

### **3.6 Laboratory**

Most of the procedures and laboratory equipment and supplies used in Exam 4 are similar to those in Exam 3. Only some important components or those that have changed are re-iterated here. Please refer to the Exam 1 and Exam 2 MOP for a detailed list. In addition, any component new in Exam 4 will also be described.

#### **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.

MESA involves the collection of 49.5 mls of blood from participants at Exam 4.

#### **II. EQUIPMENT & SUPPLIES**

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

5 ml SCAT-I tubes (1 per participant). Must be stored refrigerated until used.  
Cryovials – 0.5 ml , 1.5 ml, and 2 ml with color-coded caps

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 (21 G) with luer adapter (B-D # 7251)  
Vacutainer barrels  
Tourniquets  
Alcohol prep pads  
Gauze (2x2)  
Surgical tape - paper tape (easier on participants)  
Band-Aids  
Blood collection tubes (keep extras on hand):

2 – 10 ml Serum tubes (Fisher Scientific #22-301-710, Monoject # 8881301710)  
2 – 10 ml EDTA tubes (Fisher Scientific # 22-311-743, Monoject # 8881311743)  
1 – 4.5 ml Citrate tubes (Fisher Scientific # 22-029-309, Monoject # 8881340486)  
1 – 5 ml SCAT-I\*

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For Blind Duplicate Samples: 5 ml Serum (Fisher Scientific # 22-239-324, Monoject # 8881301413),  
5 ml EDTA (Fisher Scientific # 22-029-325, Monoject # 8881311446),  
4.5 ml Citrate (Fisher Scientific # 22-029-309, Monoject # 8881340486),  
5 ml SCAT-I\*

Blood tube rocker  
Blood tube racks  
Ice bucket and crushed ice - filled 10 min before draw  
Stopwatches or timers (ex. Fisher Scientific # 06-662-9)  
Scissors  
Pens  
Labels  
Phlebotomy / Processing Form  
Blood Spill Kit  
Biohazardous waste container  
Needle/sharps container

\* provided by CBAL

### **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, 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.

Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak-proof container. Never recap or break needles.

Hepatitis B vaccine be offered to all unvaccinated technicians handling blood, and documentation of vaccination, or technician's declining to be vaccinated, should be kept on file at the Clinical Center.

#### 2. Participant ID Labels

The Coordinating Center will supply each field center with sheets of sample ID barcode labels to use for labeling draw tubes, working tubes, cryovials, and freezer boxes. There will be a total of 34 labels: 6 labels for the draw tubes plus 3 extra labels, 2 labels for two pooling tubes, and 23 cryovial labels. Each participant set of barcode labels has the same 7-digit sample ID number (the first digit identifies the clinic – Wake Forest = 3, Columbia = 4, John's Hopkins = 5, UMin = 6, Northwestern = 7, UCLA = 8). On the labels for the cryovials the digit '4' which follows the 7-digit ID number, indicates Exam year 4. The labels for the cryovials also have a 2-digit cryovial

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number (01 to 23) that serves as a unique identifier for each cryovial within a sample ID – this is critical in tracking the repository. See Appendix for proper orientation of the barcode label on the cryovial. The extra labels can be used for labeling the freezer boxes, etc.

There will also be special QC ID labels for the blind duplicate samples. See the section on blind duplicates for further information on the procedure.

It is essential that blood samples be precisely labeled throughout the collection and processing stages to ensure that participant samples are not miscoded. To facilitate accurate labeling, it is suggested that you pre-label sets of collection tubes and cryovials prior to the participant's visit, with a crosscheck of the labels with each participant's ID # prior to the phlebotomy.

### 3. Forms

The purpose of the Phlebotomy/Processing Forms (P/P Forms) is to facilitate the efficient collection of plasma and serum samples from the participants, with maximum protection for the participant and the technician. In addition, the P/P Forms facilitate the monitoring of phlebotomy and other quality assurance parameters and provide information critical to the interpretation of the assay results and maintenance of the sample repository.

The completed Phlebotomy/Processing Forms will be included in the sample shipments to CBAL.

Both the Phlebotomy Form and the Processing Form must be labeled with the participant ID #. All forms must be completed in ink.

The Phlebotomy Completion Form will be scanned, and the information will be electronically sent to the Coordinating Center. The completed Phlebotomy Form and Processing Form will then be sent with the sample shipments to CBAL. Both forms must be labeled with the correct pre-printed barcode sample ID label. All forms must be completed in ink.

### 4. Participant Refusal of Phlebotomy

Rarely, a participant will refuse phlebotomy. Please keep a list of MESA Enrollment ID #s of any of these participants and identify which test they refused.

### 5. Venipuncture

5.1 Initial preparation for specimen collection prior to the arrival of participants is similar to that of Exam 3.

#### 5.3 Priority of tubes & Preparation of phlebotomy draw-tubes and aliquot racks

A total of approximately 49.5 ml of blood will be drawn from each participant in 6 tubes. (20% will have 7 tubes collected for a total of 54.5 ml of blood)

The order in which the tubes are collected is important. Blood collection must be drawn in the following order:

- |    |               |            |
|----|---------------|------------|
| 1. | 10 ml EDTA    | purple top |
| 2. | 10 ml Serum   | red top    |
| 3. | 4.5ml Citrate | blue top   |

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- |    |               |            |
|----|---------------|------------|
| 4. | 10 ml EDTA    | purple top |
| 5. | 10 ml Serum   | red top    |
| 6. | 5 ml SCAT-I   | red top    |
| 7. | 10 ml Heparin | green top  |
| 8. | 10 ml EDTA    | purple top |
| 9. | 5 ml EDTA     | purple top |

Note: The numbering and the priority of tubes 1-6 is the same as Exam 3. Tubes 7-9 are only for participants selected for the related ancillary studies.

Tubes #1 and #4 are 10ml EDTA tubes (Fisher Scientific # 22-311-743, Monoject # 8881311743). After centrifugation, plasma from these tubes will be pooled and aliquotted into seven aliquots that are color coded with purple caps. This plasma will be used for lipid levels and banked for future testing. For selected Blind Duplicate participants, an additional 5 ml EDTA tube (Fisher Scientific # 22-029-325, Monoject # 8881311446) will be used.

Tubes #2 and #5 are 10ml red-topped Serum tubes (Fisher Scientific #22-301-710, Monoject # 8881301710). After filling, let these tubes stand at room temperature for a minimum of 40 minutes, but a maximum of 90 minutes, to allow the blood to clot. The tubes are then centrifuged and the serum is pooled and aliquotted into seven aliquots that are color coded with red caps. The serum will be tested for glucose and stored in repository for later analysis. For selected Blind Duplicate participants, an additional 5 ml Serum tube (Fisher Scientific # 22-239-324, Monoject # 8881301413) will be used.

Tube #3 is a 4.5ml blue-topped citrate tube (Fisher Scientific # 22-029-309, Monoject # 8881340486), a silicon-coated glass tube containing 0.5ml of 3.2% sodium citrate. After centrifugation, plasma is aliquotted into 4 aliquots that are color coded with blue caps. The plasma will be banked for future testing.

Tube #6 is a 5ml red-topped "Special Coagulation" tube (SCAT-I) containing a white, powdered anticoagulant provided by CBAL. This tube contains a special combination of anticoagulants that ensure long-term stability of the plasma sample. Specifically, this tube, when filled, will contain 4.5 mm EDTA, 150 KIU/ml aprotinin and 20 uM D-Phe-Pro-Arg-chloroketone. The SCAT-1 tube must be stored refrigerated. It must be drawn after at least one other tube has been drawn. Important to note, this tube is 'non-sterile', therefore it must be drawn using a butterfly apparatus with 12 inches of tubing; alternatively, a syringe may be used for the venipuncture, and expressed through the stopper (with great care to limit turbulence) into the SCAT tube. It is critical that the SCAT-1 tube is mixed well (>30 sec of gentle inversion) before being placed on ice to await further processing/centrifugation. The four aliquots for this tube are color-coded with yellow caps and the plasma will be stored in repository for future testing.

Tube #7 is a 10ml heparin tube and #8 is a 10ml EDTA tube. They require no processing. They are to be packed in a special box with "bricks" for maintaining the temperature and shipped next day delivery.

Tube #9 is a 5ml EDTA tube. It should be mixed and immediately placed and kept at 4°C. Within 90 minutes, plasma should be separated by centrifugation, transferred into airtight 2ml cryovials, flash frozen on dry ice and stored at -70°C. If it is not convenient to flash freeze, the samples can just be placed at -70 degrees C.

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### Summary of Blood Mixing During Venipuncture

Each tube should be treated as follows:

Tube #1 and 4	EDTA – place on mixer for ~30 seconds, then place in ice bath.
Tube #3	Citrate – place on mixer for ~30 seconds, then place in ice bath.
Tube #6	SCAT-1 – place on mixer for AT LEAST 30 seconds, then place in ice bath.
Tube #2 and 5	Serum – do NOT mix; place in rack at room temperature for AT LEAST 40 minutes, but less than 90 minutes.
Tube #7 and 8	Heparin and EDTA – Rotate tubes gently up and down 5-6 times. Then put on mixer for at least 3 minutes. Maintain at room temperature until shipped same day.
Tube 9	EDTA – place on mixer for ~30 seconds, then place in ice bath.

#### 5.4 Collection of Blind Duplicate Tube

As with previous MESA Exams, 20% of the participants will have an additional tube of blood collected, for a total of seven tubes (approximately 54.5 mLs total) of blood. This sample is collected for quality control purposes. This sample is collected *last* into the Blind Duplicate Tube (#7), which may be an EDTA, Serum, Citrate, or SCAT tube.

#### 5.5 Preparation of Phlebotomy Room – is similar to that of Exam 3.

##### Setup of Draw Tube and Aliquot Racks

To facilitate accurate tracking of collected specimens, we recommend that you set up a blood collection tube rack with the set of draw tubes, pre-labeled with the provided participant ID labels. The tubes should be in the rack according to the order in which they are to be drawn, as specified above.

An aliquot rack, with pre-labeled cryovials, should be set up to correspond with each participant's blood collection tube rack; and the cryovials should be in numerical order. It may be helpful to have the red cryovials per participant in a separate rack since the red serum collection tubes are generally centrifuged at a different time from the other tubes.

##### 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 and the tubes and cryovials are labeled.
2. Check that the sample processing station is properly equipped. Every item on the checklist must be ready and in its proper position.
3. Make sure the phlebotomy room is tidy and stocked with extra smelling salts, basin, washcloths, and that the draw tube mixer is functional.
4. Label the tubes and cryovials with the participant ID (if not previously done).
5. Approximately 10 minutes before scheduled blood specimen collection, fill styrofoam ice bath  $\frac{3}{4}$  full with crushed ice.

#### 5.6 Preparation of Participants – is similar to that of Exam 3. A few points are worth re-stating here.

5.61 The participant's experience must be as pleasant as possible. Give the participant enough time to feel comfortable, both before and after the blood collection. In many

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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.

- 5.62 Participants who are concerned about the volume of blood collected should be reassured that the total amount of blood drawn is about 4 tablespoons, although it may look like more. Additionally, reassure them that blood cells die and are made continuously and what is collected will be reproduced in one to two days.
- 5.63 **Phlebotomy Form Questions.** There are five 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 three 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. Question 5 deals with fasting status. The participant should be fasting (nothing to eat or drink except water) for 12 hours with a minimum acceptance of 8 hours. If s/he has not fasted for at least 8 hours, the blood collection needs to be rescheduled.

Items 12 and 13 are related to ancillary studies. If participant is selected for an ancillary study draw, indicate "Yes" for the appropriate question and then complete the related items. If not selected for a particular ancillary study, respond "No" to Question 12 and/or 13 and leave the related items blank.

- 5.7 Venipuncture Procedure – is similar to that of Exam 3.

### **ALWAYS WEAR LATEX GLOVES AND LAB COAT**

1. Arrange draw tubes in order of draw on the table top or in the tube rack within easy reach. Assemble butterfly apparatus and Vacutainer holders, gauze, and alcohol prep prior to tourniquet application.
2. Apply tourniquet.
3. Examine participant's arms for the best site for venipuncture. Release tourniquet.
4. Cleanse venipuncture site by wiping with alcohol prep pad in a circular motion from center to periphery. Allow area to dry.
5. Reapply tourniquet and start timer and document start time. **NOTE:** If possible, 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. In our experience, however, especially with sick and/or elderly subjects, this may result in flow stopping, and the trauma of a second venipuncture. Therefore, this is a "judgment call" based upon the phlebotomist's experience and skill.
6. 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 below the venipuncture site.
7. With the needle bevel upward, enter the vein in a smooth continuous motion.
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.
9. Grasp the flange of the Vacutainer holder and gently push the tube forward until

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the butt end of the needle punctures the stopper, exposing the full lumen of the needle.

**NOTE** : Attention should be paid to minimizing turbulence whenever possible. Small steps, such as slanting the needle in 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 stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.
12. 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 of the same type without removing the needle from vein. Plasma tubes are not acceptable if < ½ full.
13. When the blood flow ceases, remove the tube from the Vacutainer holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted (if necessary). Place all tubes, except serum, on tube mixer for a minimum of 30 seconds.
14. Release tourniquet, if still applied. The ideal tourniquet time is two minutes.
15. 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. Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma. Discard needle into puncture-proof sharps container. Record on Phlebotomy form duration tourniquet was applied and end venipuncture time.
16. The Citrate, EDTA, and SCAT-I, tubes are placed on wet ice. The serum tubes are maintained at room temperature.
17. Clean up the venipuncture area (if necessary). Dispose of needle and tubing in the appropriate biohazard needle sharps containers. Complete the Phlebotomy Form.
18. Bring the filled blood collection tubes to the processing area, keeping the EDTA, citrate and SCAT-I tubes on ice and the serum tubes at room temperature.

### 5.8 Guidelines for Difficulties – are the same as Exam 1, 2 and 3.

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. 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 has "good veins" the phlebotomist can reassuringly say, "Oh, you have good veins; there should be no problem."

1. Bandaging the Arm. If the patient continues to bleed apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops. A gauze bandage can be tightly wrapped around the arm over the pad, and left on for at

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least 15 minutes.

2. 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. The phlebotomist should not attempt a venipuncture more than twice.
    - e. Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on their part.
    - f. If venipuncture is unsuccessful, this should be noted on the Phlebotomy Form.
  
  3. 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 head between knees.
    - 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 washcloth 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 sick, contact a medical staff member who will advise you on further action.
  
  4. Other Possible Problems: Not all tubes are 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.
  
  5. Other Possible Problems: Collection tube does not fill. First, try another tube of the same type. Partially filled plasma tubes are not acceptable if less than ½ full. If a tube is less than ½ filled, it should be discarded. Partial tubes for serum are acceptable, but will result in a reduced number of aliquots. If a tube is not completely filled clearly note on the Processing Form as this can effect future assays.
6. Processing Specimens

### A. Overview

Processing should be initiated as soon as possible (0 – 30 minutes) following venipuncture. The red-topped serum tubes must stand at room temperature for at least 40 minutes before centrifugation. If centrifugation of the other tubes is not immediate, the citrate, EDTA, and SCAT-I tubes should remain on ice. Personal protective equipment (non-permeable lab coats, double-gloves with at least one latex pair; splatter shields are recommended) **MUST BE** worn for processing.

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**B. Daily Preparation**

The following items should be on hand before beginning processing:

- Lab coats and gloves, splash shields, other Personal Protective Equipment as needed.
- Refrigerated Centrifuge: 2,000 g-force minimum, 4 °C, Swinging bucket.
- 10% bleach solution (or approved biohazard disinfectant)
- test tube holders (adapters) for centrifuges
- Harvard Trip Balance / Pan balance
- water bottles for balance
- freezer (-70°C or colder)
- emergency eye wash station
- biohazard trash can, with biohazard bags (*biohazardous waste puncture proof containers*)
- test tube racks / cryovial racks
- Fixed volume pipettes with tips (MLA) and adjustable pipettes (Rainin, Finn, etc) with tips.  
Volumes needed to pipette: 0.5 ml, 1.0 ml (200 to 1000 ul)
- cryogenic vials\* (0.5 ml and 1.5 ml)
- cryovial labels (from the CC)
- Revco Boxes (#5954 and #5956) and dividers (9 x 9 and 7x7)
- Styrofoam/insulated shipping boxes
- Refrigerator- for storage of special blood tubes
  - Can be a household fridge.
  - Cannot be the same as food fridge.
- Labels/lab tape for reagents
- Sharpie pens
- ID labels for cryovials and freezer boxes

\* = provided by CBAL

**C. Description of Aliquots**

Aliquot Assignments:

Collection Tube	Min Volume Needed after Centrifugation	Number of Aliquot Tubes	Color Code	Volume per Aliquot Tube
#1: 10mL EDTA	4.0mL	7 cryovials	Purple	(Combine plasma from Tubes 1 &4 before aliquoting) 7 @ 1.0mL
#4: 10mL EDTA	4.0mL			
#2: 10mL Serum	4.0mL	7 cryovials	Red	(Combine sera from Tubes 2 & 5 before aliquoting) 1 @ 0.5mL 6 @ 1.0mL
#5: 10mL Serum	4.0mL			
#3: 4.5mL Citrate	2.0mL	4 cryovials	Blue	4 @ 0.5mL
#6: 5mL SCAT	2.0mL	4 cryovials	Yellow	4 @ 0.5mL
#7: 10ml Heparin	No Processing Performed			
#8: 10 ml EDTA	No Processing Performed			
#9: 5ml EDTA	2.5mL	2 cryovials	Purple	1 @ 1.5mL 1 @ 1.0mL

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### D. ALIQUOTING: Citrate, EDTA, SCAT-1 and Serum tubes

1. Aliquoting consists of removing the serum or plasma in small amounts (e.g.: 0.5ml) by pipette and placing it into the appropriate color-coded cryovials (provided). Correctly color-coding the aliquots is important. Color-coding is predetermined and used to identify sample type such as citrated plasma vs. SCAT-I plasma, etc.
2. This process must be done while the tubes and cryovials are on ice (unless otherwise noted).
3. When aliquotting serum and plasma, 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.
4. Use a new pipette tip for each draw tube.
5. Once the sample is aliquotted cryovials should be immediately (< 10 minutes) frozen in an upright position at -70°C or promptly placed on dry ice for quick freezing.

After centrifugation, pool plasma or serum of like tubes from the same participant, (e.g.: EDTA plasma from tubes 1 & 4; Serum tubes 2 & 5). A disposable transfer pipette may be useful in transferring the plasma or serum from the centrifuged blood collection tube into a 15 ml or similar 'pooling tube'. Make sure the pooling tubes are clearly labeled with ID#s. From the pooled plasma or serum, now in the 15mL tube, pipette the appropriate volume into each cryovial for that draw tube type (i.e.: EDTA plasma = 7 purple capped cryovials).

If any tubes are accidentally mixed during pipetting so that plasma is contaminated with red cells, they may be recentrifuged.

*Upon completion of the processing steps, aliquots must be frozen at -70°C or below within 10 minutes, or place immediately on dry ice. Make sure all cryovials and tubes are frozen in the upright position.*

### E. Summary of Timing Issues

After blood drawing, time before centrifugation:

EDTA, SCAT-1, and Citrate: store on ice; preferably < 15 minutes (maximum < 30 minutes) before centrifuging.

Serum: store at room temp for at least 40 minutes, but < 90 minutes prior to centrifuging.

**After aliquotting, ALL samples must be frozen within 10 minutes or placed immediately on dry ice.**

Aliquot racks will be set up to correspond to each blood collection tube rack. Rack setup is completed the previous day. All tubes and vials are labeled with sample ID labels (if not previously done) and arranged in appropriate working order.

#### Low sample volume

If there is insufficient sample of a tube type to make the full set of aliquots, if possible fill the cryovial that is marked with an \* on the Processing page for that tube type first.

Any partially filled cryovial (less than the specified volume) should be marked with a dot on the cap and a "P" in the comment field on the Processing Form next to that cryovial number.

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### F. Centrifugation – EDTA, SCAT-1, & Citrate Tubes

If centrifugation is not immediate, tubes are stored upright on wet ice. Tubes are centrifuged 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. Maximum time elapsed before centrifugation is 30 minutes from time of collection. Please note all start times on the Processing Forms. Once centrifugation is complete, tubes are carefully placed on ice and are ready to aliquot.

1. EDTA. Place tube in the centrifuge. After centrifugation, the EDTA plasma from tubes 1-4 is pooled and aliquoted, by specified volume into cryovials #01 - 07. If tube #9 was drawn, it should be aliquoted separately. These will be purple-capped cryovials.
2. CITRATE. After centrifugation, carefully pipette 0.5ml of this plasma into each of the cryovials # 8 – 11. These will be blue-capped cryovials.
3. SCAT-1. After centrifugation, carefully pipette 0.5ml of this plasma into each of the cryovials #12 – 15. These will be yellow-capped cryovials.

### G. Serum - Centrifugation

Allow serum tubes to clot for at least 40 minutes at room temperature (maximum time before centrifugation is 90 minutes). These tubes are centrifuged at 4°C at 2,000g x 15 minutes or 3,000g x 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, pool the serum before aliquoting and place on ice. Carefully pipette 0.5ml of pooled serum into cryovial # 16. Pipette 1.0 mL of pooled serum into each of the cryovials #17-22. These will be red-capped cryovials. The remaining red cells in the serum draw tube (and the tube itself) can be discarded in the biohazardous waste.

### H. Special Circumstances

1. Blood specimens (EDTA, Citrate, SCAT-I) cannot be processed within 30 minutes of collection.  
If centrifugation cannot be performed within 30 minutes of collection, try to process specimens as soon as possible after that time. **Note time of collection and centrifugation** on the P/P form. Maintain the EDTA, citrate, and SCAT-I tubes on wet ice until centrifugation.
2. Serum and plasma cannot be frozen within 10 minutes of aliquoting.  
Every effort should be made to freeze serum and plasma cryovials at -70°C or below as soon as possible after aliquoting. If specimens cannot be placed immediately at -70°C or below, they may be temporarily (< 2 hours) stored at -20°C or placed on dry ice until transfer to -70°C or below. Dry ice is the preferred solution.

### I. Processing Completion.

The completed Phlebotomy/Processing Forms are kept in a temporary file. Enclose copies of the Phlebotomy/Processing Forms with each shipment of samples to the Central Blood Analysis Laboratory. Upon receipt at CBAL, forms and

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samples are examined for monitoring/QC purposes.

Completed, frozen cryovials from three participants are packed into one freezer box. Be sure the Phlebotomy and Processing Forms are completely filled out.

Wipe down all work areas with 10% Bleach solution (or approved biohazard disinfectant).

Label and arrange cryovials in their proper racks for the next days blood processing

### **SHIPPING BLOOD SAMPLES**

#### A. General

Blood samples are shipped only on Mondays or Tuesdays to the CBAL by an overnight carrier (Federal Express is preferred). Samples will be shipped on a pre-arranged schedule.

#### B. Packaging Samples

Sample Shipping Checklist:

- Coolers
- Rubber bands for freezer boxes
- Ziplock plastic bags for freezer boxes
- Absorbent material (i.e. paper towels, newspaper)
- Packaging tape
- Dry ice (~10 lbs per mailing container)
- Labels: Fedex address labels,
- UN3373 Diagnostic Specimen label (This is new IATA for 2005)
- Dry Ice Labels (class 9, UN1845)
- Labeled freezer boxes with participant samples
- Completed Processing Forms
- Completed Shipping Forms (to be faxed)
- Temperature “Bricks” for the inflammation samples

#### C. Procedure

For frozen shipment to the University of Vermont:

1. Line cooler with absorbent material (i.e. paper towels).
2. Place approximately 1/2 the dry ice (per mailer) on the bottom of the cooler.
3. Place another layer of absorbent material (i.e.: paper towels) on top of the dry ice – so it will be between the dry ice and the freezer boxes containing the samples. It is important to ensure there is sufficient absorbent material between the dry ice and the Ziploc bags containing the freezer boxes.
4. Collect the freezer boxes containing samples to be shipped, and check the sample ID numbers against the Processing Forms for that shipment. Each

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cryovials box contains the samples from 3 participants.

5. Place a rubber band around each cardboard freezer box containing samples before enclosing each box in a Ziploc plastic bag. Be sure to seal the Ziploc bags so they are leak proof. Then carefully place these bagged boxes containing samples in the mailer. The rubber band is important for aiding in the prevention of a cryovial spill; the sealed Ziploc bag & absorbent material are for compliance with commercial carrier specifications.
6. Another layer of absorbent material is placed on top of the sample freezer boxes.
7. The remaining dry ice is placed on top of this last layer of absorbent material.
8. The Shipping Form (same form that is faxed to VT), and Phlebotomy/Processing Forms for all samples included in that particular shipment, are placed in a plastic bag on the top of the absorbent material before the top is securely taped closed.
9. Affix shipping label(s). Place the entire box in the refrigerator if pickup is not immediate. (Samples should not be on dry ice for > 24 hours).

The completed Shipping Form, with the Fedex airbill #s, is faxed to the University of Vermont at (802) 656-8965.

This shipping protocol follows the procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904. Copies of these regulations are included with this MOP.

### D. Mailing Addresses:

University of Vermont  
Department of Pathology  
Colchester Research Facility, Room T205  
208 South Park Drive, Suite 2  
Colchester, VT 05446  
Attn: Elaine Cornell  
(802) 656-8963  
(802) 656-8965 Fax

## QUALITY ASSURANCE

### A. Overview of Field Center Monitoring

Quality assurance monitoring of the blood collection and processing protocols 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 order to prevent any sample associated problems, the CBAL has designed a system for monitoring the quality of blood collection and processing in each Field Center. The first component in the quality assurance program for Field Centers consists of the CBAL training course and certification process for each Field Center technician. Other components of the program include maintenance of equipment check logs at each field center, Field Center Supervisor checklist, review of Phlebotomy/Processing Forms by the CBAL, and analysis of problems associated with the phlebotomy. Through the monitoring of these parameters, any systematic or random problems should be identified and appropriate corrective actions 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. There will be a one time

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training session on blood collection and processing of the MESA samples. The training session will present information relating to the collection of the blood sample (i.e.: infection control, safety precautions including OSHA regulations, handling equipment, venipuncture procedure and possible venipuncture problems), and proper processing procedures for the varied array of draw tubes, including centrifugation and temperature requirements, and aliquoting the multitude of corresponding color-coded cryovials. Training will also cover additional areas considered relevant to maintaining the standard and quality of the samples collected.

### Field Center Technician Requirements.

Prior clinical phlebotomy experience is mandatory for the Field Center technicians who will be performing blood collection for the MESA study.

Field Center Technicians should have read the MESA Manual of Operations before attending the CBAL training session. 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 session and successfully complete both the written and practical examinations will be certified in MESA blood collection & processing. Once fully certified, this technician is 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 the Lab Manual of Operations.
2. Observe the process performed by a certified technician.
3. Successful completion of the practical exam (using the Certification Form/Supervisor Checklist), which involves observation by a certified personnel of the trainee completing the phlebotomy/processing procedure on a volunteer.
4. Successful completion of written exam (prepared by CBAL).

Completed written exams are mailed to the CBAL for correcting and will be kept on file there.

### C. Field Center Equipment Records

Each Field Center is responsible for maintaining daily and monthly records for equipment performance. Daily temperature checks on refrigerators, freezers and refrigerated centrifuges should be performed. Equipment temperature logs are filed on site for future reference and reported to the CBAL monthly. These equipment records can identify problems with sample quality in the aliquoting and local storage steps.

### D. Field Center Supervisor Checklist

The Field Center Supervisor checklist serves as a periodic monitoring measure. The Field Center Supervisor will observe the MESA technicians at their site while they perform the phlebotomy and processing procedures, recording their observation on the checklist. Completed Supervisor Checklists will be sent to the CBAL for monitoring purposes. Checklists need to be completed once per month per technician.

### E. Maintaining Certification

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

### F. Field Center Acknowledgement Forms

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The Vermont Central Blood Lab will analyze the condition of each shipment received and will complete an Acknowledgement Form and fax it back to the Field Centers. The Acknowledgement Form is used as a tool to track possible problems and variations from protocol on a weekly basis.

### Alert Values

The University of Minnesota will be analyzing these samples for Lipid Panel, Glucose, and Creatinine.

Alert values are as follows:

Total Cholesterol	>360 mg/dL
Triglycerides	>1000 mg/dL
HDL cholesterol	< 20 mg/dL
LDL cholesterol	> 260 mg/dL
Glucose	< 50 or > 400 mg/dL
Creatinine	> 2.0 mg/dL

### Blind Duplicates

Blind duplicate samples will be collected on 5% of the participants on four different tube types. This results in 20% of all participants having a blind duplicate sample collected for QC. The criteria for collecting a blind duplicate sample will be based on a check digit in the participant's ID number.

The following tube types will be drawn (only one tube per participant depending on the check digit):

- 5 ml EDTA (Fisher Scientific #22-029-325, Monoject # 8881311446)
- 5 ml Serum (Fisher Scientific #22-239-324, Monoject # 8881301413)
- 4.5 ml Citrate (Fisher Scientific #22-029-309, Monoject # 8881340486)
- 5 ml SCAT-I\*

\*Provided by CBAL

The blind duplicate tube is collected after the regular tubes are filled. It would be the seventh tube filled. The tubes are handled in the same way as the regular collection tubes. EDTA, Citrate, SCAT-I are placed on the mixer for approximately 30 seconds, then placed in ice, and centrifuged within 15 to 30 minutes. Serum remains at room temperature for a minimum of 40 minutes, with a maximum of 90 minutes, to clot before centrifuging.

Aliquoting Scheme:

<b>Tube Type</b>	<b>Cryovial color</b>	<b># x sample volume</b>
EDTA	purple	2 x 1.0 ml
Serum	red	4 x 0.5 ml
Citrate	blue	4 x 0.5 ml
SCAT-I	yellow	4 x 0.5 ml

Cryovials must be labeled with a QC ID#. This ID# is matched to the Participant ID#.

After aliquoting, cryovials are frozen immediately at  $-70^{\circ}\text{C}$  in an upright position. Blind Duplicate cryovials are placed in their own freezer box with a 9 x 9 grid. More than one participant's samples are included in one box. These samples are shipped a week or so after the original samples are sent out to the CBAL, so that the laboratory cannot match them with the original participant. It is important to complete the Blind Duplicate Shipping Log and include a copy in the shipping box with the frozen samples. The

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Blind Duplicate Shipping Log is also faxed to VT the day the frozen samples are shipped.

FIELD CENTER FORMS

MESA Phlebotomy/Processing Form  
MESA Shipping Log  
MESA Blind Duplicate Shipping Log  
MESA Field Center Supervisor Checklist  
MESA Field Center Technician Certification Examinations  
MESA Equipment Temperature Logs

DIAGRAMS/INSTRUCTIONALS:

Aliquoting Scheme Flow Chart  
Freezer Box Diagram  
IATA Packing Instructions 650 and 904