuncture supplies are stocked for MESA 7 as well as all ancillary studies.
2. Check tubes and cryovials are labeled for MESA 7.
3. Check the phlebotomy room is tidy and stocked with all items needed, and the draw tube rocker is functional.
4. Check that the sample processing station is properly equipped with all items needed.
5. Approximately 10 minutes before the scheduled specimen collection, fill an ice bucket ¾ full with crushed ice.

5.5 Preparation of Participants – is similar to that of previous MESA Exams. A few points are worth restating here.

5.5.1 This study depends on and requires the voluntary cooperation of the participants. These people are giving their time – and precious bodily fluids – and their only reward is the knowledge that they are contributing to progress in medicine. Thus, the experience must be as pleasant as possible. Give the participant enough time to feel comfortable, both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with, and the attitude and competence of, the technician who draws the blood. Do not under any circumstances force or coerce the participant to have blood drawn.

5.5.2 **Phlebotomy Form Questions**. There are four questions to ask the participant before the start of venipuncture. The first three questions deal with the participant's experience with venipuncture. If they answer yes to any of these questions, the phlebotomist can take extra care with the procedure. Question 4 deals with diabetes status. Check yes only if the participant is taking medication for diabetes.

5.6 Venipuncture Procedure – is similar to that of previous MESA Exams.

ALWAYS WEAR NITRILE OR LATEX GLOVES AND LAB COAT

Blood drawing is standardized for the sitting position. You may have participants clench their fists (moderately) during phlebotomy, for up to two minutes. Venipuncture is performed with a 21-gauge butterfly needle with 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. Using 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. It also allows the collection of PAXgene RNA tubes by eliminating the possibility of blood back-washing from the tube to the participant. The use of 23-gauge or 19-gauge butterfly needles is acceptable but should be noted on the Phlebotomy Form.

1. Arrange draw tubes in order of draw in a tube rack or on the table top within easy reach. Assemble butterfly apparatus and vacutainer holders, gauze pads, and alcohol prep pads prior to tourniquet application.

2. Apply tourniquet (quick-release tourniquet is recommended; please do not use a blood pressure cuff).

3. Examine participant's arms for the best site for venipuncture. Release tourniquet. (*Please do not leave tightened tourniquet on participant for a prolonged period of time prior to start of venipuncture as this is likely to yield an atypical sample*.)

4. Cleanse venipuncture site by wiping with an alcohol prep pad in a circular motion from center to periphery and allow area to dry.

5. Re-apply tourniquet and start timer. Total time tourniquet is applied is recorded on the Phlebotomy Form. (It is best to release the tourniquet as soon as possible after flow has been established.) Tightened tourniquet should be on no longer than 2 minutes recommended, or loosen tourniquet then reapply if necessary. However, this may result in cessation of blood flow, especially in sick and/or elderly participants, and may result in the need for a second venipuncture. Therefore, this is a "judgment call" based upon the phlebotomist's experience and skill. If tourniquet is reapplied, be sure to record all tourniquet times on the Phlebotomy Form.

6. Grasp the participant's arm firmly, using your thumb to draw the skin taut to anchor the vein. The thumb should be 1 to 2 inches below the venipuncture site.

7. With the needle bevel upward, enter the vein in a smooth continuous motion. Take note of the time as this is considered the start of venipuncture and is documented on the Phlebotomy Form.

8. 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. Alternatively rolled towels or similar material may be placed under the elbow for support.

9. Grasp the flange of the vacutainer holder and gently push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle. Minimize turbulence whenever possible. Small steps, such as slanting the vacutainer to have the blood run down the side of the tube instead of shooting all the way to the bottom, may result in significant improvement.

10. Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly. Try moving the needle slightly without causing discomfort to the participant.

11. Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shutoff valve and cessation of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.

12. Fill each vacutainer as completely as possible; i.e. until the vacuum is exhausted and blood flow ceases. If a vacutainer tube (for MESA 7) fills only partially (<½ full), remove the tube and

attach another of the same type without removing the needle from the vein. The EDTA and serum tubes specified for MESA 7 both have additives therefore either of these tubes less than ½ full are not acceptable. The PAXgene RNA tube usually takes at least 10 seconds to fill even though only a 2.5 mL fill volume, and if there are difficulties filling this tube, all volumes are acceptable. If a tube is not completely filled, clearly document on the Phlebotomy Form.

13. When the blood flow ceases, remove the tube from the vacutainer holder. The shutoff valve on the butterfly re-covers the point, stopping blood flow until the next tube is inserted.

14. Release tourniquet, if still applied. The ideal tourniquet time is two minutes. Tourniquet may be reapplied if necessary.

15. To remove the needle, lightly place a clean gauze pad over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with the gauze pad. Have the participant hold the gauze pad firmly for one to two minutes to prevent formation of a hematoma. Discard needle and tubing in the appropriate puncture-proof biohazard waste container. Note time needle is removed as this is considered the end of venipuncture and is recorded on the Phlebotomy Form.

16. Record on the Phlebotomy Form the duration the tourniquet was applied (also note duration if tourniquet is reapplied), and be sure both the start and end venipuncture times are documented on the Phlebotomy Form.

17. Ensure all filled draw tubes are correctly labeled with the provided MESA labels.

18. Gently invert MESA 7 serum tubes 5 times immediately after filling, then keep at room temperature for a minimum of 60 minutes, but no longer than 90 minutes, for blood to completely clot.

19. Place EDTA tubes on the tube mixer for a minimum of 30 seconds then keep tubes on wet ice until ready to centrifuge or move to the freezer for 2 mL EDTA whole blood.

20. The PAXgene RNA tube is gently inverted 8-10 times immediately after filling, then placed in the tube rack at room temperature to await freezing in a -80°C freezer.

21. For any MESA ancillary study draw tubes collected, please refer to their study specific protocol for mixing and temperature requirements as this can have a significant impact on the quality of the blood sample for the study's research aims.

22. If the participant continues to bleed, apply pressure to the site with a gauze pad. Keep the arm elevated until bleeding stops. If necessary, tightly wrap a gauze bandage around the pad and leave in place for at least 15 minutes.

23. Clean up the venipuncture area (if necessary). Check the Phlebotomy Form is completed.

24. Take the filled blood collection tubes to the processing area, keeping the EDTA tubes on ice, and the serum at room temperature. The PAXgene RNA tube may remain at room temperature until freezing upright in a -80°C freezer.

5.7 Guidelines for Difficulties – are the same as in previous MESA Exams.

Handling participants who are extremely apprehensive about having blood drawn. 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 and return later for the blood draw. 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 has "good veins" the phlebotomist can reassuringly say, "Oh, you have good veins; there should be no problems."

Procedures for Difficult Draw. If a blood sample is not forthcoming, the following manipulations may be helpful.

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

b. 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.

c. 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.

d. Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on their part.

e. It is permitted to use a heating pad to facilitate a better blood draw, however the phlebotomist would have to wait 10-15 minutes after the heating pad is removed, before performing the venipuncture.

f. If venipuncture is unsuccessful, note on the Phlebotomy Form.

WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD COLLECTION.

a. Have the person remain in the chair, if necessary have him/her sit with their head between their knees until his/her color returns and he/she feels better.

b. Provide the person with a basin if he/she feels nauseous.

c. Have the person remain seated until he/she feels better.

d. Place a cold wet cloth on the back of the person's neck.

e. If the person faints, use smelling salts to revive by crushing the ampoule and waving it under the person's nose for a few seconds.

f. If the person continues to feel ill, contact a medical staff member for advice.

Other Possible Problems – Not all Draw Tubes Collected.

(Blood flow ceases, difficult venipuncture, etc.). Always fill collection tubes in the order specified. Make notations of difficulties on the Phlebotomy Form. If the participant is willing, another attempt should be made to complete the draw collecting only those tubes that were not filled in the first venipuncture following the same tube order.

Other Possible Problems – Draw Tube does Not Fill.

First, try another tube of the same type. EDTA and serum tubes for MESA 7 both have additives therefore tubes less than ½ full are not acceptable. The PAXgene RNA tube usually takes at least 10 seconds to fill, and if there are difficulties filling this tube, all fill volumes are acceptable. EDTA and serum tubes between ½ full and completely full are acceptable but will probably yield a reduced number of aliquots. If any draw tube is not completely filled, clearly note on the Phlebotomy Form as this could affect future assays.

6. MESA Classic - Processing Specimens

A. Overview

Processing of the MESA Classic samples should be initiated as soon as possible (0-30 minutes) following venipuncture. Personal protective equipment (non-permeable lab coats, double-gloves – nitrile or latex) is required during processing (splatter shields/face protection recommended), and adhere to any additional safety regulations recommended or required by your institution.

Double check each participant's pre-labeled cryovials are correctly organized in the cryovial racks, cryovials are of the correct size, and all cryovials are correctly labeled ready for processing the samples – including proper orientation of the barcode label on the cryovial so the barcodes can be scanned upon receipt at the CBAL (refer to the Appendix for the diagram showing correct barcode label orientation on the cryovial).

For details on mixing, temperature requirements, and processing of ancillary study samples, refer to the specific ancillary study lab protocol sections in the MESA 7 Manual of Operations.

B. Daily Preparation

The following items should be on hand before beginning processing of the MESA Classic samples:

- Lab coats, ample supply of nitrile or latex gloves, splash shields, and other Personal Protective Equipment as needed.
- Emergency eye wash station.
- Biohazard waste container, with large and small biohazard bags *(puncture-proof biohazardous waste containers).*
- Refrigerated Centrifuge with a Horizontal (swing-out head) rotor – 2000 g-force minimum, 4°C.
- Test tube holders (adapters) for centrifuges.
- Harvard Trip Balance / Pan balance.
- Water bottles for balance.
- -80°C or colder freezer.
- Refrigerator 4°C for temporary storage of collected urine in case aliquoting is delayed: -Can be a household style fridge; But not a fridge being used for food storage.
- Lab mats and wipes.
- Clock.
- Stop watch or timer.

- 10% bleach solution or other approved biohazard disinfectant.
- Ice bucket with crushed ice, filled before start of processing.
- Fixed volume pipettes with tips (MLA). Volumes to be pipetted: 0.5 mL, 1.0 mL, and 1.5 mL. (Calibrated adjustable pipets may be used.)
- Draw tube racks.
- Cryovial/tube racks for 0.5 mL and 2.0 mL, and pooling tubes.
- Fisher cryovials* (0.5 mL and 2.0 mL) with appropriate color caps (blue, red, yellow, and green). Pre-labeled and have extras on hand.
- Pooling tubes (50 and 15 mL conical tubse or other suitable tube for pooling plasma or serum from draw tubes).
- MESA Participant ID barcode labels (supplied by the CC).
- MESA Phlebotomy Form (or other means for processing technician to access venipuncture start and end times).
- MESA 7 Processing Form (and any MESA ancillary study Processing Forms needed).
- Labels/lab tape for reagents.
- Pens and Sharpie pens.
- Cryovial freezer boxes – Thermo Scientific #5954 (Fisher Cat# 11-678-24A) fiberboard 2" storage boxes with 9 x 9 grids, and Thermo Scientific #5956 (Fisher Cat# 11-678-24B) fiberboard 3" storage boxes.

*= provided by CBAL

C. Summary of Timing till Centrifuging for MESA Classic Tubes:

After blood collection, time before centrifugation:

EDTA: store on ice; preferably < 15 minutes (maximum < 30 minutes) before centrifuging.

Serum: store at room temperature for at least 60 minutes, but < 90 minutes prior to centrifuging.

After aliquoting, freeze ALL samples within 10 minutes or place immediately on dry ice. Do not freeze in -20C freezer unless necessary for temporary storage (<1 hour).

D. Centrifugation:

Instructions for centrifuging MESA Classic EDTA and Serum tubes.

- EDTA tubes should be kept upright on wet ice until centrifuging. Centrifuging these tubes should be initiated as soon as possible (0-30 minutes) following venipuncture. Record start time of centrifuging on the MESA Classic Processing Forms.

- Serum 10 mL tubes. Keep at room temperature for at least 60 minutes, but no longer than 90 minutes, to allow them to clot. Record start time of centrifuging on the MESA Classic Processing Forms.

- Centrifuge EDTA and serum tubes at 4°C at least at 2,000g x 15 minutes or 3,000g x 10 minutes for a total of 30,000 g-minutes. Immediately after tubes are centrifuged, carefully place them upright on wet ice in preparation for pooling and aliquoting.

- PAXgene RNA and 2 mL EDTA WB tubes. Do NOT centrifuge these tubes. Filled tubes may remain at room temperature until as soon as able to freeze upright in a -80°C freezer.

- For any MESA ancillary studies involving the collection of additional sample please refer to the study specific protocol for processing details. _Note_: _Sites collecting MESA Epigenomics - the CPT draw tubes are slightly taller than the standard 10 mL draw tube. It is important to check they fit the centrifuge buckets and can swing freely **BEFORE** starting the centrifuge._

E. Description of MESA 7 Aliquots:

MESA 7 Blood Aliquots:

Collection Tube	Sample Type	Number of Aliquot vials	Color Code	Volume per Aliquot Vial
5 x 10 mL EDTA draw tubes (#1-4 & 8)	Plasma (pooled)	42 (Cryos# 01-10 & 23-54)	Blue	0.5 mL plasma in 0.5 mL cryovials
		12 (Cryos# 11-22)	Blue	0.25 mL plasma in 0.5 mL cryovials
	pRBC	5 (Tube#75-79)	White	~5 mL pRBC in 10 mL transfer tubes
10 mL & 5 mL Serum draw tubes (#5 & #6)	Serum (pooled)	14 (Cryos# 55-56)	Red	0.5 mL serum in 0.5 mL cryovial
2 mL EDTA WB (#7)	(do NOT centrifuge)	(do NOT aliquot)		Remains in draw tube.
1 x PAXgene RNA (#9)	(do NOT centrifuge)	(do NOT aliquot)		Remains in draw tube.

ALIQUOTING: MESA Classic EDTA and Serum Tubes

- Aliquoting involves removing the serum or plasma in small amounts (e.g. 0.5 mL) by pipette and placing it into the appropriate labeled and color-coded cryovials. Color-coding is predetermined and used as part of the sample identification. If the correct color cap is not immediately available, be sure the sample type is clearly identified on the cryovial label. Correct cap color when possible.

- The aliquoting process must be done while the tubes and cryovials are on wet ice (unless otherwise noted).

- When pooling plasma from the five EDTA tubes (or pooling serum from the two serum tubes), be careful not to disturb the top of the cell layer with the pipette tip as this will result in platelet, white cell, and red cell contamination.

- Whenever pipetting from a draw tube, always use a new pipette tip for each draw tube.

- Always use a new pipette tip for each pooling tube.

- Pool like tubes from the same participant (i.e. pool the five 10 mL EDTA tubes from the same participant, and pool the two serum tubes from the same participant) before aliquoting.

- After pooling, and just before aliquoting, gently mix the pooled sample.

- Freeze all aliquots immediately (< 10 minutes) in an upright position at -80°C or colder. (Promptly place aliquots on dry ice for quick freezing if a -80°C freezer is not immediately available.)

- If any draw tubes are accidentally mixed during pipetting so that plasma is contaminated with red cells, the tubes may be re-centrifuged at the same speed and duration as the original spin.

- If there is insufficient sample volume within a sample type to make the full set of aliquots, fill the lowest numbered cryovials for that sample type first. Any partially filled (less than the specified volume) cryovial should be marked with a "P" on the cryovial label and a "P" in the comment field of the Processing Form next to that cryovial number. Note: Do not have multiple partially filled cryovials for a sample type. It is more important to have aliquots filled to their specified volume with a reduced number of total aliquots, then to have multiple partially filled aliquots.

Upon completion of the processing steps, aliquots must be frozen at -80°C or below within 10 minutes, or place immediately on dry ice. Make sure all cryovials are correctly labeled and are frozen in the upright position.

<u>EDTA plasma → Cryovials# 01-54</u>. Keep draw tubes, pooling tube, and cryovials on wet ice during processing. After centrifuging, pool plasma from the five 10 mL EDTA tubes in a labeled 50 mL tube (or other suitable pooling tube). After the plasma is removed from the draw tubes, pour remaining packed cells into each of appropriately labeled 10 mL transfer tubes. Gently invert the pooling tube containing the plasma several times to ensure it is thoroughly mixed. Aliquot, by the volumes specified in the table above, into cryovials# 01-54 and color-code with blue caps. Double check the specified sample volume is being aliquoted into the correctly labeled cryovial. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. After aliquoting is complete, discard the empty pooling tube in the biohazard waste container.

<u>Serum → Cryovials# 55-68</u>. Keep draw tubes, pooling tube, and cryovials cool on wet ice during processing. After centrifuging, pool serum from the 10 mL and 5 mL draw tubes in a 15 mL tube (or other suitable pooling tube). After the serum is removed from the draw tubes, the draw tubes with the remaining coagulated cells can be discarded in the appropriate biohazard waste container. Gently invert the pooling tube containing the serum several times to ensure thoroughly mixed. Aliquot 0.5 mL into cryovials# 55-68, and color-code with red caps. Double check the specified sample volume is being aliquoted into the correctly labeled cryovial. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. After aliquoting is complete, discard the empty pooling tube in the biohazard waste container.

<u>EDTA pRBC → # 75-79.</u> The packed red blood cells from the EDTA draw tubes are poured over into labeled transfer tubes with cryovial labels #s 75-79. Freeze transfer tubes in an upright position at -80°C or colder within 10 minutes of aliquoting. After pour over is complete, discard the empty draw tubes in the biohazard waste container.

<u>EDTA 2 mL WB → # 80.</u> The EDTA whole blood tube is **NOT** aliquoted (and **NOT** centrifuged). Double check the draw tube is labeled with its correct MESA barcode label – the

barcode label includes the 5-digit Biospecimen ID + 1-digit visit year '7' + the 2-digit repository identifier '80'. The sample remains in the draw tube, and is frozen upright in a -80°C freezer.

PAXgene RNA → # 81. The PAXgene RNA tube is **NOT** aliquoted (and **NOT** centrifuged). Double check the draw tube is labeled with its correct MESA barcode label – the barcode label includes the 5-digit Biospecimen ID + 1-digit visit year '7' + the 2-digit repository identifier '81'. The sample remains in the draw tube, and is frozen upright in a -80°C freezer.

NORTHWESTERN Specific Aliquot Instructions:

MESA 7 Blood Aliquots:

Collection Tube	Sample Type	Number of Aliquot vials	Color Code	Volume per Aliquot Vial
4 x 10 mL EDTA draw tubes (#1-4) and 1 x 6 mL (#7)	Plasma (pooled)	34 (Cryos# 01-10 & 23-54)	Blue	0.5 mL plasma in 0.5 mL cryovials
		12 (Cryos# 11-22)	Blue	0.25 mL plasma in 0.5 mL cryovials
	pRBC	3 (Tube#75, 76 & 79)	White	~3-5 mL pRBC in 10 mL transfer tubes
10 mL & 5 mL Serum draw tubes (#5 & #6)	Serum (pooled)	14 (Cryos# 55-56)	Red	0.5 mL serum in 0.5 mL cryovial
2 mL EDTA WB (#7)	(do NOT centrifuge)	(do NOT aliquot)		Remains in draw tube.
8 mL Heparin CPT	See MESA Epi MOP for details	See MESA Epi MOP for details	See MESA Epi MOP for details	See MESA Epi MOP for details
1 x PAXgene RNA (#9)	(do NOT centrifuge)	(do NOT aliquot)		Remains in draw tube.

ALIQUOTING: MESA Classic EDTA and Serum Tubes

- Aliquoting involves removing the serum or plasma in small amounts (e.g. 0.5 mL) by pipette and placing it into the appropriate labeled and color-coded cryovials. Color-coding is predetermined and used as part of the sample identification. If the correct color cap is not immediately available, be sure the sample type is clearly identified on the cryovial label. Correct cap color when possible.

- The aliquoting process must be done while the tubes and cryovials are on wet ice (unless otherwise noted).

- When pooling plasma from the five EDTA tubes, be careful not to disturb the top of the cell layer with the pipette tip as this will result in platelet, white cell, and red cell contamination.

- Whenever pipetting from a draw tube, always use a new pipette tip for each draw tube.

- Always use a new pipette tip for each pooling tube.

- Pool like tubes from the same participant (i.e. pool the five 10 mL EDTA tubes from the same participant) before aliquoting.

- After pooling, and just before aliquoting, gently mix the pooled sample.

- Freeze all aliquots immediately (< 10 minutes) in an upright position at -80°C or colder. (Promptly place aliquots on dry ice for quick freezing if a -80°C freezer is not immediately available.)

- If any draw tubes are accidentally mixed during pipetting so that plasma is contaminated with red cells, the tubes may be re-centrifuged at the same speed and duration as the original spin.

- If there is insufficient sample volume within a sample type to make the full set of aliquots, fill the lowest numbered cryovials for that sample type first. Any partially filled (less than the specified volume) cryovial should be marked with a "P" on the cryovial label and a "P" in the comment field of the Processing Form next to that cryovial number. Note: Do not have multiple partially filled cryovials for a sample type. It is more important to have aliquots filled to their specified volume with a reduced number of total aliquots, then to have multiple partially filled aliquots.

  Upon completion of the processing steps, aliquots must be frozen at -80°C or below within 10 minutes, or place immediately on dry ice. Make sure all cryovials are correctly labeled and are frozen in the upright position.

EDTA plasma → Cryovials# 01-46. Keep draw tubes, pooling tube, and cryovials on wet ice during processing. After centrifuging, pool plasma from the 4x10 mL and 1x6 mL EDTA tubes in a labeled 50 mL tube (or other suitable pooling tube). After the plasma is removed from the draw tubes, pour remaining packed cells into each of appropriately labeled 10 mL transfer tubes. Gently invert the pooling tube containing the plasma several times to ensure it is thoroughly mixed. Aliquot, by the volumes specified in the table above, into cryovials# 01-54 and color-code with blue caps. Double check the specified sample volume is being aliquoted into the correctly labeled cryovial. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. After aliquoting is complete, discard the empty pooling tube in the biohazard waste container.

Serum → Cryovials# 55-63. Keep draw tube and cryovials cool on wet ice during processing. After centrifuging carefully remove serum aliquots from draw tube (serum can be transferred to a transfer tube using a transfer pipette if helpful). Aliquot 0.5 mL into cryovials# 55-63, and color-code with red caps. Double check the specified sample volume is being aliquoted into the correctly labeled cryovial. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. After aliquoting is complete, discard the empty draw tube (and transfer tube if applicable) in the biohazard waste container.

EDTA pRBC → # 75-79. The packed red blood cells from the EDTA draw tubes 1, 2, and 7 are poured over into labeled transfer tubes with cryovial labels #s 75, 76, and 79. EDTA draw tubes 3 and 4 with packed red blood cells are given to the MESA Epi technician for further processing. Freeze transfer tubes in an upright position at -80°C or colder within 10 minutes of aliquoting. After pour over is complete, discard the empty draw tubes in the biohazard waste container.

EDTA 2 mL WB → # 80. The EDTA whole blood tube is **NOT** aliquoted (and **NOT** centrifuged). Double check the draw tube is labeled with its correct MESA barcode label – the barcode label includes the 5-digit Biospecimen ID + 1-digit visit year '7' + the 2-digit repository identifier '80'. The sample remains in the draw tube, and is frozen upright in a -80°C freezer.

PAXgene RNA → # 81. The PAXgene RNA tube is **NOT** aliquoted (and **NOT** centrifuged). Double check the draw tube is labeled with its correct MESA barcode label – the barcode label includes the 5-digit Biospecimen ID + 1-digit visit year '7' + the 2-digit repository identifier '81'. The sample remains in the draw tube, and is frozen upright in a -80°C freezer.

F. Special Circumstances

- EDTA and Serum cannot be processed within time limits of collection.
  If unable to centrifuge filled draw tubes within specified time limits following collection, process them as soon as possible. Clearly document the time of collection and centrifugation on the Processing Form. Keep the EDTA tubes upright and cool on wet ice, and serum tubes upright at room temperature until centrifugation.

- Serum and plasma cryovials cannot be frozen within 10 minutes of aliquoting.
  If cryovials cannot be frozen at -80°C or colder within 10 minutes of aliquoting, do it as soon as possible. They may be temporarily (< 2 hours) placed on dry ice (preferred, but be sure to keep the cryovials in an upright position). If cryovials are not frozen at -80 within 10 minutes of being aliquoted, record storage conditions, storage temperature, and length of time at that temperature on the Processing Form.

G. Processing Completion.

- Record, by checking in the boxes on the Processing Form, all cryovials that are filled. Note any partials. Check processing start times are recorded, and forms are completely filled out legibly in ink. Double check the Biospecimen ID listed on the Processing Form matches that listed on the Phlebotomy form for the same MESA participant.

- The MESA 7 Processing Forms are kept in a temporary file ready for easy access at time of packaging the frozen shipment to CBAL. Copies of the MESA 7 Processing Forms are enclosed with each shipment of samples to the CBAL. Upon receipt at CBAL, forms and samples are examined for monitoring and quality control purposes.

- Frozen cryovials #01-74 are packed into one 2" freezer box; use 10x10 (or 9x9 if available) box grid. Frozen transfer tubes and draw tubes are placed into one 3" freezer box with 4 participants per box; use 7x7 box grid. Place the provided MESA ID freezer box labels for each participant sample set on the front cover of the appropriate freezer boxes. Store these freezer boxes, with the samples, in a -80°C freezer until ready to ship to CBAL. (Refer to Appendix, Freezer Box Diagrams for shipping frozen samples to CBAL.)

- Wipe down all work areas with 10% bleach solution or approved biohazard disinfectant.

- Label and arrange cryovials in their proper racks for the next day's blood processing.

IV. SHIPPING – MESA CLASSIC - BLOOD AND URINE SAMPLES

A. General

In MESA Exam 7, sites ship frozen MESA 7 samples to CBAL every other week on a pre-arranged schedule – shipping schedule is being developed. Frozen samples are shipped only on Mondays or Tuesdays to CBAL by an overnight carrier (Federal Express is preferred).

B. Packaging Samples

Specimen shipping checklist:

- Frozen MESA 7 samples (blood and urine) – Cryovials# 01-74 in labeled 2" freezer boxes, and transfer tubes and draw tubes in labeled 3"freezer boxes.
- Styrofoam shipping containers with outer cardboard sleeve (supplied by CBAL).
- Rubber bands for freezer boxes.
- Zip top plastic bags for freezer boxes.
- Absorbent material in sufficient quantity to absorb the entire liquid contents of the package.
- Packaging tape.
- Dry ice (~10-15 lbs per shipping container)
- Shipping Labels (FedEx address labels)
- Category B labels (UN3373 BIOLOGICAL SUBSTANCE CATEGORY B)
- Dry Ice Labels (Dry Ice UN1845 class 9 – Miscellaneous Dangerous Goods Label)
- Completed MESA Processing Forms
- Completed Shipping Forms
- Completed MESA Blind Duplicate Shipping Form if any QC samples included in shipment.

C. Procedure

This shipping protocol follows procedures mandated by the International Air Transport Association's Dangerous Goods Regulations – Packaging Instructions 650 and 954.

For frozen shipments to the University of Vermont:

1. Line Styrofoam shipper(s) with absorbent material (i.e. absorbent pads).
2. Place approximately ½ the dry ice (~5-7 lbs) on the bottom of the shipping container.
3. Place another layer of absorbent material on top of the dry ice – so it will be between the dry ice and the zip top plastic bag enclosing freezer boxes containing samples.
4. Collect the freezer boxes containing samples to be shipped, and check the participant ID numbers against the Processing Forms and Shipping Forms for that shipment. Add MESA Lung hair follicle and nasal cell samples if not already included in participant cryovial box.
5. Place each freezer box (lid secured with a rubber band) in a leak-proof zip top plastic bag with an absorbent sheet, then carefully place these bagged boxes in the shipping container. The rubber band helps prevent freezer boxes from opening and spilling contents; the zip top bag serves as an additional form of containment, and the absorbent material is essential in the event of a thaw and spill.
6. Place another layer of absorbent material on top of the bagged freezer boxes containing the samples.
7. Add remaining dry ice on top of this last layer of absorbent material in the shipping container.
8. Place the Processing Forms for all the samples included in the shipment, along with a copy of the corresponding Shipping Form(s) in a zip top bag.
9. If there are any boxes of QC samples included in the shipment, the corresponding Blind Duplicate Shipping Form, listing the QC IDs, is placed in the zip top bag with the regular MESA Classic Shipping Form, and MESA Classic Processing Forms.

10. Place any bagged hair shaft samples collected in a zip top bag. Place both the hair shaft bag and form bag on top of the Styrofoam shipper lib before sealing the cardboard shipper.
10. Affix shipping label(s) to the shipping container. (Package samples as close to time of FedEx pick-up as possible to minimize the length of time on dry ice.)
11. E-mail notification of the shipment, including the FedEx airbill number(s) and number of participant sample sets shipped, the day samples are packaged to: Jessica.lanzer@med.uvm.edu , Elaine.Cornell@uvm.edu and Rebekah.Boyle@uvm.edu

    Mailing Address:
    University of Vermont,
    360 South Park Drive,
    Colchester, VT 05446
    Attn: Rebekah Boyle
    (802) 656-8938

V. QUALITY ASSURANCE

A. Overview of Field Center Monitoring

Quality assurance monitoring of the blood collection and processing is important for the identification of any deviations from the standardized methods. Differences in the manner of blood collection or processing could potentially create a statistically significant difference in assay results. In an effort to prevent any sample associated problems, a system, similar to previous MESA Exams, is being implemented to aid in monitoring the quality of blood collection and processing at each Field Center. Components of the quality assurance program for Field Centers are:

1. CBAL training course presented via Zoom for Phlebotomy and Processing and certification process for Field Center technicians,
2. Equipment maintenance/temperature logs at each Field Center,
3. Field Center Supervisor Checklist,
4. Sample Acknowledgement Forms.

Monitoring of these parameters will aid in identifying any systematic or random problems and appropriate corrective actions can be taken.

B. Field Center Technician Training & Certification

Standardization of venipuncture and blood processing procedures is of utmost importance for the quality of the blood samples and subsequent data analysis. CBAL will conduct a one-time training session on blood collection and processing of the MESA samples. This training session will be held via Zoom and will be record for future use. The training session will present information relating to the collection of the blood samples (i.e. infection control, safety precautions including OSHA regulations, handling equipment, venipuncture procedure and possible venipuncture problems), and proper processing procedures of the draw tubes, including mixing, centrifuging, time and temperature requirements, and aliquoting.

Field Center Technician Requirements:

- Field center technicians who will be performing blood collection for the MESA study must have prior clinical phlebotomy experience.

- Reading the MESA Manual of Operations before attending the CBAL training session is mandatory.
- Certification in MESA blood collection and processing is required before working with actual participants and blood samples.

Field Center Technician Certification:

Field center technicians who attend the CBAL training and successfully complete both the written and practical examinations will be certified in MESA blood collection & processing. (Completed written exams will be corrected and kept on file at the CBAL.) Fully certified technicians are qualified to certify other technicians at their site in the complete or partial process with final approval from the CBAL.

The steps for certification are:

1. Read and understand the appropriate chapters in the MESA 7 Lab Manual of Operations.
2. Successful completion of the written exam (prepared by CBAL).
3. Successful completion of the practical exam (using the Supervisor Checklist in the Manual) which requires observation by certified personnel of the phlebotomy/processing procedure completed on a volunteer.

C. Field Center Equipment Records:

Each Field Center is responsible for the maintenance of daily and monthly records for equipment performance. Daily temperature readings on refrigerators, freezers, and refrigerated centrifuges are recorded on individual equipment temperature logs. Any temperature deviations are to be addressed. Equipment temperature logs are filed on site for future reference and reported to the CBAL quarterly per year. These equipment records can identify potential concerns with sample quality in the processing and local storage steps.

D. Field Center Supervisor Checklist:

The Field Center Supervisor Checklist serves as a monitoring measure. Each Field Center Supervisor is required to observe the MESA technicians in the performance of the phlebotomy and processing procedures, recording their observation on the checklist. For the first three months, complete checklists each month per technician, then at least once every other month for the remainder of the study (see schedule below).

Completed Supervisor Checklists are sent to the CBAL for monitoring purposes.

MESA 6 – Supervisor Checklist Schedule											
Month	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Nov	Dec	Jan
Complete Checklist		X	X	X		X		X		X		X

E. Maintaining Certification

A technician should perform phlebotomy and/ or processing on a minimum of one participant, every month in order to maintain certification.

F. Field Center Acknowledgement Forms

Sample Acknowledgement Forms are generated by the CBAL and e-mailed to the sites after each field center’s shipment of samples is received and processed by the CBAL. These forms provide feedback to

the sites, serving as a tool to track possible problems or deviations from protocol. Shipment condition, any concerns noticed after review of the Shipping Forms and Processing Forms, and physical inspection of the specimens, are summarized on the Sample Acknowledgement Form.

G. Blind Duplicates

- A blind duplicate sample for quality control will be reserved from ~20% of participants (10% each for EDTA and serum). Participants selected for EDTA QC activity will automatically be selected for urine QC. Participants selected for serum QC activity will automatically be selected for hair and nasal brushing QC.
- Participants will be selected for QC purposes based on the last digit of their **7-digit MESA participant ID number**. Participants whose last digit of their MESA ID number is "6" will be selected for EDTA and urine QC activity. For serum, hair, and nasal brushing QC activity, participants are selected whose last digit of their **7- digit MESA participant ID number** is "7".
- Participants to be selected for blind duplicate purposes will have pre-assigned QC ID numbers. Blind duplicate labels for the appropriate QC sample type(s) for a participant will be provided.
- EDTA QC activity:
  After filling EDTA repository Cryovials# 01-04, aliquot 0.5 mL of EDTA plasma into a 0.5 mL cryovial labeled with the participant's appropriate blind duplicate label. After the Blind Duplicate cryovial is aliquoted, continue aliquoting the remaining EDTA repository cryovials starting with Cryovial# 05. EDTA blind duplicate cryovials are color coded with blue caps and the last two digits of the 8-digit barcoded QC cryovial number are "01".
- Urine QC activity:
  Participants selected for EDTA QC activity are automatically selected for urine QC. After filling urine repository Cryovials# 69-70, aliquot 1.5 mL urine into a 2.0 mL cryovial labeled with the participant's urine blind duplicate label. Resume aliquoting the remaining urine repository cryovials starting with # 71. Urine QC cryovials are color-coded with yellow caps and the last two digits of the 8-digit barcoded QC cryovial number are "03".
- Serum QC activity:
  After filling serum repository Cryovials# 55-58, aliquot 0.5 mL serum into a 0.5 mL cryovial labeled with the participant's appropriate blind duplicate label. Once the Blind Duplicate cryovial is filled, continue aliquoting the remaining serum repository cryovials starting with # 59. Serum blind duplicate cryovials are color-coded with red caps, and the last two digits of the 8-digit barcoded QC cryovial number are "02".
- Freeze filled blind duplicate cryovials immediately (within 10 minutes of aliquoting), and be sure they are frozen in the upright position.
- Place blind duplicate samples (from multiple participants and all QC types) in a 2" freezer box (with a 9x9 or 10x10 box grid). Complete the Blind Duplicate Shipping Form listing the cryovials in the order they are loaded in the freezer box and include the freezer box in the frozen shipment of MESA Classic samples to CBAL according to instructions listed in section "**IV. Shipping – MESA Classic - Blood And Urine Samples**".

VI **APPENDIX**:

1. MESA Exam 7 Phlebotomy Form
2. MESA Exam 7 Processing Form
3. MESA Exam 7 Shipping Form
4. MESA Exam 7 Blind Duplicate Shipping Form
5. MESA Exam 7 Label Set (example)
6. MESA Barcode Label Orientation Diagram
7. Aliquoting Scheme Flow Chart & Processing Guides (Blood & Urine)
8. Freezer Box Diagram for Shipping Samples
9. Field Center Equipment Temperature Logs
10. Field Center Supervisor Checklist

APPENDIX Item 1: MESA Exam 7 Phlebotomy Form (Urine /Phlebotomy/Hair)

MESA Exam 7

Biosample Collection

Participant ID #: ☐☐☐☐☐☐☐ Acrostic: ☐☐☐☐☐☐☐

Phlebotomist ID: ☐☐☐☐ Date: ☐☐ / ☐☐ / ☐☐☐☐ (Month / Day / Year)

QC ID:

Biosample ID: ☐☐☐☐☐

PARTICIPANT QUESTIONS

	Yes	No	Don't know
1. Do you bleed or bruise easily?	○	○	○
2. Have you ever been told you have a disorder relating to blood clotting or coagulation?	○	○	○
3. Have you ever experienced fainting spells while having blood drawn?	○	○	○
4. Do you have diabetes for which you take insulin or oral hypoglycemics?	○	○	○

PROCEDURE

5. Time at start of urine collection: ☐☐ : ☐☐ (Hr : Min) ○ AM ○ PM

6. Was urine sample filled?

- ○ Yes → Volume ☐ *mL* (*min 50 mL*) *Skip to #7*
- ○ No → Why was urine sample not taken?
  - ○ Participant unable to void
  - ○ Refused
  - ○ Other: ☐
- ○ Partial → ☐☐ mL

7. Time at start of venipuncture: ☐☐ : ☐☐ (Hr : Min) ○ AM ○ PM

8. Was any blood drawn?

- ○ Yes, full sample
- ○ Yes, partial sample
- ○ No, refused
- ○ No, hard to stick
- ○ No, other: ☐

9. Elapsed time until tourniquet released: ☐☐☐ seconds *(120-seconds optimum)*

Exam 7

Biosample Collection

10. Time at end of venipuncture: ☐☐ : ☐☐ (Hr, Min) ○ AM ○ PM

11. Quality of venipuncture: ○ Traumatic ○ Clean

Mark all that apply
- ○ Vein collapsed
- ○ Excessive duration of draw
- ○ Vein hard to get at
- ○ Hematoma
- ○ Multiple sticks
- ○ Leakage at venipuncture site

If tube is not full, but is at least half full, please indicate "Partial" and enter the volume to the nearest mL.

Q12 for Wake, Columbia, Hopkins, Minnesota, UCLA. NWU skip to Q13.

12. volume per tube:

	Filled: Yes	Filled: No	Filled: Partial	Specify volume (mL): *min 1/2 full*
a. EDTA 10 mL	○	○	○	☐
b. EDTA 10 mL	○	○	○	☐
c. EDTA 10 mL	○	○	○	☐
d. EDTA 10 mL	○	○	○	☐
e. Serum 10 mL	○	○	○	☐
f. Serum 5 mL	○	○	○	☐
g. EDTA 2 mL	○	○	○	☐
h. EDTA 10 mL	○	○	○	☐
i. PAXgene 2.5 mL	○	○	○	☐

(continued)

Exam 7

Biosample Collection

For NWU only:

13. Blood volume per tube:

	Filled: Yes	Filled: No	Filled: Partial	Specify volume (mL): *min 1/2 full*
a. EDTA 10 mL	○	○	○	☐
b. EDTA 10 mL	○	○	○	☐
c. EDTA 10 mL	○	○	○	☐
d. EDTA 10 mL	○	○	○	☐
e. Serum 10 mL	○	○	○	☐
f. EDTA 2 mL	○	○	○	☐
g. EDTA 6 mL	○	○	○	☐
h. CPT 8 mL	○	○	○	☐
i. PAXgene 2.5 mL	○	○	○	☐

Hair Collection

14. Was hair collection completed?

○ Yes →

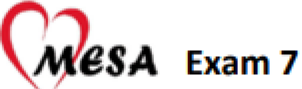
○ No

14a. How many follicles were collected (1-10)? ☐☐

14b. How many shafts were collected (1-10)? ☐☐

14c. Location of hair collection:

- ○ Front or head
- ○ Left side
- ○ Right side
- ○ Top
- ○ Back



Exam 7

Biosample Collection

15. Is the participant selected as a quality control subject for serum, hair, and nasal brushing?

- ○ NO
- ○ YES →

	NO	YES
Was serum QC sample collected?	○	○
Was hair QC sample collected?	○	○
Was nasal brushing QC sample collected?	○	○

Biosample QC ID: ☐☐☐☐☐

16. Is the participant selected as a quality control subject for EDTA and urine?

- ○ NO
- ○ YES →

	NO	YES
Was EDTA QC sample collected?	○	○
Was urine QC sample collected?	○	○

Biosample QC ID: ☐☐☐☐☐

Comments: ____

APPENDIX Item 2: MESA Exam 7 Processing Form (Classic)

Version 2/23/2022

MESA Exam 7 Classic Processing Form

MESA PPT ID: _____________

Processor ID:_________

Date: ______/______/_________

Biospecimen ID Label

Processing Start Time: _____:_____ EDTA | Processing Start Time: _____:_____ Serum

Cryo #	Type	Color	Sample Vol	Check if Done	Comment*	Cryo #	Type	Color	Sample Vol	Check if Done	Comment*
1	EDTA	B	0.5 mL			43	EDTA	B	0.5 mL		
2	EDTA	B	0.5 mL			44	EDTA	B	0.5 mL		
3	EDTA	B	0.5 mL			45	EDTA	B	0.5 mL		
4	EDTA	B	0.5 mL			46	EDTA	B	0.5 mL		
5	EDTA	B	0.5 mL			47	EDTA	B	0.5 mL		
6	EDTA	B	0.5 mL			48	EDTA	B	0.5 mL		
7	EDTA	B	0.5 mL			49	EDTA	B	0.5 mL		
8	EDTA	B	0.5 mL			50	EDTA	B	0.5 mL		
9	EDTA	B	0.5 mL			51	EDTA	B	0.5 mL		
10	EDTA	B	0.5 mL			52	EDTA	B	0.5 mL		
11	EDTA	B	0.25 mL			53	EDTA	B	0.5 mL		
12	EDTA	B	0.25 mL			54	EDTA	B	0.5 mL		
13	EDTA	B	0.25 mL			55	Serum	R	0.5 mL		
14	EDTA	B	0.25 mL			56	Serum	R	0.5 mL		
15	EDTA	B	0.25 mL			57	Serum	R	0.5 mL		
16	EDTA	B	0.25 mL			58	Serum	R	0.5 mL		
17	EDTA	B	0.25 mL			59	Serum	R	0.5 mL		
18	EDTA	B	0.25 mL			60	Serum	R	0.5 mL		
19	EDTA	B	0.25 mL			61	Serum	R	0.5 mL		
20	EDTA	B	0.25 mL			62	Serum	R	0.5 mL		
21	EDTA	B	0.25 mL			63	Serum	R	0.5 mL		
22	EDTA	B	0.25 mL			64	Serum	R	0.5 mL		
23	EDTA	B	0.5 mL			65	Serum	R	0.5 mL		
24	EDTA	B	0.5 mL			66	Serum	R	0.5 mL		
25	EDTA	B	0.5 mL			67	Serum	R	0.5 mL		
26	EDTA	B	0.5 mL			68	Serum	R	0.5 mL		
27	EDTA	B	0.5 mL			69	Urine	Y	1.5 mL		
28	EDTA	B	0.5 mL			70	Urine	Y	1.5 mL		
29	EDTA	B	0.5 mL			71	Urine	Y	1.5 mL		
30	EDTA	B	0.5 mL			72	Urine	Y	1.5 mL		
31	EDTA	B	0.5 mL			73	Urine	Y	1.5 mL		
32	EDTA	B	0.5 mL			74	Urine	Y	1.5 mL		
33	EDTA	B	0.5 mL			75	pRBC	W	~5		
34	EDTA	B	0.5 mL			76	pRBC	W	~5		
35	EDTA	B	0.5 mL			77	pRBC	W	~5		
36	EDTA	B	0.5 mL			78	pRBC	W	~5		
37	EDTA	B	0.5 mL			79	pRBC	W	~5		
38	EDTA	B	0.5 mL			80	EDTA WB	DT	2 mL		
39	EDTA	B	0.5 mL			81	PAXgene RNA	DT	~9 mL		
40	EDTA	B	0.5 mL			82	Hair Follicles	G	N/A		
41	EDTA	B	0.5 mL			83	Hair Shafts	N/A	N/A		
42	EDTA	B	0.5 mL			84	Nasal Cells	G	0.7 mL		

* P = partial volume

B=Blue, R=Red, Y=yellow, W=White, G=Green, DT=Draw Tube

Comments:

____

____

____

LCBR: ________________ *Frozen: Y N*

Mesa7ClassicProcessingForm_022322

APPENDIX Item 2 Continued: MESA Exam 7 Processing Form (Northwestern)

Version 2/23/2022

MESA Exam 7 Northwestern Processing Form

MESA PPT ID: ______________

Processor ID:_________

Date: ______/______/__________

Biospecimen ID Label

Processing Start Time: _____:______ EDTA | Processing Start Time: _____:______ Serum

Cryo #	Type	Color	Sample Vol	Check if Done	Comment*	Cryo #	Type	Color	Sample Vol	Check if Done	Comment*
1	EDTA	B	0.5 mL			39	EDTA	B	0.5 mL		
2	EDTA	B	0.5 mL			40	EDTA	B	0.5 mL		
3	EDTA	B	0.5 mL			41	EDTA	B	0.5 mL		
4	EDTA	B	0.5 mL			42	EDTA	B	0.5 mL		
5	EDTA	B	0.5 mL			43	EDTA	B	0.5 mL		
6	EDTA	B	0.5 mL			44	EDTA	B	0.5 mL		
7	EDTA	B	0.5 mL			45	EDTA	B	0.5 mL		
8	EDTA	B	0.5 mL			46	EDTA	B	0.5 mL		
9	EDTA	B	0.5 mL			55	Serum	R	0.5 mL		
10	EDTA	B	0.5 mL			56	Serum	R	0.5 mL		
11	EDTA	B	0.25 mL			57	Serum	R	0.5 mL		
12	EDTA	B	0.25 mL			58	Serum	R	0.5 mL		
13	EDTA	B	0.25 mL			59	Serum	R	0.5 mL		
14	EDTA	B	0.25 mL			60	Serum	R	0.5 mL		
15	EDTA	B	0.25 mL			61	Serum	R	0.5 mL		
16	EDTA	B	0.25 mL			62	Serum	R	0.5 mL		
17	EDTA	B	0.25 mL			63	Serum	R	0.5 mL		
18	EDTA	B	0.25 mL			69	Urine	Y	1.5 mL		
19	EDTA	B	0.25 mL			70	Urine	Y	1.5 mL		
20	EDTA	B	0.25 mL			71	Urine	Y	1.5 mL		
21	EDTA	B	0.25 mL			72	Urine	Y	1.5 mL		
22	EDTA	B	0.25 mL			73	Urine	Y	1.5 mL		
23	EDTA	B	0.5 mL			74	Urine	Y	1.5 mL		
24	EDTA	B	0.5 mL			75	pRBC	W	~5		
25	EDTA	B	0.5 mL			76	pRBC	W	~5		
26	EDTA	B	0.5 mL			79	pRBC	W	~3		
27	EDTA	B	0.5 mL			80	EDTA WB	DT	2 mL		
28	EDTA	B	0.5 mL			81	PAXgene RNA	DT	~9 mL		
29	EDTA	B	0.5 mL			82	Hair Follicles	G	N/A		
30	EDTA	B	0.5 mL			83	Hair Shafts	N/A	N/A		
31	EDTA	B	0.5 mL			84	Nasal Cells	G	0.7 mL		
32	EDTA	B	0.5 mL								
33	EDTA	B	0.5 mL								
34	EDTA	B	0.5 mL								
35	EDTA	B	0.5 mL								
36	EDTA	B	0.5 mL								
37	EDTA	B	0.5 mL								
38	EDTA	B	0.5 mL								

* P = partial volume

B=Blue, R=Red, Y=yellow, W=White, G=Green, DT=Draw Tube

Comments:

____

____

____

LCBR ________________ *Frozen: Y N*

Mesa7_NWUProcessingForm

APPENDIX Item 3: MESA Exam 7 Shipping Form

MESA EXAM 7 - SHIPPING FORM

Date of shipment: ____________
Center: ______________________ Prepared by: ____________

FedEx Air Bill#: ____

(Include in shipment AND e-mail: Jessica.lanzer@med.uvm.edu, Elaine.Cornell@uvm.edu, and Rebekah.Boyle@uvm.edu)

MESA ID#	VISIT DATE	BOX #

MESA_Exam7_ ShippingForm

APPENDIX Item 4: MESA Exam 7 Blind Duplicate Shipping Form

MESA EXAM 7: - BLIND DUPLICATE SHIPPING FORM

Date of shipment: _______________

Center: ________________________ Prepared by: _____________

FedEx Air Bill#: ____

(Include in shipment AND e-mail shipment notification to: Jessica.lanzer@med.uvm.edu, Elaine.Cornell@uvm.edu, and Rebekah.Boyle@uvm.edu)

Blind Dupe / QC ID	Sample Type	Color (Blue, Red, Yellow)

MESA7_BlindDupe_ShippingForm

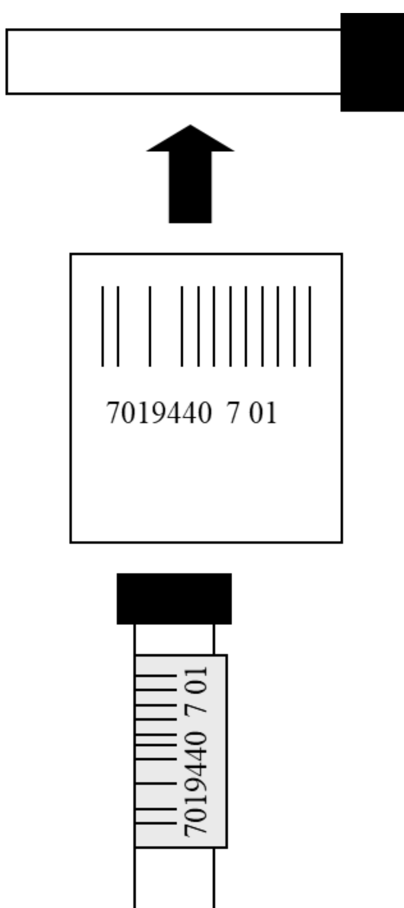
APPENDIX Item 5: MESA Exam 7 Label Set

Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 01	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 02	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 03	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 04	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 05	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 06	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 07	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 08
Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 09	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 10	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 11	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 12	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 13	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 14	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 15	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 16
Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 17	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 18	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 19	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 20	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 21	Apply this end first MESA Exam 7 EDTA 0.25mL 38801 7 22	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 23	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 24
Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 25	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 26	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 27	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 28	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 29	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 30	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 31	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 32
Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 33	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 34	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 35	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 36	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 37	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 38	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 39	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 40
Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 41	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 42	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 43	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 44	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 45	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 46	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 47	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 48
Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 49	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 50	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 51	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 52	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 53	Apply this end first MESA Exam 7 EDTA 0.5mL 38801 7 54	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 55	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 56

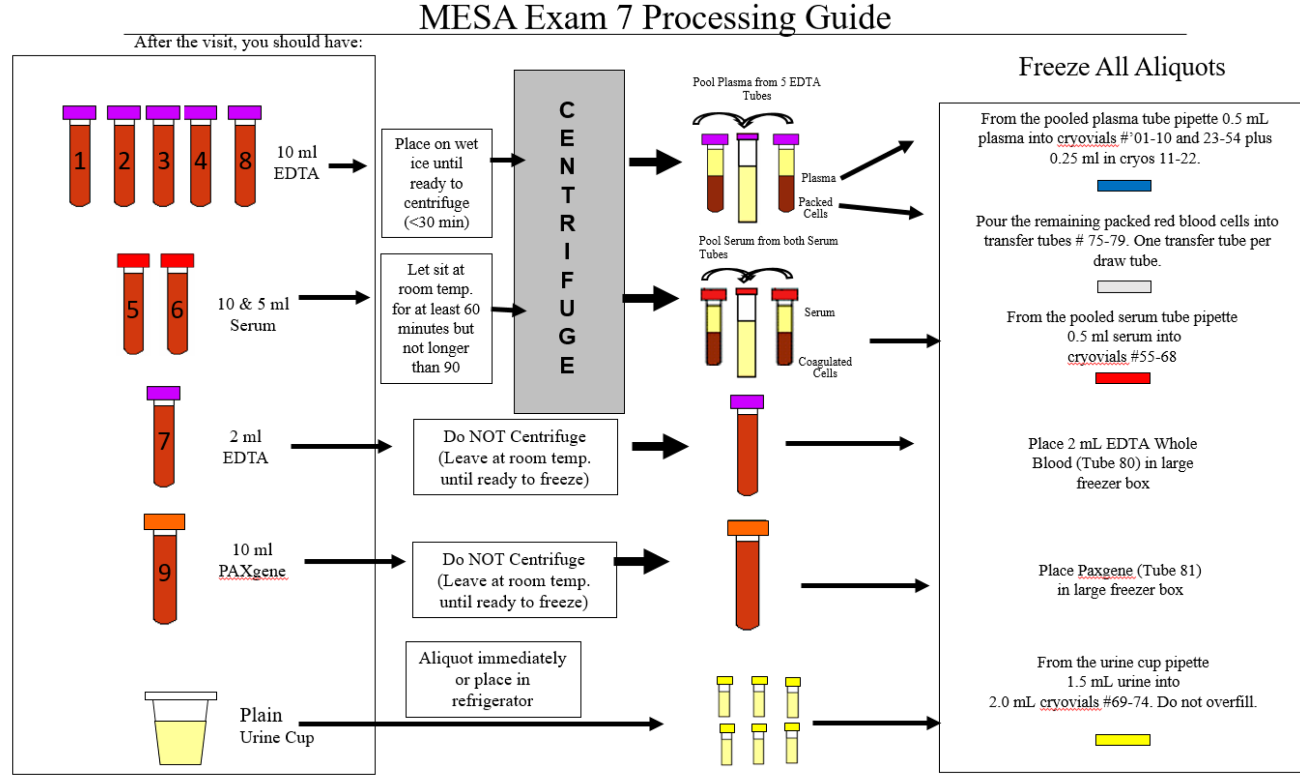
Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 57	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 58	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 59	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 60	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 61	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 62	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 63	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 64
Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 65	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 66	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 67	Apply this end first MESA Exam 7 Serum 0.5mL 38801 7 68	Apply this end first MESA Exam 7 Urine 1.5mL 38801 7 69	Apply this end first MESA Exam 7 Urine 1.5mL 38801 7 70	Apply this end first MESA Exam 7 Urine 1.5mL 38801 7 71	Apply this end first MESA Exam 7 Urine 1.5mL 38801 7 72
Apply this end first MESA Exam 7 Urine 1.5mL 38801 7 73	Apply this end first MESA Exam 7 Urine 1.5mL 38801 7 74	Apply this end first MESA Exam 7 pRBC 38801 7 75	Apply this end first MESA Exam 7 pRBC 38801 7 76	Apply this end first MESA Exam 7 pRBC 38801 7 77	Apply this end first MESA Exam 7 pRBC 38801 7 78	Apply this end first MESA Exam 7 pRBC 38801 7 79	Apply this end first MESA Exam 7 EDTA WB (Draw) 38801 7 80
Apply this end first MESA Exam 7 PAXgene RNA (Draw) 38801 7 81	Apply this end first MESA Exam 7 Hair Follicles 38801 7 82	Apply this end first MESA Exam 7 Hair Shafts 38801 7 83	Apply this end first MESA Exam 7 Nasal Cells 38801 7 84				

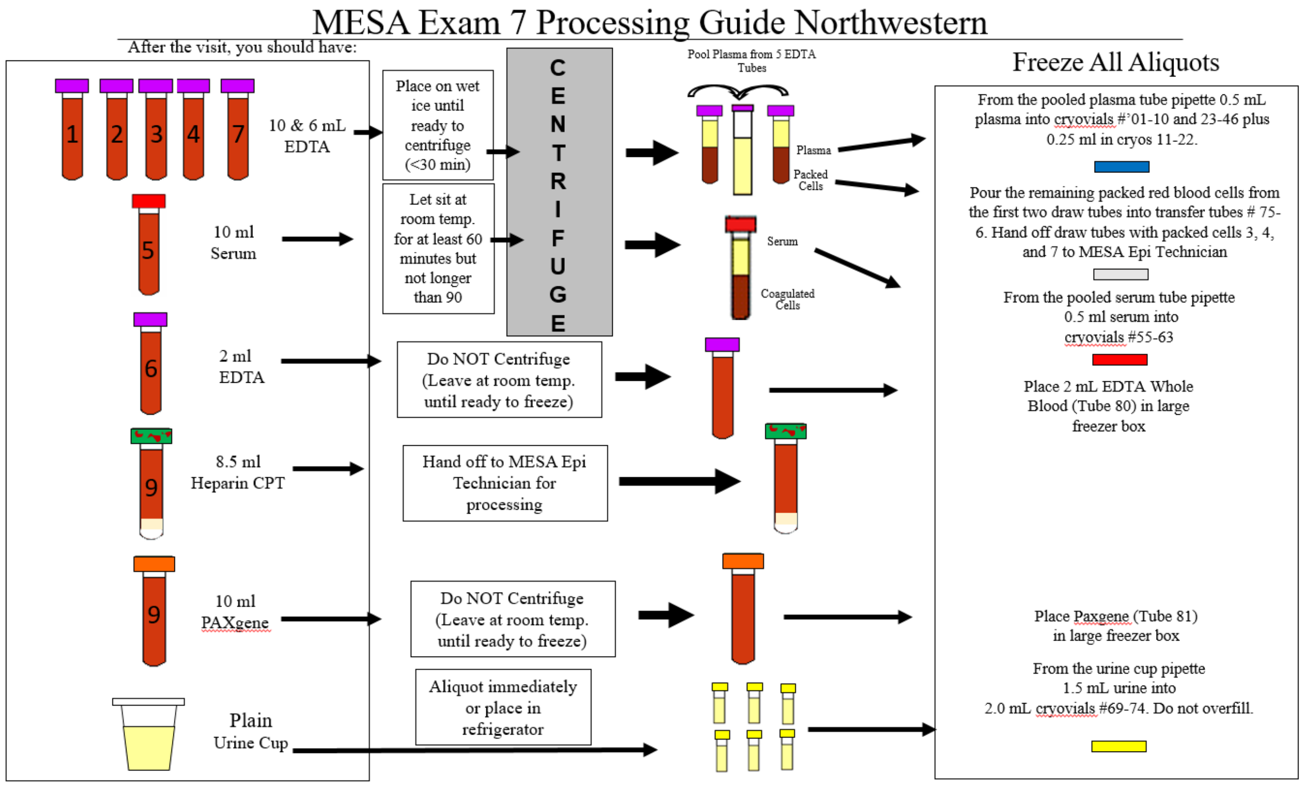
Apply this end first
MESA
Exam 7
Phleb Form
38801
Apply this end first
MESA
Exam 7
Process Form
38801
Apply this end first
MESA
Exam 7
Spirometry Form
38801
Apply this end first
MESA
Exam 7
EDTA (10mL)
38801
Apply this end first
MESA
Exam 7
EDTA (10mL)
38801
Apply this end first
MESA
Exam 7
EDTA (10mL)
38801
Apply this end first
MESA
Exam 7
EDTA (10mL)
38801
Apply this end first
MESA
Exam 7
Serum (10mL)
38801
Apply this end first
MESA
Exam 7
Serum (5mL)
38801
Apply this end first
MESA
Exam 7
EDTA (10mL)
38801
Apply this end first
MESA
Exam 7
Urine Collection
38801
Apply this end first
MESA
Exam 7
EDTA Pooling Tube
38801
Apply this end first
MESA
Exam 7
Serum Pooling Tube
38801
Apply this end first
MESA
Exam 7
Shipping Form
38801
Apply this end first
MESA
Exam 7
Freezer Box
38801
Apply this end first
MESA
Exam 7
Freezer Box
38801
Apply this end first
MESA
Exam 7
Freezer Box
38801
Apply this end first
MESA
Exam 7
(extra)
38801
Apply this end first
MESA
Exam 7
(extra)
38801
Apply this end first
MESA
Exam 7
(extra)
38801
Apply this end first
MESA
Exam 7
(extra)
38801

APPENDIX Item 6: MESA Barcode Label Orientation Diagram



APPENDIX Item 7: Aliquoting Scheme Flow Chart & Processing Guides (Blood & Urine)





APPENDIX Item 8: Freezer Box Diagram for Shipping Samples

Cryovial Box

Cryo '01	Cryo '10	Cryo '19	Cryo '28	Cryo '37	Cryo '46	Cryo '55	Cryo '64	Cryo '69	
Cryo '02	Cryo '11	Cryo '20	Cryo '29	Cryo '38	Cryo '47	Cryo '56	Cryo '65	Cryo '70	
Cryo '03	Cryo '12	Cryo '21	Cryo '30	Cryo '39	Cryo '48	Cryo '57	Cryo '66	Cryo '71	
Cryo '04	Cryo '13	Cryo '22	Cryo '31	Cryo '40	Cryo '49	Cryo '58	Cryo '67	Cryo '72	
Cryo '05	Cryo '14	Cryo '23	Cryo '32	Cryo '41	Cryo '50	Cryo '59	Cryo '68	Cryo '73	
Cryo '06	Cryo '15	Cryo '24	Cryo '33	Cryo '42	Cryo '51	Cryo '60		Cryo '74	
Cryo '07	Cryo '16	Cryo '25	Cryo '34	Cryo '43	Cryo '52	Cryo '61			
Cryo '08	Cryo '17	Cryo '26	Cryo '35	Cryo '44	Cryo '53	Cryo '62		Cryo '82	
Cryo '09	Cryo '18	Cryo '27	Cryo '36	Cryo '45	Cryo '54	Cryo '63		Cryo '84	

Tall Tube Box

PPT 1 Tube '81		PPT 2 Tube '81		PPT 3 Tube '81		PPT 4 Tube '81
PPT 1 Tube '80		PPT 2 Tube '80		PPT 3 Tube '80		PPT 4 Tube '80
PPT 1 Tube '75		PPT 2 Tube '75		PPT 3 Tube '75		PPT 4 Tube '75
PPT 1 Tube '76		PPT 2 Tube '76		PPT 3 Tube '76		PPT 4 Tube '76
PPT 1 Tube '77		PPT 2 Tube '77		PPT 3 Tube '77		PPT 4 Tube '77
PPT 1 Tube '78		PPT 2 Tube '78		PPT 3 Tube '78		PPT 4 Tube '78
PPT 1 Tube '79		PPT 2 Tube '79		PPT 3 Tube '79		PPT 4 Tube '79

APPENDIX Item 9: Field Center Equipment Temperature Logs

MESA Exam 7 - Field Center Equipment Temperature Log

Equipment: ________________________________ Year: ____________________

Equipment ID#: _____________________________ Site: _____________________

Month: ____________			
Date	Temp °C	Tech	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

Month: ____________			
Date	Temp °C	Tech	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
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30			
31			

APPENDIX Item 10: Field Center Supervisor Checklist - Phlebotomy

MESA Exam 7 Phlebotomy - Supervisor Checklist

DATE: ☐ mo ☐ day ☐ year

Field Center: ☐

Technician Name/ID: ☐

Supervisor: ☐

Please check the appropriate box if technician performance is satisfactory for each line item. Please note any comments or remedial action taken in 'Comments' section if performance was not satisfactory.

Preparation:

1. ☐ Phlebotomy area properly prepared and stocked with supplies (tube rocker, ice bucket, extra draw tubes & labels, etc.).
2. ☐ Phlebotomy Form is correct Blood Draw Type based on participant's consent.
3. ☐ Draw tubes labeled with participant ID and in the correct order for the Blood Draw Type.
4. ☐ Questions on Phlebotomy Form asked and answers recorded.

Venipuncture:

5. ☐ Script properly delivered
6. ☐ Non-permeable lab coat, gloves, and face shields used.
7. ☐ Correct preparation of venipuncture site.
8. ☐ Venipuncture smoothly executed.
9. ☐ Tubes filled in correct draw tube priority order.
10. ☐ Any replacement tubes correctly labeled.
11. ☐ Tourniquet released within 2 minutes; tourniquet maybe reapplied if necessary.
12. ☐ Proper appropriate care of venipuncture site after needle is removed.
13. ☐ Needle & tubing appropriately disposed.

Handling of filled draw tubes:

14. ☐ The correct tubes inverted and placed on the rocker for the time limits specified in the MOP.
15. ☐ Filled tubes placed in the correct racks - on ice or at room temperature – ASAP per MOP.
16. ☐ EDTA or Serum tubes <1/2 full discarded

P/P Form:

17. ☐ Check correct Participant ID barcode labels are on both Phlebotomy and Processing forms.
18. ☐ Venipuncture starts and end times legibly recorded on the Phlebotomy form.
19. ☐ Elapsed tourniquet time(s) noted on form (if reapplied, note additional elapsed tourniquet times).
20. ☐ Form completely filled out, and any comments recorded in the Comments section.

Urine:

22. ☐ Urine collection container correctly labeled and urine section on Phlebotomy Form completed.

Comments: ______________________________

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Supervisor Signature ______________________________

APPENDIX Item 10 con't: Field Center Supervisor Checklist – Processing

MESA Exam 7 Laboratory Processing - Supervisor Checklist

DATE: ☐ ☐ ☐ **Field Center:** ________

mo **day** **year**

Technician Name/ID: ________

Supervisor: ________

Please check the appropriate box if technician performance is satisfactory for each line item. Please note any comments or remedial action taken in 'Comments' section if performance was not satisfactory.

Preparation:

1. ☐ Aliquot racks organized and cryovials checked that they are correctly labeled. Blind duplicate aliquot cryovials labeled when appropriate.
2. ☐ Personal protective equipment in use (Non-permeable lab coats, gloves, and face shields used).

Blood Processing:

3. ☐ Time checked to ensure tubes are processed within the correct time limits post venipuncture per protocol.
4. ☐ Equipment is checked to ensure all tubes requiring centrifuging are centrifuged at the correct temperature and speed.
5. ☐ All EDTA plasma pooled before aliquoting into correctly labeled and color-coded cryovials.
6. ☐ Serum tubes pooled before aliquoting into correctly labeled and color-coded cryovials (no serum pooling step at Northwestern).
7. ☐ New pipet tip used for each Participant's sample type and aliquots kept on ice during aliquoting.
8. ☐ Filled cryovials checked off on the Processing Form and frozen upright @ -80 °C within 10 minutes. Partial (< specified vol) cryovials are marked with a "P" on the label and Processing Form.

Processing Completion:

9. ☐ Urine is kept refrigerated until aliquoting into correctly labeled tubes.
10. ☐ Processing area and equipment is cleaned with appropriate disinfectant.
11. ☐ Processing Form completely filled out, including recording all blood and urine aliquots obtained and if any are less than the required volume (partials). Any comments noted in comment section.

Comments: ____

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Supervisor Signature ____