MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS

EXAM 5

Field Center Procedures

Manual of Operations

February 10, 2010

Multi-Ethnic Study of Atherosclerosis

Exam 5 ~ Field Center Procedures ~ Manual of Operations

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SECTION 1: OVERVIEW AND CLINIC EQUIPMENT

1.1 MESA Exam 5 Overview

Welcome to Exam 5 of the Multi-Ethnic Study of Atherosclerosis (MESA)! This is a continuation of a vitally important national research project that will influence diagnosis and treatment of cardiovascular diseases over the next several decades. The study enrolled 6814 participants at six field centers and consisted of four examinations, periodic surveillance phone calls, and collection of data from Events (new diagnosis of a clinical cardiovascular disease)

As we continue on, keep the above in mind when you perform the procedures on those gracious and willing participants. The ultimate value of this study depends on *you* and on the quality of the data that you collect.

1.1.1. Purpose and Objectives

1.1.1.1 The purpose of MESA is to study subclinical cardiovascular disease (CVD). Subclinical diseases are those detected by non-invasive procedures, such as MRI, CT, ECG and ultrasound, before they have produced any clinical signs or symptoms.

1.1.1.2 The primary objectives of MESA are to determine:

- What factors influence the progression of mild subclinical disease to more severe subclinical disease?
- What factors influence the progression of subclinical disease to clinical disease?

1.1.1.3 The secondary objectives of MESA are to:

- Assess ethnic, age, and gender differences in subclinical CVD prevalence and risk of progression
- Describe the interrelationships of established risk factors for subclinical CVD with new risk factors that are identified by the MESA study
- Develop population-based methods, suitable for application in future screening and intervention studies, to identify asymptomatic individuals who are at high risk for subclinical CVD.

1.1.2. Brief Description of the MESA study

1.1.2.1 MESA is a large and complex long-term study. Of the 6814 men and women ages 45–85, approximately 38% of the cohort are Caucasian, 28% African-American, 23% Hispanic, and 11% Asian, predominantly of Chinese descent.

1.1.2.2 The cohort was recruited from the following six field centers (site ID number in parentheses):

- Wake Forest University, Winston-Salem, NC (3)
- Columbia University, New York, NY (4)
- Johns Hopkins University, Baltimore, MD (5)

- University of Minnesota, Minneapolis-St. Paul, MN (6)
- Northwestern University, Chicago, IL (7)
- UCLA, Los Angeles, CA (8)

1.1.2.3 For this exam, there are 6 Reading Centers and Labs. These centers are located at:

- Harbor-UCLA (CT RC)
- Johns Hopkins University (MRI RC)
- University of Wisconsin (Ultrasound RC)
- Wake Forest University (ECG RC)
- University of Minnesota (Lipid Lab)
- University of Vermont (Central Blood Lab)
- University of Vermont (Lab Repository)

1.1.2.4 The Coordinating Center at the University of Washington (Seattle) continues to coordinate all aspects of the study, including development of the Manual of Operations, development of data entry and management software, quality control, and statistical analysis of the data for publication in academic journals.

1.1.2.5 Funding for MESA is provided by the National Heart, Lung, and Blood Institute (NHLBI), a division of the National Institutes of Health. The Project Office from NHLBI also provides scientific leadership to the study.

1.1.2.6 The original MESA project took 10 years to complete. The first 18 months of the study were devoted to protocol development, staff training, and pilot testing. MESA Exam 1 (i.e. the "Baseline Exam") took place over a two-year period, beginning in late July 2000. The second and third exams each required about 18 months to complete, and the fourth exam required just under two years to complete. During these four exams, participants were also contacted by telephone every 6–9 months to determine whether any medical events have occurred. The remaining 18 months of the study consisted of close out and data analysis for publication.

1.1.3. Events

In MESA, an "event" is the development of a medical condition requiring participant hospitalization or other specified types of treatment, or the death of a participant. In MESA, we are particularly interested in collecting data about myocardial infarction (MI or "heart attack"), stroke, transient ischemic attack (TIA or "mini-stroke"), angina, congestive heart failure (CHF), peripheral vascular disease (PVD), and death. When an event occurs, we will collect a separate set of data based on hospital and physician records and on interviews with participants or their proxies.

1.1.4. How to use this manual

This manual contains step-by-step instructions for completing all of the components in **Exam 5** of the MESA study. Many of the steps and clinic procedures are the same as in previous exams; thus where appropriate the previous manuals of operation may also be consulted.

You should carefully study all sections that relate to procedures that you will be performing, and you should keep this manual handy as a reference. If you have questions about anything in the manual, please direct them to your Study Coordinator.

1.2 Supplies and Equipment

Most supplies and equipment needed for Exam 5 are similar to that of previous exams. Each clinic should be equipped with the following supplies and equipment:

1.2.1 Exam Equipment & Supplies on hand at Field Centers

- Clean sheets, pillow cases, blankets
- Gowns or scrubs & slippers
- Examining table disposable paper
- Snacks
- Consent forms (and participant folders/files)
- Three Dinamap Blood Pressure Machines, Model PRO 100 (including printer paper, power cable, and power converter)
- 2 MAC1200 ECG machines and modems
- MAC1200 ECG paper, GE/Marquette disposable silver chloride electrodes
- 1 HEARTSQUARE
- Detecto Platform Balance, # 1915-339W Scale in lbs/kg
- Stadiometer
- Two Gulick II anthropometric tapes in cm, Model 67021
- Wall mounted mirror for verifying tape measurement
- Doppler machine, # EN 50 Nicolet LE 100 5 megahertz vascular probe
- Aneroid blood pressure sphygmomanometers (2-3)
- Blood pressure cuffs (pediatric, adult, large, and thigh)
- Stop watches
- Four 50-pound weights (certified prior to MESA visit) to calibrate scale
- Germicidal cleanser and wipes
- Nidek Auto Lensmeter (model LM-990A)
- Nidek Autorefractor/Keratometer (model ARK-760A)
- 3 foot Cable from lensmeter to autorefractor (LM-03-0808)
- 10 foot Cable from autorefractor to computer (TRS-10-0809)
- Computer
- Surge Protector
- Spectacles of known prescription (NEI will provide to each clinic)
- Penlight
- Blower brush
- Mirror (for use in inserting & removing contacts)
- Plastic tray (to place contact lens case in while removing or inserting contacts)
- Unisol 4 saline solution – NOTE: You must write the date opened on the bottle. The bottle should be discarded 30 days after opening.
- Clarity lens cleaner
- Contact lens cases
- Alcohol wipes
- Chin rest tissue
- ARK printer paper
- Lensmeter printer paper
- Lint-free Kimwipes
- Absorbond lens wipers

- Canon CR6-45NM fundus camera with a digital Canon D-60 camera back
- Laptop computer, with EyeQSL software, provided by Digital Healthcare Inc.
- Motorized instrument table
- Pneumatically adjustable stools for both photographer and subjects.
- Lens cotton – One 4 oz. Box (long-grain Red Cross sterile cotton batting)
- Lens cleaning alcohol – One 8oz. bottle (100% alcohol)
- Spare view lamp (#BH3-3277) – One
- Canon camera fuses (125V, 4 amp) – Two
- Instrument table and stools

1.2.2 Examination Equipment Provided by the Coordinating Center

- Valhalla Model BCS-2 Body Composition Scale and Printer
- 5 tablet PC computers
- 1 desktop computer
- 3 audio recorders (digital)
- Test eyes/steel balls (ARK9-00-ARKJ-4)

1.2.3 Phlebotomy and Laboratory Supplies being Provided by the Central Laboratory

- 5 mL SCAT-1 tubes
- 8 mL Cell Preparation tubes
- Cryogenic vials (0.5 mL, 2.0 mL)
- Nalgene Freezing container #2 'Mr. Frosty' (for cell prep) (Nalge#5100-001)
- 15 mL centrifuge tubes (for cell prep)
- Freezing Media A and B
- PBS (phosphate buffered saline)
- Acetic acid (for urine)
- ACD/dextran (for red cell membranes)

1.2.4 Phlebotomy and Laboratory Equipment Already Purchased and Available at Field Centers

- Two Centrifuges with temperature control, 2,000 g-force minimum, swinging bucket, and test tube holders (adaptors), e.g., IEC Centra CL3R with CL3R swinging bucket rotor (#243) and aerocarrier tube holder for IEC 243; adapters need are #6561E, 6561E, 6562E, 6566E.
- Harvard Trip Balance / Pan balance, **VWR** # 12344-051
- Water tubes for balancing the centrifuge, **VWR # 21008-102**
- Freezer (-70 C or colder)
- Refrigerator for storage of special blood tubes, media, etc (can be a household fridge, should not be used to store food, from Sears or a similar store)
- Test tube racks / cryovial racks (Simport # T315)
- Fixed volume pipettes with tips (MLA) and regular adjustable pipettes (Rainin, Finn, etc) with tips. Volumes needed to pipette: 225 ul, 0.5 ml, 1.0 ml (200 to 1000 ul), 3 to 5 ml, 9 ml, and 14 ml.
- Graduated cylinder, **Lab Safety** 25 ml Nalgene 9A-22763
- Blood tube rocker (Thermolyne Labquake Tube Shakers C400-110)
- Blood tube racks, **Fisher Scientific** # 60914764

- Stopwatches or timers, **VWR** # 62344-756

1.2.5 Phlebotomy and Laboratory Supplies

- Butterfly needles (21 G) with luer adapter
- Vacutainer barrels
- Tourniquets #CK1126
- Alcohol prep pads # 10-3001
- Gauze (2x2) Kendall # 1806
- Surgical tape - paper tape 3 M durapore
- Band-aids (first –aid) # 1290033
- Blood collection tubes:
  - 10 ml Serum (red-top) tubes
  - 10 ml EDTA (purple-top) tubes
  - 4.5 ml Citrate (blue-top) tubes
- Polypropylene (or polystyrene) disposable test tubes (10-15 mL) for pooling samples
- Revco Boxes and dividers (Revco box # 5954 with Revco 10x10 grid # 5958)
- Isopropyl alcohol
- Distilled water
- 10% bleach solution (or approved biohazard disinfectant)

1.2.6 Laboratory Shipping Supplies

- Styrofoam/insulated boxes (Polyfoam Packer # 355-CS for frozen shipments, #33-12KD for refrigerated shipments)
- Ziplock bags for freezer boxes
- Dry ice
- Ice (gel) packs
- Absorbent material (old newspaper or paper towels) for layering between dry ice
- Elastic bands for freezer boxes
- Packing tape

SECTION 2: OVERVIEW OF EXAM 5 COMPONENTS

2.1 Summary of Exam 5 components

2.1.1 Exam 5 Procedures:

1. Anthropometry
2. Seated Blood Pressure
3. Phlebotomy
4. Electrocardiogram
5. Ankle-Brachial Index
6. Retinal Photographs
7. Refraction Test
8. Cognitive Function Tests
9. Questionnaires (health information, psychosocial, pollution exposure)
10. Cardiac MRI Scan
11. Ultrasound IMT Scan
12. Cardiac CT Scan

2.1.2 Exam 5 Screens/Forms

2.1.2.1 Clinic Examination Data Forms and Questionnaire

1. Clinic Reception (computer screen)
2. Clinic Check-off Sheet (computer screen)
3. Anthropometry (computer screen)
4. Seated Blood Pressure (computer screen)
5. Ankle-Brachial Index (computer screen)
6. Personal History (computer screen)
7. Medical History (computer screen)
8. Medications (computer screen)
9. Dietary Supplements (computer screen)
10. Health & Life (computer screen)
11. Eye History Questionnaire (computer screen)
12. Physical Activity (computer screen)
13. Cognitive Function – CASI (computer screen)
14. Cognitive Function – Digit Span (computer screen)
15. Cognitive Function – DSST (computer screen and paper)
16. Air Questionnaire (computer screen)
17. Food Frequency Questionnaire (scannable form)
18. MRI Exclusion Form (computer screen)

2.1.2.2 Reading Center Completion Forms

1. Phlebotomy Completion (computer screen and paper)
2. Urine Completion (computer screen)
3. Lab Processing (paper form)
4. Vision (Refraction) Completion (computer screen)
5. Retinal Completion (computer screen)
6. MRI Completion (scannable form)
7. CT Exam Completion (scannable form)
8. Ultrasound IMT Completion (scannable form)

2.1.3 Surveillance Phone Call Forms and Questionnaires:

1. Participant tracking (paper form and computer screen)
2. Contact Log (paper form)
3. Contact Cover Sheet (computer screen)
4. General Health (computer screen)
5. General Health – Death (computer screen)
6. Death Information (computer screen)
7. Specific Medical Conditions (computer screen)
8. Specific Medical Procedures (computer screen)
9. Other Admissions (computer screen)

SECTION 3: CLINIC QUESTIONNAIRES & EXAMINATION

3.1 Pre-Exam Activities

3.1.1 Preparatory Activities Prior to Participant Arrival

Participants due to have an Exam 5 visit should be contacted at minimum one week prior to the planned appointment date. During this contact, the Eleventh Follow-up Phone interview will be completed (see section 7), an Exam 5 appointment scheduled, and if applicable, appointments for CT and/or MRI. In some cases, the exam appointment may be scheduled by a different person during a separate call.

The purpose of completing the Pre-Exam 5 Phone Call questionnaire is to obtain the most recent information on the participants' health status as well as new events, and to schedule the exam.

Preparatory steps prior to clinic visits are the same as for MESA Exam 1–3. In addition, the **Contact History Sheet** and **Participant Data Report** (a summary of data from the participant's Exam 1–3 visits) should be printed, the Sixth Follow-up Phone Call forms should be available, and both should be reviewed for completeness prior to the participant's arrival. Any information missing should be noted and obtained.

3.1.2 Calendars, Reports, and Forms

3.1.2.1 The clinic manager should review the clinic visits scheduled for the following day and check that the necessary materials and equipment are in place.

3.1.2.2 For each participant scheduled for the following day, print the **Contact History Sheet** and the **Participant Data Report** and have his/her most recent Surveillance Phone Call forms available.

3.1.2.3 Print a set of Exam 5 forms that will be scanned for each participant scheduled. These will be pre-printed with participant IDs and can be printed as far in advance as you would like. Because the ID number is not pre-printed on the Chinese forms, the participant ID will need to be hand-written on each form. For each participant, gather all the forms required for a visit, including the informed consent and medical release, and place into the participant's binder. Make sure his/her ID matches the binder.

3.1.3 Supplies and Equipment

3.1.3.1 Set up vacutainer and aliquoting tubes for the basic Exam 5on the racks and attach the pre-printed labels to the tubes. Place any additional pre-printed labels near the racks to be affixed the next day once consent for the related procedures has been obtained. Place all phlebotomy supplies on the blood drawing table (21 g, luer adaptor, vacutainer barrel, tourniquet, alcohol pad, gauze 2x2, surgical tape, Band-Aid).

3.1.3.2 Make sure that the examination rooms are clean and have clean linen.

3.1.3.3 Prepare the participants' gowns or (scrubs) and slippers.

3.1.3.4 Prepare the examination room for seated BP, anthropometry measurement,

etc. Check all instruments that will be used for the examination.

3.1.3.5 Make sure a snack will be available for the participants.

3.1.4 Staffing

3.1.4.1 Prepare staff assignment sheet and make sure everyone knows his/her responsibilities. This is particularly important if you schedule a large number of participants on a given day.

3.1.4.2 If participants have been scheduled for CT or MRI, make sure that the respective technicians have the participant schedule and forms, and that the scanning centers are ready to receive the participants.

3.1.5 Instruction to Participants before the Clinic Visit

Mail instructions to the participant 7–10 days before the clinic visit and explain them over the telephone when you schedule the visit. If possible, make a reminder call to the participant the day before the clinic visit and reiterate the instructions. (If the participant is acutely ill—e.g. "flu" or bronchitis—when you make this reminder call, *tell him/her not to come to the clinic*. Arrange to contact him/her again to reschedule when he/she has recovered.) Before the examination, make sure the participants understand the following instructions.

1. Participants must fast (except water) for at least 12 hours before the examination. Instruct them to consume dinner at least 12 hours before their scheduled appointment at the clinic. Only water and prescription medications are permitted from dinner until the start of the examination the next morning.

2. Participants should avoid heavy exercise during the 12 hours before the visit.

3. Participants should not smoke on the morning of the visit.

4. Participants should bring all current medications, both prescription and over-the-counter, including vitamin preparations, dietary supplements, injectable medicines such as insulin, inhalers, patches, and herbal remedies to the clinic. If the participant forgets to bring the medications, schedule another clinic visit to obtain this information or collect the information when the participant returns for imaging procedures.

5. Participants should bring the name and complete address of their personal physician or health plan, particularly if they wish to have examination results sent to that provider. They should also bring the current contact information for proxies and contact people.

3.2 MESA Exam 5 Guidelines

3.2.1 Examination Guidelines

The MESA Exam 5 will be scheduled over an 18-month period, beginning April 1, 2010. The examination will include several questionnaires and procedures (i.e., anthropometry, blood pressure measurement, fasting blood collections, etc.). Selected participants will have one or more of CT scan, MRI scan, spirometry test, and ultrasound scan. We estimate that the complete examination including CT and/or MRI will require between 5 and 9. The examination may be performed in one day or over several days. However, every effort should be made to perform all the components of the examination within a *four-week period*. Clinics may vary the exam sequence if needed; but all components of Exam 5 must be completed.

1. **A pregnancy test should be performed within 48 hours before the CT exam in all women of child-bearing potential who are scheduled to have a CT exam**. This could be done in the clinic or in the Radiology Department.
2. Questionnaires and clinic procedures should be performed before the CT and/or MRI exams.
3. Anthropometry and blood collection should be performed while the participant is fasting. (If participant is not fasting, record date and time he/she last ate or drank.) Blood pressure measurement should be done before venipuncture. CT, MRI, ultrasound, spirometry, and questionnaires do not require fasting.
4. Blood drawing should be done after a 12-hour fast and before 12:00 noon.

3.2.2 Examination Order

Guidelines for clinic order are listed below. Many elements are left to the discretion of the individual field center.

1. Anthropometry, blood pressure, and urine collection should be done immediately following the greeting and informed consent, and *before* venipuncture.
2. Resting blood pressures should be obtained after the subject has been in the seated position for at least five minutes.
3. Venipuncture should be performed in the fasting state after blood pressure measurement. If a participant comes to the clinic non-fasting, perform exam components that do not require fasting, and schedule the participant for another clinic visit for fasting blood collection.
4. Questionnaires and other exam procedures may be administered at any time during the examination. During the interviews, make every effort to avoid distractions, ensure privacy, and maintain confidentiality for the participant. Do not conduct interviews during the snack or in the waiting area in the clinic.

3.2.3 Guidelines for Examination of Diabetic Participants

1. Diet-controlled diabetics must fast overnight and are treated the same as non-diabetics.
2. Diabetics taking oral hypoglycemic medications or insulin must fast overnight (unless a bedtime snack was prescribed by their physician) and to come to the

clinic without taking their hypoglycemic medication. They should bring their morning medication dose with them to the clinic. Schedule all known diabetics taking oral hypoglycemic medications or insulin for examination as early as possible (before 9 a.m.). Draw fasting blood samples promptly on arrival at the clinic (after measuring blood pressure). Immediately following venipuncture, serve breakfast and instruct participants to take hypoglycemic medication as prescribed.

3.3 Participant Selection, Clinic Reception, & Clinic Check Off Screens

3.3.1 Participant Selection Screen

I. PURPOSE

The C*heck In and Reception p*rocess is the first thing the participant experiences upon arrival and sets the tone for the entire visit. It is the means by which the computer is prepared for entry of all subsequent information.

II. METHODS

1 General Instructions

1.1 The Participant Section process is the first step in conducting the Exam 5 visit. The day begins with no participants checked in.

1.2 Participants are selected from the Master List and added to the Checked In List as they arrive.

1.3 No forms or screens will be available for a given participant until he or she is checked in on the computer.

2. Specific Instructions for Completing the Participant Selection Screen

2.1 Print the face sheet for the participant in advance. It will include ***Participant ID***, ***Name, Acrostic, Birth Date, QC ID***, and ***Language spoken***, and any procedures for which the participant has been selected.

2.2 The clinic reception process is very important in setting the participant's frame of mind for the rest of the exam day. Greet each participant warmly as soon as he/she arrives at the clinic. (If a participant arrives at the clinic acutely ill—e.g., "flu" or bronchitis—do not continue with the clinic examination. Make arrangements to contact him/her to reschedule the appointment after he/she has recovered.)

2.3 Enter the participant ID number and acrostic in the fields provided in the upper left part of the screen. (Typing of this information in full will avoid ID number errors and accidental selection of forms for the wrong participant.)

2.4 Once the correct identifying information has been entered, click the Continue button and the participant will be added to the Checked In List, which is the large white area on the lower half of the screen.

2.5 The computer will go to the Check-off screen, which at this point will provide only the Check-in/Consent

3.3.2 Check-in/Consent (Clinic Reception)

I. PURPOSE

The *Check-in/Consent* process is the means by which important information is collected as

soon as the participant arrives. The participant's consent status is recorded on this screen, so it must be completed before any procedures can be performed. In addition, it serves the purpose of informing clinic staff of specific procedures the participant has been selected to undergo.

II. METHODS

1 General Instructions

1.1 Instruct the participant to read the **informed consent** documents carefully, answer the questions at the end, and sign it (see section on informed consent). Once the participant has completed and signed the informed consent, the specific permissions must be entered in the Clinic Reception screen (can be done while participant changes clothes). Following this, the procedures to be performed will be displayed on the computer and the clinic visit will begin.

1.2 Ask the participant if he/she has any questions. After you have answered any questions, give the participant a gown (or robe) and slippers and take him/her to a dressing room to change. Provide a locker or other safe place for the participant's clothing and any other items that need to be stored. This concludes the Clinic Reception phase of the clinic visit.

2. Specific Instructions for Completing the Check-in/Consent Screen

2.1 The consent screen will first ask if the consent for the main Exam 5 has been signed. Once the participant has signed the consent, Click "Yes" and additional items will be displayed on the screen.

2.2 The ***Visit Date*** and check in time will automatically be filled with the current date and time; in the event that the actual visit took place on a different date, this field may be changed. Whenever possible, the second appointment date for Exam 5, if any, should be entered in the screen. If the second appointment cannot be scheduled at this time, a clinic staff person should go back and enter the second appointment date as soon as the information is available.

2.3 Associated permissions (HIPAA consent, medical records release, etc.) should be completed on this screen. Click Next Page to continue with the consent options.

2.4 Indicate participant consent for any ancillary studies for which he or she has been selected. On this screen, also indicate other permissions related to sharing of data and samples.

2.5 In the ***Local Medial/Hospital Identification Number*** field, record the participant's local hospital or medical record number (if any). This number is not required, however.

2.6 In the ***Reception Interview*** area, ask the participant when he/she last ate or drank. Record the information in military (24:00 hours) time. Record the time, again in military time, when this form is being completed. The computer will calculate and display the amount of time elapsed since the participant last ate or drank. If the two times given are less than 8 hours apart; reschedule the visit or the fasting component of the exam.

2.7 Ask if the participant has been ill in the last seven days (e.g., cold, flu, fever, vomiting.) If the participant responds "**Yes,**" inform the participant that the clinic exam cannot be completed at this time, thank him/her and reschedule another visit.

2.8 Once all consent responses have been entered, the remaining forms for the visit will be available.

3.3.3 Clinic Check Off Screen

I. PURPOSE

The *Clinic Check off Screen* is the means to ensure that all parts of Exam 5 are completed or scheduled. All Exam 5 forms, along with any ancillary study procedures for which the participant was selected and gave consent, will be displayed on this screen to allow for the tracking of the participant's progress through Exam 5. Activities for checking out the participant at the end of the visit are also included on this screen.

II. METHODS

1. General Instructions

1.1 The screen is used to access all the data entry screens and is not accessible until the consent process has been completed.

1.2 Make sure to schedule dates for the participant's CT, MRI, and/or Ultrasound IMT examinations, if appropriate.

1.3 Provide the participant with appointment reminders for the CT, MRI, and/or Ultrasound IMT exams, if appropriate.

1.4 Produce the Exit Report and give to participant.

1.5 Ask the participant if he/she has any questions at exam exit time. After you have answered any questions, thank the participant. This concludes the Clinic check off screen and the clinic visit day for the participant.

2. Specific Instructions for Navigating the Clinic Check Off Screen

2.1 Print the Phlebotomy sheet and give/send to phlebotomist immediately.

2.2 To enter the data for a given procedure, click on that procedure on the Check-off screen; the selected computer form will open and will be ready for data entry.

2.3 Visit day progress order should be as shown on the screen for the first five steps/procedures. The remainder of the procedures could be performed in any order.

2.4 For participants selected to have CT, spirometry, and/or MRI in Exam 5, schedule CT and/or MRI examinations if you have not done so and prepare appointment reminders to give to the participant on exit. Record the date and time for the appointment/s in the appropriate field on the screen.

2.5 When the participant has finished the exam, check the list of forms to be sure all are Complete. If there are forms or procedures remaining, schedule a second visit to complete the exam.

2.6 Click Check Out/Exit. This screen will allow you to print an exit report for the participant.

3.4 Interviews - Questionnaires

3.4.1 Interviewing Guidelines and Techniques

I. GENERAL INTERVIEW INFORMATION

1. Interviewer bias is any preference or inclination that creates a systematic difference between responses obtained by different interviewers. It can be affected by:
   - Respondent's perception of the interviewer and his/her reaction to that
   - Interviewer's perception of the respondent and his/her reaction to that

2. Characteristics of a good interview

   2.1 The interviewer creates a friendly, but businesslike atmosphere.

   2.2 The respondent is at ease. Keep these factors in mind:
   - the respondent may view a female interviewer as less threatening.
   - the respondent may view a much older interviewer as judgmental.
   - the respondent may view a much younger interviewer as inexperienced

   2.3 The interviewer obtains the answer to the question that is asked by:
   - proper use of probes and repeating a question rather than interpreting it.

   2.4 The interviewer obtains clarification of confusing answers.

   2.5 The interviewer gives only neutral responses to the respondent's answers.

   2.6 The interviewer accurately records responses.

3. Specific skills required for interviewers

   3.1 The ability to ask questions at the correct pace and in a conversational tone.

   3.2 A thorough knowledge of the questions and response categories (this will keep the interview flowing smoothly).

   3.3 Knowledge of how and when to use probes.

   3.4 The ability to think as an interviewer and to temporarily put aside other roles (e.g., researcher, health care provider).

   3.5 The ability to maintain a positive attitude about the interview (this lets the respondent know that the interview is important).

   3.6 The ability to keep some level of control over the interview process (e.g., by rewarding the respondent for answering questions but not for other behavior).

II. INTERVIEWING TECHNIQUES

1. Standardized Interviewing Technique

1.1 MESA is a collaborative study being conducted through six field centers located throughout the United States. In order to produce data that can be considered collaborative, MESA study designers must develop and use standardized approaches to train interviewers and collect information about participants. Standardization is achieved by using scripts in training, training supervisors centrally, establishing qualifications for supervisors, reviewing collected data, taping and reviewing interviews, and, finally, observing interviewers in the field.

1.2 It is critically important that interviewers read the sections in this manual that are applicable to the questionnaires they will be administering.

III. THE INTERVIEW

All interviews should be tape recorded for quality control purposes if the participant is willing to allow it.

The following procedures are recommended for a successful interview:

1. Prior to the visit prepare all materials (e.g., appropriate report, identification, stamped-self-addressed envelopes) that will be necessary for the interview.

2. Find an area where both you and the participant can talk and use the computer comfortably with minimal distractions.

3. Make sure that the participant understands the questions and that you are interpreting the responses accurately. Do this by restating what you think the participant is telling you. At the same time, be careful not to impose your interpretations on the interview questions or the participant's comments.

4. Convey your interest in the participant's thoughts and feelings, but do your best to keep him/her focused on the interview questions. When the participant strays from a question, try to use what he/she is saying to redirect the conversation back to the interview questions. Give positive reinforcement for direct answers. If necessary, set time limits at the outset of the interview to encourage the participant to stay on track.

5. Participants may try to convince you to answer certain questions for them. Let the participants know that you are interested in *their* answers.

6. Be aware of any hearing and vision impairments and their effects on the participant's understanding of the interview questions. If necessary, read the interview questions to participants who have visual impairments or limited reading ability.

7. <u>Encourage, but do not force, participants to answer to all questions</u>.

8. If non-participants are present during the visit, address the participant directly and do not encourage conversation with other parties. If necessary, ask that you and the participant be left alone for a brief time to complete the questionnaire.

9. Be able to adapt to interruptions. Let the participant know that you are willing to continue the interview after the interruptions are completed.

10. Make the interview a positive experience for the participant. React favorably to answers and give compliments, when appropriate.

11. Give the participant clear information about when the next clinic visit will be conducted and follow through with the plans that you make.

3.4.2 Participant Tracking

I. PURPOSE

The Tracking Form is an update of information collected during previous surveillance interviews. It allows us to collect information (name, address, telephone number, and email address) on the participant, his/her health care provider(s), and any proxies or contacts he/she may designate. We will use this information to contact and communicate with the participant and his/her physician(s), proxies, or other contacts. In Exam 5, this information needs to be reviewed and updated with any changes since form completion during the most recent follow-up contact.

Participant tracking is typically administered as part of the eleventh follow-up interview process. If this has not occurred, it should be administered during the clinic exam.

II. MATERIALS/EQUIPMENT

The participant's personal address book, a phone book, and a computer will all be useful in helping participants find and record the information asked for during this process.

III. DEFINITIONS

1. Proxy. A person designated by the participant to knowledgeably answer questions about the participant, in the event that he/she is unable to answer. A proxy may, but does not have to, live with the participant and should be familiar with the status of the participant's health.

2. Contact. A person designated by the participant who may be relied upon to know the participant's whereabouts. A contact does not live with the participant, but always knows how to get in touch with him/her.

3. Email address. A computer address where electronic mail is received e.g. bozo@clownmail.com.

4. Second surname. Another last name used by the participant. Some participants (e.g., some members of the Hispanic population) use two last names. Also, some married women use both their maiden name and their husband's last name.

IV. METHODS

General instructions

1.1 The most recent data for this questionnaire is pre-printed on the eleventh surveillance tracking form and was probably collected within the past month. If this is the case, tracking does not need to be repeated. The following instructions should be followed for participants whose eleventh surveillance interview has not yet been done.

1.2 Current information is essential for maintaining contact with participants and for communicating with their proxies, contacts, and health care providers. You should emphasize to the participants that the tracking form needs to be updated as completely and accurately as possible. Also encourage participants to designate as

proxies ***only*** those people who are familiar with the status of their health, because it is the proxies who will answer health-related questions if the participant is unable to.

1.3 The interviewer should verify and clearly print, in ink and in capital letters, all changes in the appropriate spaces. The participant should not use a nickname in place of a full, legal name. He/she should provide an area code with each phone number, even if within the local calling area. Boxes/spaces for items of information that are not applicable should be left blank. You should verify health care provider information using a local telephone directory. Obtain missing information over the phone.

Specific Instructions:

If the eleventh follow-up surveillance interview was not done prior to the Exam 5 visit, ask the participant as outlined below to obtain participant information:

Section A. Participant Information:

Begin with, "***Please VERIFY your name, address, telephone number(s), and email address (if you have one).***" Note any changes in section "A", "Changes" column. Then, ask the participant the following questions:

A1. *"Do you plan to change your name within the next year?"* (Note that this is question as well as questions A2 & A3 are not written in the form)
If "**no**", continue to question A2.
If "yes", ask, "***what will your new last name be?"***
Record the information in section "A", "Changes" column. Then, continue to the next question, A2.

A2. "*Do you plan to be out of this area for an extended period of time (a month or longer) within the next year?"*
If "**no**", continue to question A3.
If "yes", ask, "***approximately when will you leave and when will you return?"***
Record the month/year for both in section "A", "Changes" column. Then, continue to the next question, A3.

A3. *"Will there be a change in your local address within the next three months?"*
If "**no**", continue to section B, contacts/proxies.
If "yes", ask, "***what will your new address be?"***
Record the street address, city, state, and ZIP code in section "A", "changes" column. Then, continue with section B.

Section B. Contacts/Proxies Information:

"*Please update the following information on people who are familiar with the status of your health AND who could help us contact you, if necessary. If possible, please include one person who lives with you and one who does not."*

The participant should provide as much information as possible. Assist him/her, if necessary, in obtaining information. Record any changes regarding 'proxies' in the lines to the right of that particular 'proxy'.

If a 'proxy' was used to obtain the information on this form, the interviewer should check the appropriate box under the name of the 'proxy' person.

If the 'proxy' used to obtain the information on this form is not listed among the current proxies, ask the 'proxy' for his/her name, relationship to the participant, complete address, and telephone number. Fill the information in the spaces provided at the end of section B.

Encourage the participant to list contacts that do not all live at one address to obtain at least one contact that might be available if an entire family/household moves away.

Section C. Health Care Providers Information:

"Please update the following information about your health care providers i.e. a clinic, doctor, nurse, or physician's assistant who provides your usual medical care?"

If the participant does not have a health care provider then the form is complete. Thank the participant.

If the participant has a health care provider, record any changes in the "Changes" column. The form is now complete. Thank the participant.

3.4.3 Medical History

I. PURPOSE

The Medical History identifies the participant's medical conditions and provides other information that may:

- be used to adjust for co-morbidity;
- characterize the participant's access to medical care

II. METHODS

General instructions:

This is an **interviewer-administered** questionnaire. Questions should be read to the participant verbatim as they appear on the screen to ensure standardization. In addition, any introductory and transitional wording should be read verbatim.

Questions to be skipped due to participant responses will automatically be skipped by the computer. At the end of each screen, click the Save button to record the responses in the database and move to the next screen.

For most questions, possible responses are "Yes", "No," "Don't Know," and/or "Not Applicable" or "N/A" (not applicable). A few other questions have choices as indicated. The interviewer should read all choices to the participant and have the participant choose the appropriate response/s for each question.

Do not probe to make interpretations about a participant's specific symptoms. Ask questions as written and record answers as given by the participant.

Specific instructions:

Begin the questionnaire by reading to the participant the following introduction:
The following are some questions about your medical history. Questions refer to things that happened since your last MESA visit on _____. Please answer to the best of your knowledge.

1. ***How would you say your health currently compares with other persons of your age?***
   Select "better," "same," or "worse." Choose the appropriate response to the best estimate.

Questions 2–4 pertain to how the participant feels about him/herself when compared to others of his/her own age. The participant should be encouraged to estimate and answer "Yes" or "No." The participant may choose "Don't Know" if he/she cannot give a yes or no answer or does not know anyone his/her own age. [NOTE: These questions will be skipped if they were already answered in the eleventh follow-up interview.]

2. ***When walking on level ground, do you get more breathless than people your own age?***
   Select "yes," "no," or "don't know."

3. ***When walking up hills or stairs, do you get more breathless than people your own age?***
   Select "yes," "no," or "don't know."

4. ***Do you ever have to stop walking because of breathlessness?***
   Select "yes," "no," or "don't know."

5. ***Do you ever get pain in either leg or buttock while walking?***
   If "no," **skip to question 6**..
   If "yes," **ask the following:**

   a. ***Does this pain ever begin when you are standing still or sitting?***
      Select "yes" or "no."

   b. ***In what part of your leg or buttock do you feel the pain?***
      Choices include "pain includes calf/calves" or "pain does not include calf/calves."

   c. ***Do you get it if you walk uphill or hurry?***
      Select "yes" or "no."

   d. ***Do you get it if you walk at an ordinary pace on the level?***
      Select "yes" or "no."

   e. ***Does the pain ever disappear while you are walking?***
      Select "yes" or "no."

f. ***What do you do if you get it when you are walking?***
Select "stop or slow down" or "continue on."

g. ***What happens to the pain if you stand still?***
If "not relieved," **proceed to question h.**
If "relieved," ask ***how soon?***
Select "10 minutes or less" or "more than 10 minutes."

h. ***Is this pain predominantly in the right side, left side, or in both legs?***
Select one of the choices.

6. ***Are you taking aspirin on a regular basis?*** Examples of "regular" are daily, every other day, and weekly. If the participant says less than once a week, record "no."
Select "yes," "no," or "don't know.
If yes, **ask the following**: ***"how many days a week?"***
Record number of days/week.

7. ***Do you usually bring up phlegm on most days for 3 or more months during the year?***
Select "yes" or "no.
If yes, **ask the following**: ***"For how many years have you had this cough?"***
Record number of years. If the participant says less than one year, record "00."

8. ***Do you usually have a cough on most days for 3 or more months during the year?***
Select "yes" or "no.
If yes, **ask the following**: ***"For how many years have you brought up phlegm from your chest like this?"***
Record number of years. If the participant says less than one year, record "00."

9. ***Do In the last 12 months, have you had wheezing or whistling in your chest?***
Select "yes" or "no.
If "no," **skip to question 10**.
If "yes," **ask the following:**

a. ***In the last 12 months, how often have you had this wheezing or whistling?***
Select choice indicated by participant

b. ***In the last 12 months, have you had an attack of wheezing or whistling in chest that has made you feel short of breath?***
Select choice indicated by participant

Question 10 is asked to determine if the participant has had some type of inflammatory condition.

10. ***In the past two weeks, have you had any of the following:***

a. Fever
b. Cold, flu, or sore throat
c. Urinary infection (also called "bladder infection")
d. Seasonal allergy (such as hay-fever ***)***

e. Bronchitis
f. Sinus infection or sinusitis
g. Pneumonia
h. Gums bleeding while brushing or flossing (includes "periodontal disease" and "gingivitis")
i. Tooth infection
j. Flare-up of gout
k. Flare-up of arthritis
Select "yes," "no," or "don't know" for each item.

Questions 11-15 pertain to conditions the participant has been told he or she has by a doctor since the last MESA visit. The participant should choose "Yes" or "No" if he/she is fairly sure about the diagnosis and "Don't Know" if he/she believes he/she might have been told about the diagnosis but is not sure. If the person is cared for primarily by a health care provider other than a physician, such as a nurse practitioner, try to determine that the diagnosis was made in a medical setting and, if so, include the response.

Has a doctor told you that you have developed any of the following since your last MESA visit on____?

11. ***Diabetes*?** [NOTE: These questions will be skipped if they were already answered in the eleventh follow-up interview.]
Select "Yes", "No", or "Don't Know".
If "no," **skip to question 12**.
If "yes," **ask the following:**

a. ***Are you currently taking medicine for your diabetes?***
Select "Yes", "No", or "Unsure".
If "No or "Unsure"," **continue to question 12**.
If "yes," **ask the following:**
What kind of medicine are you taking for your diabetes?
Select choice indicated by participant

12. ***Emphysema* or *Chronic Obstructive Pulmonary Disease (COPD)?***
Select "Yes", "No", or "Don't Know".

13. ***Asthma*?**
Select "Yes", "No", or "Don't Know".
If "No or "Don't Know"," **skip to question 14**.
If "Yes," **ask the following:**
For some people, asthma symptoms completely go away as they grow older. Later in life, however, asthma may recur. At approximately what ages did you experience each of the following events?

Age developed first asthma symptoms
Age doctor first diagnosed asthma
Age at start of 10 year (or more) period without asthma symptoms
Age at first recurrence of asthma symptoms

For each event, enter the age it was experienced by the participant. If not known, select either the alternate choice or "Don't Know"

14. ***Liver problems?***
    Select "Yes", "No", or "Don't Know".
    If "No or "Don't Know"," **skip to question 15**.
    If "Yes," **ask the following:**
    Did you have liver failure?
    Select "yes" or "no.

15. ***Kidney disease?***
    Select "Yes", "No", or "Don't Know".
    If "No or "Don't Know"," **skip to question 16**.
    If "Yes," **ask the following:**
    Did you have kidney failure, require dialysis or transplantation?
    Select "yes" or "no.

16. ***Has your doctor or health care provider ever told you that you had a kidney stone?***
    Select "Yes", "No", or "Don't Know".
    If "No or "Don't Know"," **skip to question 17**.
    If "Yes," **ask the following:**

    a. ***How old were you during your first stone episode?***
       Enter age at first episode

    b. ***How many kidney stones have you had in the past?***
       Select choice indicated by participant

17. ***Have any first degree relatives (i.e. mother, father, siblings, children) ever had a kidney stone?***
    Select "Yes", "No", or "Don't Know".

At this point, men are done with the questionnaire. [Computer will skip to end of form.]

Reproductive History
—for women only—

Questions 18-20 should be asked only of women who are NOT post-menopausal. The computer will skip over these questions for post-menopausal women

If participant has previously reported removal of both ovaries, screen will skip to question 19. If this has not been previously reported, begin with question 18.

18. ***Have you had surgery to remove your ovaries?*** (Removal of the ovaries might have been in conjunction with a hysterectomy.)
    If "no" or "don't know," record and continue with question 19.
    If "yes," ask the following:
    At what age? Record the response.
    How many ovaries were removed? Select 1 or 2. **If both ovaries removed,**

computer will skip to question 21.

If participant has previously reported hysterectomy, screen will skip to question 20. If this has not been previously reported, proceed with question 19.

19. ***Have you had a hysterectomy (surgery to remove your uterus/womb)?***
(Hysterectomy might have been done in conjunction with removal of the ovaries.)
If "no" or "don't know," record and continue with question 20.
If "yes," ask the following:
At what age? Record the response in the boxes **and skip to question 21.**

If participant has previously reported going through menopause, screen will skip to question 21. If this has not been previously reported, proceed with question 20.

20. ***Have you had a menstrual period in the past 12 months?***
If "no" or "don't know," record and skip to question 21.
If "yes," ask the following:
How many periods have you had in the last 12 months? Record number of periods reported.

21. ***Since your last MESA visit, have you taken hormone replacement therapy?***
If "no," **questionnaire is completed.**
If yes, ask the following:
    a. ***Are you currently using hormone replacement therapy?"***
    If "yes," ***At what age did you begin?*** Record age and proceed to question b.
    If "no," ***At what ages did you take hormones?*** Provide age started and age stopped and proceed to question b.

    b. ***Which type of therapy were you on?"***
    Select "estrogen alone," or "estrogen with progestin," or "other types of hormone replacement"
    (Common estrogen-only preparations are Premarin or Estratab; common estrogen+progestin regimens are Premarin plus Provera, Estratab plus Provera, Prempro, or Premphase.)

At the end of the form, click the Save button on the last screen and the program will return you to the Clinic Checkoff screen to select the next form or procedure.

3.4.4 Eye History

I. PURPOSE

The Eye History questionnaire identifies the participant's present and past history of eye conditions and use of medications. The information will help in the assessment and diagnosis of the retinal photography findings.

MESA Retina or Retinal Photography aims at evaluating the relation of retinal microvascular characteristics (e.g., retinal arteriolar narrowing, arterio-venous nicking, and retinopathy) to subclinical cardiovascular disease, clinical disease, and their risk factors. It is proposed to test new hypotheses that link retinal microvascular characteristics and arteriolar caliber to a wide array of subclinical cardiovascular measures (including left ventricular function defined from cardiac magnetic resonance imaging, peripheral arterial function defined from radial artery tonometry, and endothelial function defined from flow-mediated vasodilation of the brachial artery), clinical cardiovascular outcomes (including coronary heart disease, congestive cardiac failure and stroke), and their risk factors (including hypertension and diabetes).

II. METHODS

1. General Instructions

This is an **interviewer-administered questionnaire**. Questions should be read to the participant verbatim as they appear on the form to ensure standardization. In addition, any introductory and transitional wording should be read verbatim.

If the topic should arise, remind the participant that all information is strictly confidential and will be used only for research purposes. Explain that information about the Eye History may be important in understanding their health. The information may, in turn, help us to better understand the causes of heart disease.

In general, for each question, possible responses are: "Yes" (and choosing either right, left, both eyes, or yes, don't remember which eye), "No", "Don't Know", "Refused" or "Not Applicable". Have the participant choose the appropriate responses for each question. Do not probe to make interpretations about a participant's specific symptoms. Ask questions as written and record answers as given.

2. Specific Instructions

Begin by reading the introductory statement then proceed with the questions. Remind the participant to ask for clarification at anytime when any question is unclear.

The Eye History asks about your present and past history of eye conditions and use of eye medications. The information will help us interpret the retinal photographs.

1. ***Have you ever been told by an eye doctor that you have or had a cataract in either of your eyes?*** Another term for a cataract is "opacity of the lens of the eye".

If "no", "don't know" or "refused" to give a response, go to question #2.
If "yes", ask, ***"in your left eye, right eye, or both eyes"***.

Bubble in the appropriate choice and continue with question #1a.

1a. ***Did you have a cataract operation?***

Cataract operation/surgery is defined as removal of the lens using any surgical techniques.
If "no", "don't know" or "refused" to give a response, go to question #2.
If "yes", ask, ***"in your left eye, right eye, or both eyes".***
Bubble in the appropriate choice and continue with question #1b.

1b. ***For the eye(s) above, when was your first cataract operation?***

Choices includes: "no operation", "don't know", "refused", and "year" of surgery.
If the participant had a cataract operation, they should give the year of his/her first or only operation for each eye.
Write the year of the first operation in the blocks provided or bubble in other response in the appropriate circle and continue with question #2.

2. ***For the past 3 months, or longer, have you experienced or been told you have dry eyes, where your eyes feel like something is in them, itch, burn, feel gritty, that is not related to allergies?***

If "no", "don't know" or "refused" to give a response, go to question #3.
If "yes", ask, *"****in your left eye, right eye, or both eyes"****.*
Bubble in the appropriate choice and continue with question #2a.

There are many manifestations of "dry eye" besides the symptoms in the question, including excess tearing. Most people will be familiar with this condition. They need not have been told by an eye doctor they have this condition.
Emphasize "for the past 3 months or longer" when you ask this question.
Emphasize "not related to allergy" when clarifying response.

If the participant has occasional episodes of dry eye, the response would be "yes" only if on average it is a complaint at least 4 out of 7 days for the past 3 months or longer.

2a. ***Have you been using artificial tears for your dry eyes for the past three months or more?***

Responses are: "yes", "no", "don't know" or "refused" to give a response.

Again, note that the question requires current (4 out of 7 days) use of artificial tears for the past 3 months or longer. In addition, there are a number of over-the-counter artificial tears which are used for this condition.
Visine, taken for allergies, does not qualify as an artificial tear unless specifically prescribed for dry eye.
Drops taken for contact lens do not qualify as artificial tears for dry eye.

3. ***Has a doctor ever said you had diabetes, or high blood sugar or sugar in your urine?***

If "no", "don't know" or "refused" to give a response, go to question #4.
If "yes", ask, *"****was it confirmed as diabetes or suspected to be diabetes?"***
Bubble in the appropriate choice and continue with question #3b.

This question asks whether the participant has ever been told by a health provider that he/she has diabetes, high blood sugar, or sugar in the urine. The diagnosis must be confirmed by a physician and cannot be a conjecture on the part of the participant about the conditions possibly being present.

Persons who state that they once had sugar in the urine or were told about some sugar in the urine should be asked, "Were you ever told that you have diabetes?" If diabetes is suspected, mark "suspect/possible/borderline"; if not, "no" should be marked. Gestational diabetes is not considered as being diagnosed to have diabetes and the correct response for women who give this history in the absence of a history of diabetes mellitus or "suspect diabetes" is "no".

3a. ***Have you ever had laser treatment applied to the retina, the back of your eye, because of diabetic retinopathy?***
Responses are: "yes", "no", "don't know" or "refused" to give a response.
If "yes", ask, *"**in your left eye, right eye, or both eyes"**.*
Bubble in the appropriate choice. Continue with question #3b.

"Yes", when laser photocoagulation or laser treatment for diabetic retinopathy is specified. Xenon photocoagulation would also be coded as a "yes".

Some older persons may be confused, mistaking the bright light of either a fundus camera, slit lamp, or direct ophthalmoscope for laser treatment (these are diagnostic examinations and are not laser treatment). If you think the participant doesn't understand the question, ask him/her who did the treatment, or ask the participant to describe the treatment.

3b. ***Have you ever had an injection in your eye, because of diabetic retinopathy?***
Responses are: "yes", "no", "don't know" or "refused" to give a response.
If "yes", ask, *"**in your left eye, right eye, or both eyes"**.*
Bubble in the appropriate choice. Continue with question #4.

4. ***Have you ever experienced or been diagnosed with age-related macular degeneration?***
If "no", "don't know" or "refused" to give a response, skip to question #7.
If "yes", ask, *"**in your left eye only, right eye only, or both eyes"**.*
Bubble in the appropriate choice and continue with question #6a.

This question should be asked exactly as written, using no probes. If the participant does not understand it, repeat the question, and if the participant is still not sure about the correct answer, mark "don't know". Macular degeneration can be present without affecting vision. If subject says they have early stages of age-related macular degeneration, mark "Yes" for specific eye.

4a ***Have you ever had laser treatment for macular degeneration?***
Responses are: "yes", "no", "don't know" or "refused" to give a response.
If "yes", ask, *"**in your left eye only, right eye only, or both eyes"**.*
Bubble in the appropriate choice and continue with question #7.

4b. ***Have you ever had an injection in your eye, to treat your macular degeneration?***
Responses are: "yes", "no", "don't know" or "refused" to give a response.

If "yes", ask, *"**in your left eye, right eye, or both eyes"***.
Bubble in the appropriate choice. Continue with question #5.

5. ***Have you ever had laser vision correction or refractive surgery to treat nearsightedness or myopia? If so, which eye had the surgery to improve your distance vision?***
This question is asked exactly as it is worded here. **NOTE:** We are only interested in laser correction surgery or radial keratotomy (RK) surgery used to improve nearsightedness. Some other terms to describe this surgery include: PRK, RK, Lasik, Excimer, or Refractive surgery. You may need to probe a response of "laser surgery," since this may possibly be for another reason (for example, for treating diabetic retinopathy.
If "no", "don't know" or "refused" to give a response, go to question #6.
If the answer is "Yes," bubble in the appropriate choice and continue with queston # 5a.

5a. ***Which eye(s) had the surgery to improve your distance vision?***
Record which eye(s) was/were operated on.

6. ***How would you rate your vision without correction (without eye glasses or contact lenses)?***
The question is asked exactly as it is worded here. Give the choices "Excellent", "Good", "Fair", "Poor", "Can't see at all".
Bubble in the appropriate choice and continue with question #7.

7. ***Do you have an optometrist or ophthalmologist that you go to?***
If "yes", continue with question #7a.
If "no", "don't know" or "refused" to give a response, bubble in the appropriate circle.
This questionnaire is now completed.

7a. ***If yes, would you give his/her name and telephone number?***
Record the participant's optometrist or ophthalmologist's name and phone number in the space provided. This questionnaire is now complete.

A reviewer should review the form for any incompleteness. Then complete the questionnaire form by filling in the fields in the box labeled, "For MESA Field Center Use Only". Record the interviewer ID, reviewer ID number, and Data entry ID.

3.4.5 Medications

I. BACKGROUND AND RATIONALE

1. The Medications Form is designed to enable collection of data on participants' use of all types of medications, both prescription and non-prescription, including supplements. Information about participants' use of medications is collected at the initial (baseline) clinic visit and at follow-up visits. The participant is asked to bring to the clinic containers for all medications used during the two weeks prior to the visit. The interviewer then transcribes the name of each medication, its strength, and for prescription medications, frequency of administration from the containers onto the data collection form. As the information is entered, the interviewer queries the participant about actual usage of each medication.

2. Collecting this information will allow us to describe medication use and any changing patterns of use over time, and may help us ascertain the effect of medications on the progression of atherosclerosis in this study population. It will be important to know what medications each participant is taking, in order to assess and perhaps attempt to explain subsequent participant events and any change in the degree of disease detected at follow-up visits.

II. MATERIALS AND EQUIPMENT

Current version of the Physician's Desk Reference (PDR)

III. DEFINITIONS

1. Time frame: All prescription and over-the-counter medications and supplements used during the *two weeks prior* to the clinic visit should be included.

2. Prescription medication: Medication for which a prescription was written by a physician, physician assistant, or nurse practitioner and dispensed by a pharmacist or a physician.

3. Non-prescription or over-the-counter medication: Medication or supplements purchased without a prescription.

4. It should be noted that occasionally a physician would write a prescription for a non-prescription medication. In that case, the medication should be recorded as prescription. If, however, the physician *recommends* a medication, rather than actually writing a prescription for it, it should be recorded as non-prescription.

IV. METHODS

This is an **interviewer-administered questionnaire**. Questions should be read to the participant verbatim as they appear on the form to ensure standardization. In addition, any introductory and transitional wording should be read verbatim.

1. Obtaining medication containers. A letter is sent to the participant before the clinic visit that includes instructions regarding medication containers. The participant is asked to bring to the clinic containers for all supplements, prescription, non-prescription medications and herbal medicines taken during the two weeks prior to the clinic visit.

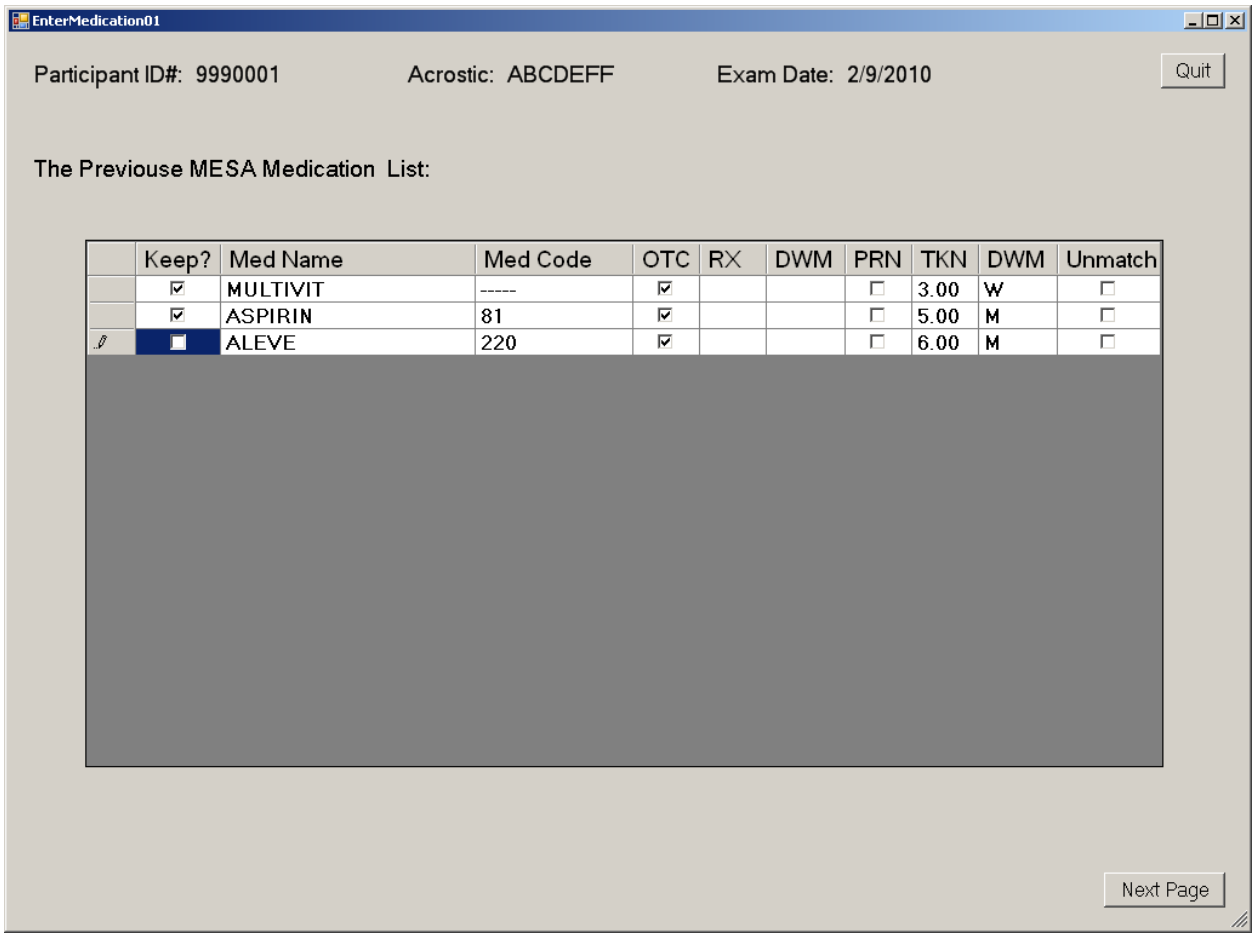
2. Medication use interview. Prior to beginning the interview, place all medications in front of the participant. The list of medications the participant reported at the previous exam will be available in the EDC program. Check each medication brought in by the participant against this list. For each one that appears on the list, check the "Keep?" box at the left of that particular medication. *IMPORTANT NOTE: You can only mark a medication to keep if the name is identical to what appears on the list.* For each medication you keep, edit the record with any updated information. Any other medications must be recorded as new medications on the Medications form.

   When asking the participant about a particular medication, show the container to the participant, keeping the other medications in view. Always conclude the interview by asking the participant if any other medications have been taken during the previous two weeks. If the participant remembers other medications, record the name, strength and frequency administered for each one in as much detail as possible. *If you are unsure about the accuracy of the participant's responses, schedule a telephone interview to verify the prescription label information.* At the end of the visit, make sure to return all medications and other personal belongings to the participant. Guidelines for completing the Medications Form follow:

   Section A. Medication Reception

   To begin, select the "Medications" from the list on the Clinic Checkoff Screen and read the following script to the participant:.

   As you know, the Multi-Ethnic Study of Atherosclerosis will be describing all medication its participants are using, both prescription and over-the-counter. These include pills, liquid medications, skin patches, eye drops, creams, salves, inhalers (puffers), and injections, as well as cold or allergy medications, vitamins, herbal remedies, and other supplements. The letter you received about this appointment included a plastic medications bag for all your current medications and asked you to bring them to the clinic. Have you brought this bag with you? Are these all the medications that you have taken in the past two weeks?



Screen 1

If "yes", ask to see the medications and use the provided medications to enter information into the Medications Form..On screen 2, select "Yes" for "Enter all meds?"

If "no", make arrangements to obtain medications at another time but record any available information as described above as best possible by interviewing the participant for the information. In screen 2, select "Incomplete" from the "Enter all meds?" section

If "refused", record reason for refusal in Comments Section on screen 2

If "took no medicines" select this from the "Enter all meds?" section in screen 2, *form is complete.*

If no medications were reported on a previous visit, the screen will report that the participant has no previous medications, and any medications brought to this visit will be entered as new medications. If medications were previously reported, they reported by the participant at their last MESA Exam will be listed below the script. Compare the provided medications with the list of previous medications and select "Keep" for any medications from the list that the participant is still currently taking. Select "Next Page." Please note the following:

1. Medication containers may be unavailable to the interviewer for a variety of reasons. Regardless of the reason, however, the interviewer should make an attempt to obtain the information necessary to complete the medication form.

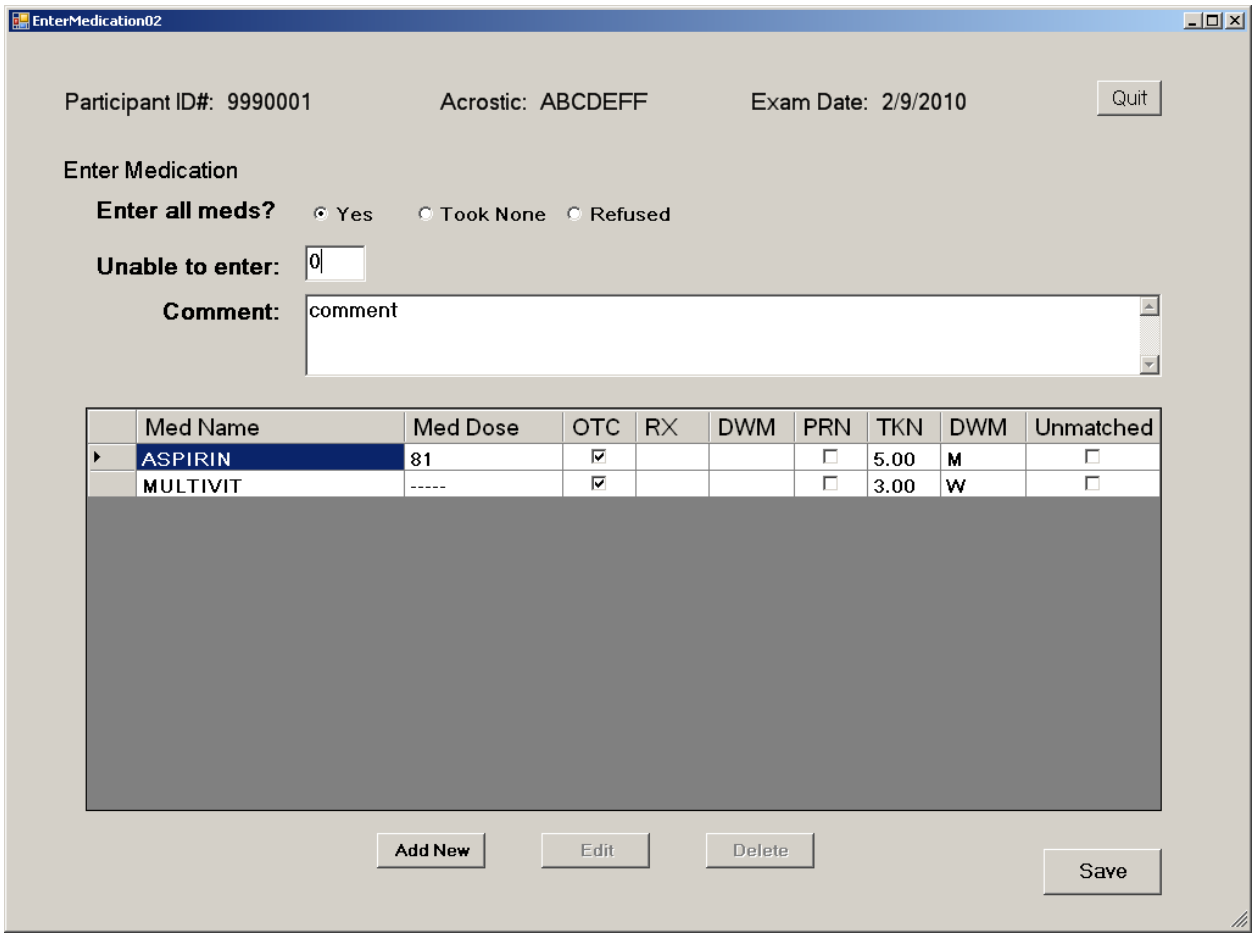
2. If the participant forgets to bring medication(s) to the clinic, the interviewer is responsible for obtaining the necessary information at a second visit or by telephone interview.

3. If the participant remembers additional medication(s) taken during the previous two weeks, the interviewer should record as much information about the medication as possible at the time of the visit and then follow up with a telephone interview to check for accuracy and completeness.

4. If the medication containers are unavailable because the participant refuses to bring them to the clinic, the interviewer should document the reason for refusal in the Comment Section. The interviewer should then attempt to obtain the participant's cooperation in obtaining the data, either by a second visit or by telephone.

5. If the participant brings a list of medications, instead of the medication containers, record all pertinent information from the list and note this in the Comments Section. If the interviewer has any doubt about the accuracy of the list, a follow-up telephone call should be scheduled to confirm what has been recorded.

6. Whenever medication information is collected by phone or from a list brought in by the participant instead of from the prescription container, try to verify the spelling of the name and the strength prescribed by referring to the PDR or some other source of accurate medicine listings.



Screen 2

Section B. Modifying the List of Medications

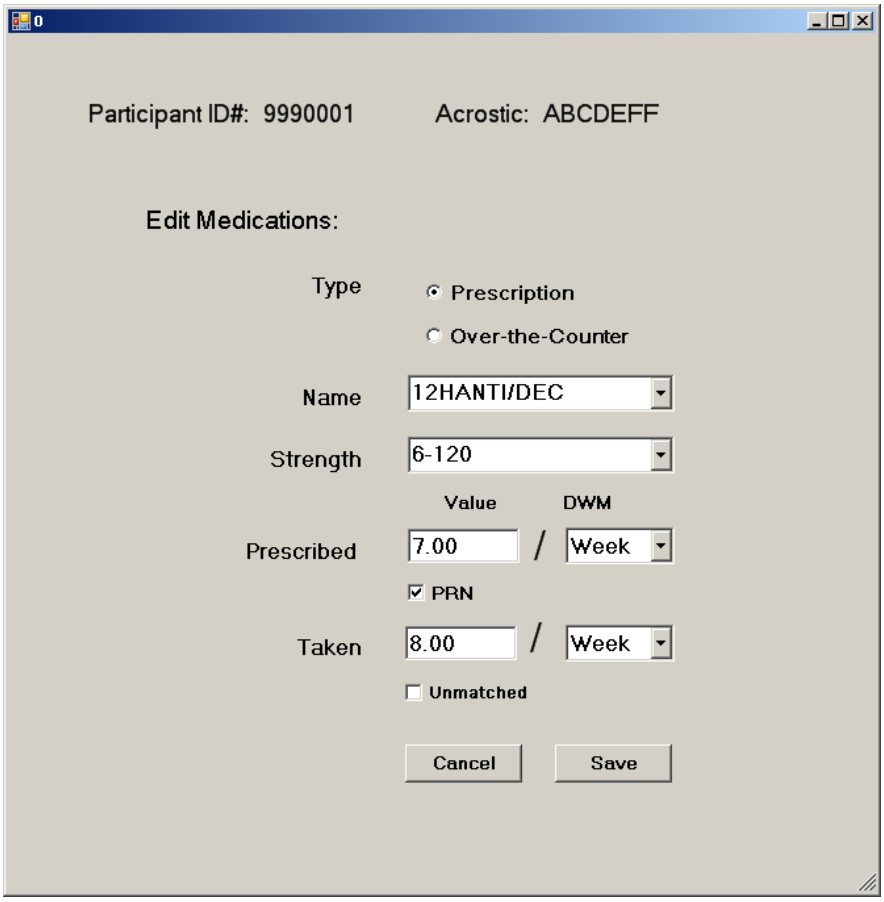
New Medications: For medications that were not reported at the last MESA Exam, select "Add New" at the bottom of screen 2, which will take you to screen 3. From the Type section, select either "Prescription" or "Over-the-Counter."

For Prescription Medications, record the name and dosage information from each new medication container onto the Medications Form using the following guidelines:

1. Medication name. Select medication name from the drop down box, or type in the name if it is not listed. The name of each medication should be recorded *exactly* as it is written on the container. Medication names that are misspelled or otherwise recorded incorrectly will cause data entry and analysis problems because they will not match the drug database. Do not record flavors of products or whether the preparations are sugar-free or sodium-free. If the medication name is longer than the spaces available on the form, type as much as possible and then record the complete medication name in the Comments Section. If it is not possible to type the medication name, insert an asterisk (*) and explain in the Comments Section.

2. Combination Medications contain two or more drugs. Some combination medicines,

such as Dyazide, come in only one fixed combination (hydrochlorothiazide 25mg and triamterene 50mg). These combination medicines do not usually list strength. Record the name in the "Medication Name" space and leave the "Strength" column blank.



Screen 3

Other combination medications are available in more than one fixed dose combination. For example, Inderide, which is a combination of propranolol and hydrochlorothiazide, is available as propranolol 40mg and hydrochlorothiazide 25mg, or propanolol 80mg and hydrochlorothiazide 25mg. These combination medications usually list the strength as in "Inderide 40/25" or "Inderide 80/25." For these medications, record the name in the "Medication Name" space and the strength combination (e.g., 40/25) in the "Strength" space.

3. Strength. Select the strength from the drop down list. If the strength is not listed, type in the information using the following guidelines:

   - Record the strength of each medication in milligrams (mg) whenever possible, beginning with the first space on the left in the "Strength" column.

- When strength is in milligrams, *do not* record the abbreviation "mg;" record *only* the amount of drug (e.g., if the strength is "250 mg," record only "250").

- When strength is not recorded as milligrams, record *all* numbers, digits, and characters used to denote strength, including:

  - milliliter (ml)
  - per milliliter (/ml)
  - milliequivalent (mEq)
  - hour (hr)
  - per hour (/hr)
  - percent (%)

- When strength is separated by a "/" (e.g. 40/25, as in combination medications), record them in this section.

- When strength is given in grains (gr), convert to milligrams using the following formula: (number of grains) x 65 = number of milligrams. (1 gr = 65 mg.)

- When strength is given in micrograms (mcg or µg), convert to milligrams using the following formula: (number of micrograms) ÷ 1000 = number of milligrams. (1000 mcg = 1 mg.)

- When strength is given in milligrams per milliliter (mg/ml), as is often the case with liquid medicine, record as in the following example: Ampicillin 125 mg / 5 ml is recorded as "125/5 ml." (Note omission of "mg.")

- When strength is given as a percentage (%), record as such.

- When strength is given in units (U) or units/milliliter (U/ml), as is often the case with Insulin, record as in the following examples: "100/ml" or "100U/ml."

- When it is not possible to record the strength, such as when it is not recorded on the medication label, record an asterisk (*) and explain in the Comments Section.

- Note: Do not record in the "Strength" column the number or quantity of medication items (e.g., number of tablets or tablespoons). See "Number Prescribed," below.

4. Number Prescribed. This column is designed to capture information on the number of pills (or milliliters, drops, units, etc) *prescribed* as opposed to the number actually taken. Information on the number prescribed should be taken from the medication labels.

   - Record the total number of medication items (e.g., "tablets") prescribed per the given time period (e.g., day, week, or month). Select the appropriate letter in the "Number Prescribed" column to show whether the prescribed number is per day (D), per week (W), or per month (M).

   - If the instructions include a range in the number of medication items and/or

times/day (or week or month) they are to be taken, record the lowest number of each. For example, if the label says, "take 1–2 tablets 3–4 times per day," record as "3 tablets/day" (i.e., 1 tablet 3 times/day = 3 tablets/day); or, if the label says, "take 1–2 tablets every 4 hours while awake," record as "5 tablets/day" (i.e., 1 tablet every 4 hours from 7 a.m. to 11 p.m.).

- When it is not possible to record the number of medication items prescribed per day, record an asterisk (*) and explain in the Comments Section.

- When instructions read "take as directed," record "1" as the number prescribed per day.

- When dosing instructions are complex (e.g., "take 1 pill every other day, alternating with 2 pills every other day"), record the *average* number per day (or week or month).

5. Number Prescribed: Specific Medications.

   - Pill/Tablets/Capsules: Record the total number prescribed per day (or week or month).

   - Solutions: Record the total number of milliliters prescribed per day (or week or month). Use the following conversions:
   - 1 teaspoon = 5 ml
   - 1 tablespoon = 15 ml
   - 1 ounce = 30 ml

   - Eye Drops: Record the total number of drops prescribed per day (or week or month). For example, "two drops in right eye, three times a day" = 6 drops, or "one drop in each eye, twice a day" = 4 drops.

   - Inhalers (puffers): Record the total number of sprays or puffs prescribed per day (or week or month).

   - Insulin: Record the total number of units injected per day (or week or month).

   - Creams/Lotions/Ointments: Record the total number of applications prescribed per day (or week or month).

   - Patches: Record the total number to be applied to the skin per day (or week or month).

   - Nitroglycerin Ointment: Record the total number of inches to be applied to the skin per day (or week or month).

6. PRN ("as needed") Medication is generally used for allergy, pain, or sleep; sublingual nitroglycerin is also used PRN.

   - Use the "PRN" box to indicate whether the medication is prescribed to be taken on an "as needed" basis.

- The words "as directed" do *not* mean the same as "as needed."

7. Number Taken. This column is designed to capture information on the number of pills (or milliliters, drops, units, etc) *actually taken* as opposed to the number prescribed. Information on the number *actually taken* should come directly from the participant. People do not always take their medications as prescribed. It is important to record information about both the number prescribed and the number actually taken as accurately as possible.

   - Ask the participant, **"On the average during the last two weeks, how many of these pills (or other medication items) did you take a day (or week or month)."**

   - Record the average number of pills (or other medication items) taken per day (or week or month) during the last two weeks.

   - Record "0" if none of the medication items was taken during the previous two weeks. This includes instances in which a prescription was filled but none of the medication was taken during the past 2 weeks.

   - When the number taken cannot be determined, record two asterisks (**) and explain in the Comments Section.

   - Circle the appropriate letter (D, W, M) to show whether the prescribed medication was taken per day, per week, or per month.

For Over-the-Counter Medications

Select "Over-the-Counter" in screen 3 and complete this section following instructions for Prescritpion Medication, above, but disregarding the instructions pertaining to "Number Prescribed" and "PRN Medication."

For Chinese and Other Traditional Medicines

Whenever possible, *in the comment section*, record traditional medicine use in the same fashion as with other medicine i.e. name, dosage, frequency. If this is not possible, record the *purpose* of the medicine.

Edit Medication

To edit information for medications that were reported at a previous MESA Exam, select the medication you wish to edit from the list and select "Edit" from the bottom of screen 2. Information for the medication will be displayed in screen 3 which can be edited according to the guidelines in the New Medication section above. After editing, select "Save" to return to screen 2.

Delete Medication

If a medication is listed in screen 2 in error, select the medication from the list and select "Delete" from the bottom of screen 2. Verify that you wish to delete the medication and select "Continue."

When finished entering all medications, select “Save: from the bottom of screen 2 to return to the list of forms.

3.4.6 Dietary Supplements Inventory

I. BACKGROUND AND RATIONALE

1. Information about participants' use of dietary supplements was collected at the initial baseline (Exam 1) clinic visit and is being collected again at Exam 5. The participant is asked to bring to the clinic containers for all dietary supplements used during the two weeks prior to the visit (the same as requested for medications). After conducting the medication use inventory with the participant, the interviewer then conducts the supplement use inventory recording the type and name of each supplement (e.g. multivitamin Centrum Silver, single nutrient supplement vitamin C, or botanical supplement ginseng), the amount taken per week, and duration of use. As the information is entered, the interviewer queries the participant about actual usage of each supplement.

2. Collecting this information will allow us to describe supplement use and any changing patterns of use over time, and may help us ascertain the effect of supplements (along with other diet related factors) on the progression of atherosclerosis in this study population

II. MATERIALS AND EQUIPMENT

III. DEFINITIONS

1. Time frame: All supplements used during the *two weeks prior* to the clinic visit should be included.

2. Dietary supplement: any multivitamin, single vitamin, or botanical taken during the timeframe of interest mentioned above. While supplements are commonly in pill form, other formulations are possible (e.g. liquid). If a prescription is written for the substance, it is considered a prescription medication for the purposes of this study (and would need to be recorded as a medication (see section 3.4.5).

3. Multivitamin: any supplement containing two or more individual nutrient or botanical ingredients. Common examples are Centrum Silver and One-A-Day multivitamins

4. Single Nutrient Supplement: any supplement containing only one nutrient. Single nutrient supplements are typically single vitamin supplements such as Vitamin C tablets/pills, but may also include single mineral tablets/pills such as Iron tablets/pills.

5. Botanical/Other Supplement: any other supplement that does not readily fit into a medication definition or definitions of multivitamins or single nutrient supplements (such as ginko, ginseng, or saw palmetto).

IV. METHODS

This is an **interviewer-administered questionnaire**. Questions should be read to the participant verbatim as they appear on the form to ensure standardization. In addition, any introductory and transitional wording should be read verbatim.

1. Obtaining supplement containers. A letter is sent to the participant before the clinic visit that includes instructions regarding supplement containers. The participant is asked to bring to the clinic containers for all supplements (and prescription, non-prescription medications and herbal medicines) taken during the two weeks prior to the clinic visit.

2. Supplement use interview. Prior to beginning the interview, place all supplements in front of the participant.. Separate each supplement into multivitamin, single nutrient supplement, and botanical/other supplement groups.

When asking the participant about a particular supplement, show the container to the participant, keeping the other supplement containers in view. Always conclude the interview by asking the participant if any other supplements have been taken during the previous two weeks. If the participant remembers others, record each one in as much detail as possible. *If you are unsure about the accuracy of the participant's responses, schedule a telephone interview to verify the prescription label information.* At the end of the visit, make sure to return all supplement containers and other personal belongings to the participant. Guidelines for completing the Dietary Supplements Inventory Form follow.

Whenever supplement information is collected by phone or from a list brought in by the participant instead of from the container, try to verify the spelling of the name (if not familiar and not an exact match with an entry in the supplements inventory application, call the Coordinating Center or refer to the manufacturer website or search online).

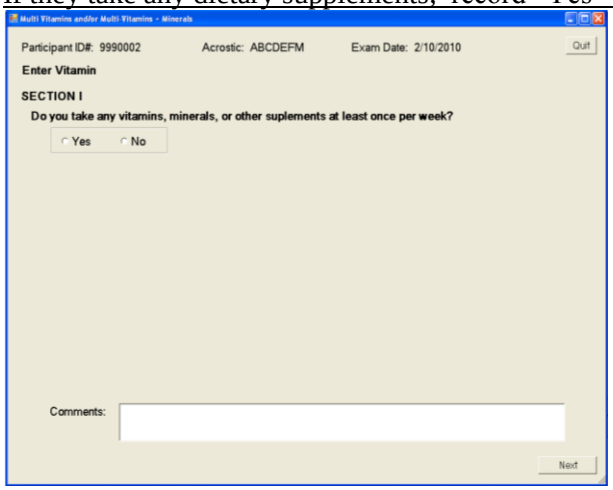
Section A. Medication Reception

To begin, select "Dietary Supplements" from the list on the Forms Page and read the following script to the participant.

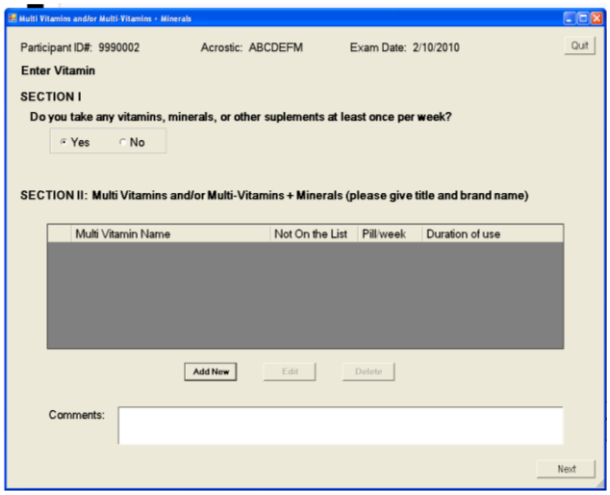
We would like to record dietary supplements that participants are using. These include, vitamins, botanicals, and other supplements in pill, tablet or liquid form. Have you brought all of the dietary supplements you have taken in the past two weeks?

Screen 1

If they take any dietary supplements, record "Yes" in section 1.

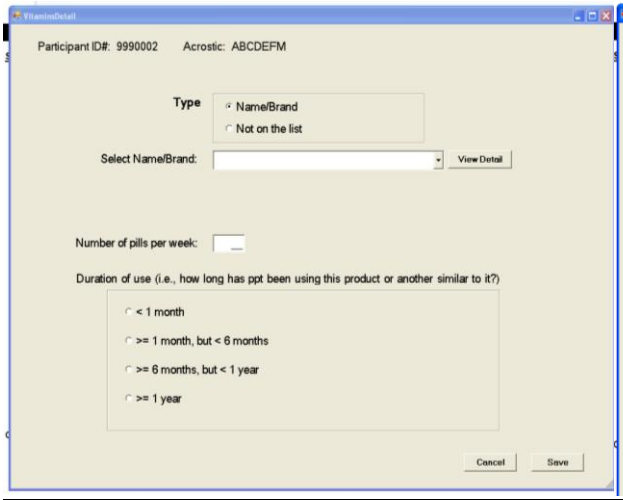


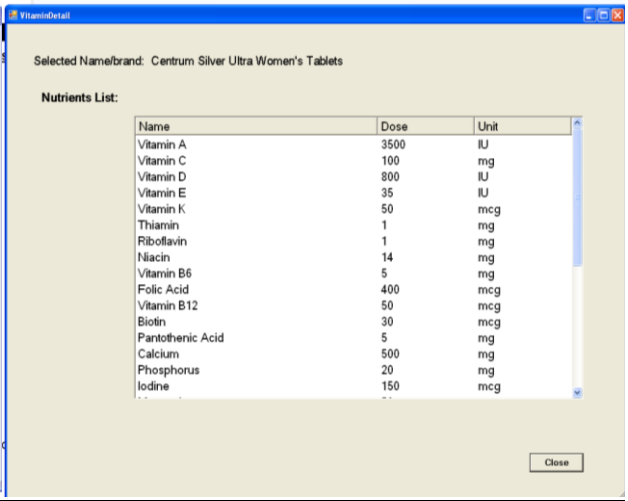
If any of the dietary supplements are multivitamins, select "Add New" in section II.



Select Name Brand and begin typing the name of the multivitamin into the Select Name/Brand box on the screen.

If the multivitimin is in the list, select it. If you are uncertain whether the name on the label is a match to name in the drop down list, select the "View Detail" button and check the individual ingredient amounts on the screen that comes up against the ingredient amounts listed on the label.



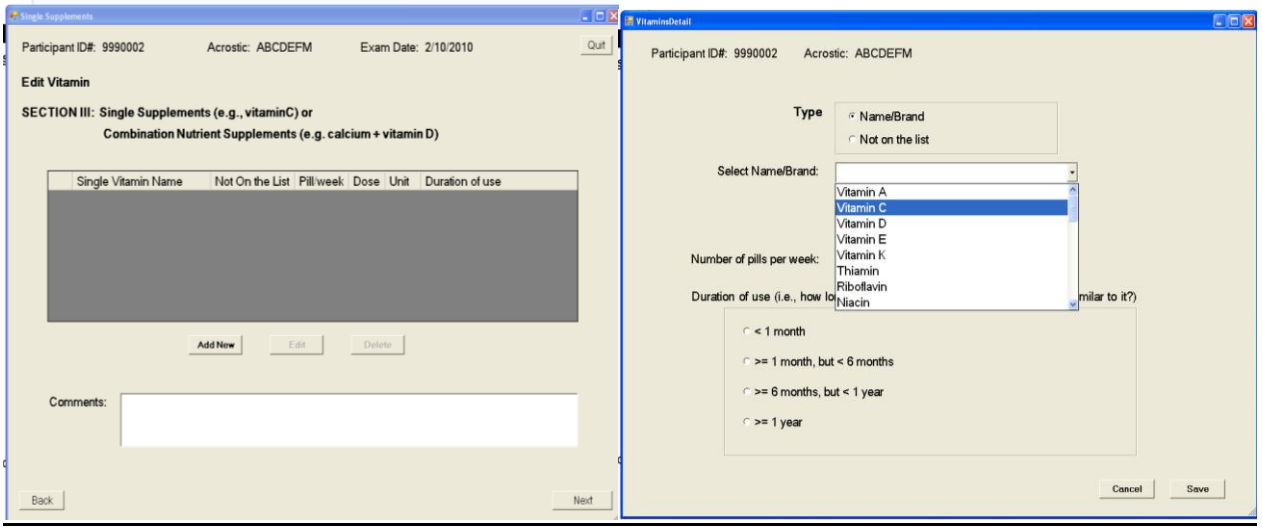


If a match to the multivitamin cannot be located in the Name/Brand drop down list, then select "Not on the list" in the Type and record a new multivitamin name in the Enter other field. Coordinating Center will review the information provided and will add any multivitamins that can be verified to the list.

Record the number of pills taken per week.

Record Duration of use and select "Save".

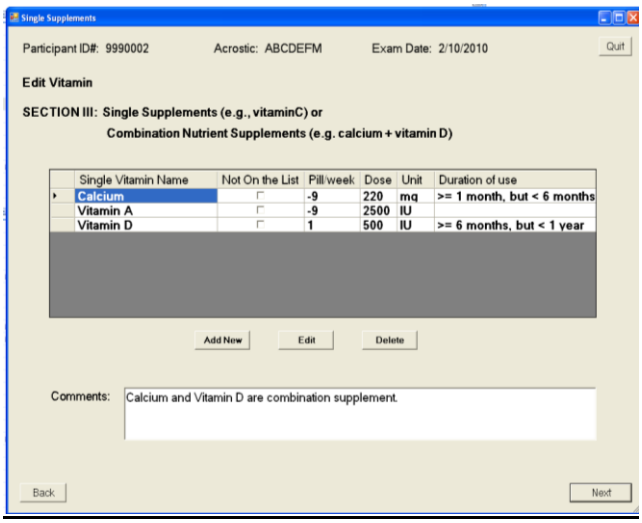
For each multivitamin taken, record an entry by selecting "Add New" and following the same steps noted above. When all are entered, select "Next" which will take you to Section III (Single Supplements).



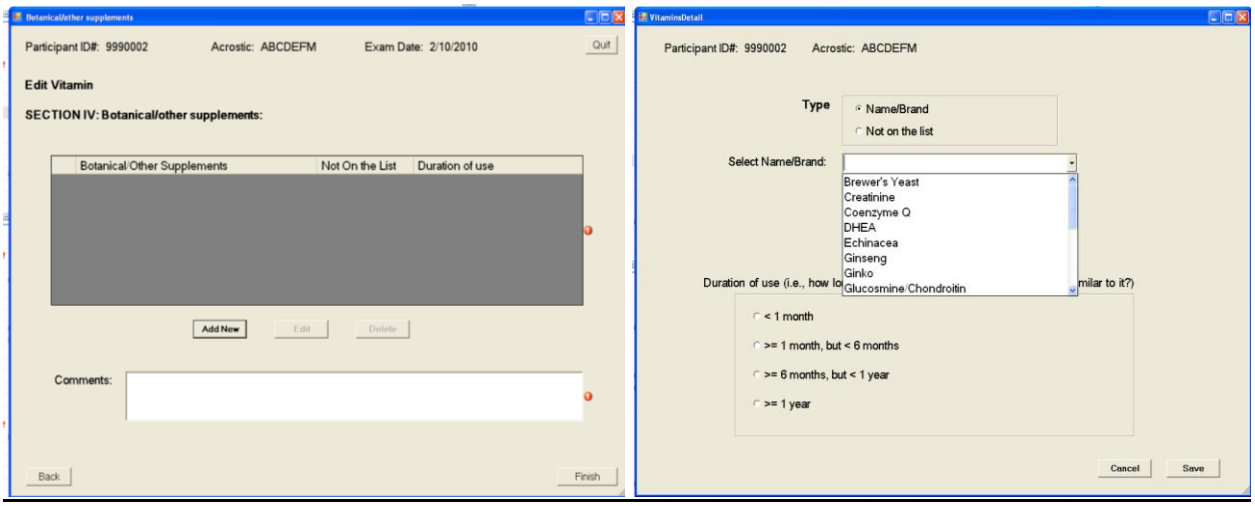
If the participant is taking one or more single (or combination) supplements, select "Add New" in Section III and select "Name/Brand" for type. Select the vitamin or mineral in the list and then record dose, unit (please report using the default unit if provided on the labeling), the number of pills per week, the duration of use, and then select "Save".

If the vitamin or mineral is not in the list, please select "Not on the list", record the vitamin or mineral name in the "Enter other" field and then record dose, unit (please report using the default unit if provided on the labeling), the number of pills per week, the duration of use, and then select "Save".

If the participant is taking a combination supplement (e.g. calcium and vitamin D), please enter as two separate single supplements.



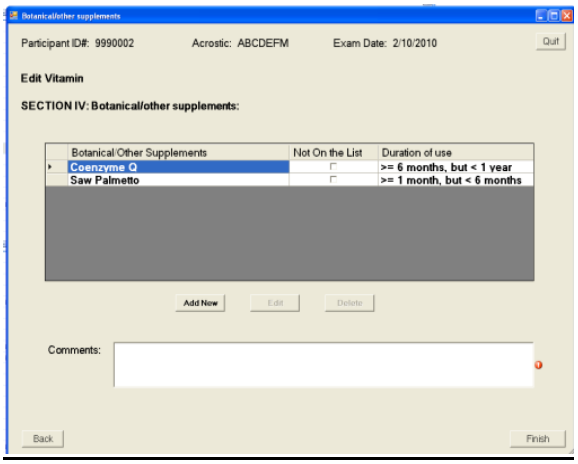
Continue recording single supplements following the steps outlined above until all are recorded. When all single supplements have been recorded, select "Next" which will take you to Section IV (Botanical/other supplements).



If the participant is taking one or more single (or combination) botanical supplements, select "Add New" in Section III and select "Name/Brand" for type. Select the botanical or other supplement in the list and then record the duration of use, and select "Save".
If the botanical/other supplement is not in the list, please select "Not on the list", record the name in the "Enter other" field, duration of use, and then select "Save".

If the participant is taking a combination botanical supplement, please enter as separate supplements.

Continue recording botanical/other supplements following the steps outlined above until all are recorded. When all have been recorded, select "Finish" which will take you to back to the main menu for this participant.



1. Supplement containers may be unavailable to the interviewer for a variety of reasons. Regardless of the reason, however, the interviewer should make an attempt to obtain the information necessary to complete the medication form.

2. If the participant forgets to bring supplements to the clinic, the interviewer is responsible for obtaining the necessary information at a second visit or by telephone interview.

3. If the participant remembers additional supplements taken during the previous two weeks, the interviewer should record as much information about the supplement as possible at the time of the visit and then follow up with a telephone interview to check for accuracy and completeness.

4. If the containers are unavailable because the participant refuses to bring them to the clinic, the interviewer should document the reason for refusal in the Comment Section. The interviewer should then attempt to obtain the participant's cooperation in obtaining the data, either by a second visit or by telephone.

5. If the participant brings a list of supplements, instead of the containers, record all pertinent information from the list and note this in the Comments Section. If the interviewer has any doubt about the accuracy of the list, a follow-up telephone call should be scheduled to confirm what has been recorded.

6. Whenever supplement information is collected by phone or from a list brought in by the participant instead of from the container, try to verify the spelling of the name (if not familiar and not an exact match with an entry in the supplements inventory application, call the Coordinating Center or refer to the manufacturer website or search online).

For other traditional supplements

The botanical/other supplements section of the form allow entry of new dietary supplements not already listed. Supplements that are common in MESA subcohort cultures should be entered here.

3.4.7 Personal History

I. PURPOSE

The Personal History questionnaire is used to collect information on socio-economic status (SES) and smoking and drinking habits, all of which are related to an individual's risk of cardiovascular disease.

II. METHODS

General instructions:

This is a **self-administered questionnaire**. Provide the participant with a laptop computer with the Personal History screen already open and give brief instructions for completion. If the participant is unable to self-administer the questionnaire, then a MESA staff member will administer the interview.

Ask the participant to try to answer all questions. Remind him/her to request assistance from a staff member if anything is unclear. Most participants should be able to complete the questionnaire on their own. However, if the participant expresses or appears to have difficulty reading or comprehending the questions, or if he or she has difficulty using the computer, offer to help and make arrangements for an interviewer administered version in the appropriate language. Instruct participant to give the laptop back to the interviewer when the form is completed. (Instruction will also be displayed on screen.)

Specific instructions:

- Instruct the participant to read each question and its instructions carefully then enter his or her response.
- Show the participant how to click on choices or enter responses.
- Explain that the computer will help by automatically skipping the questions that don't need to be answered because of a prior response
- If he/she is unsure about an exact answer (e.g., for "average number of drinks per week"), tell him/her to give a best estimate.
- In questions where the participant is asked about number of times used, instruct him/her to fill in "00," if use is less than one.

Participant Information (questions 1–3)

The participant will begin the questionnaire with the introduction below:

This form is intended to collect information about your background and lifestyle which may impact your risk of cardiovascular disease. Please complete all items except those which you are specifically instructed to skip. If you are unsure about the answer to a specific question, please estimate the answer to the best of your ability. If you have a question about a particular item, ask a staff member for clarification.

1a. ***Has your employment status changed since your MESA clinic visit on [Exam 4 visit date]?***
Choose Yes or No.

If no, the computer will skip to question #2.
If yes, the computer will continue with 1b.

1b. ***Choose one of the following that best describes your current occupation.***
Click on the most appropriate choice.

2. ***Do you do volunteer work?***
Choose Yes or No.

3. ***Where do you usually go for medical care?***
Participant should select (or type in) the place he/she goes most often for medical care. Participant should mark "other" only if the response clearly does not fit one of the given responses. For example, an urgent care clinic would be included in the "doctor's office or clinic" category. If "other" is selected, the participant should type in the type of place he/she goes for medical care.
4. ***To help pay for your medical care, do you now have:***
Participant should select (or type in) all applicable items.

Alcohol usage and smoking (questions 5–17)

All participants should answer questions 5, 10, 14, 15, 16, and 17. People who drink should complete questions 6–7; former and current smokers should complete question 11, 12, and 13; and current non-smokers should complete questions 14, 15, 16, and 17. If the participant feels uncomfortable with these questions, please reassure him/her that all collected information is strictly confidential. This section begins with the following introductory script:

The following questions are about your use of alcohol and tobacco. They will help us better understand the role of smoking and alcohol use in the risk of cardiovascular disease.

5. ***Do you presently drink alcoholic beverages?***
Choices are "yes" or "no". **If response is no, the computer will skip to question 10.**

6. ***How many glasses of red wine do you usually have per week?***
(1 serving = 3.5 oz glass, 1 bottle = 750ml = 8 glasses)
Enter the average number of drinks per week. Enter "00" if less than one glass of red wine per week.

7. ***How many glasses of white wine do you usually have per week?***
(1 serving = 3.5 oz glass, 1 bottle = 750ml = 8 glasses)
Enter the average number of drinks per week. Enter "00" if less than one glass of white wine per week.

8. ***How many cans, bottles, or glasses of beer do you usually have per week?***
(1 serving = 12 oz glass, 1 bottle = 355ml = 1 glass)
Enter the average number of 12-ounce drinks per week. Enter "00" if less than one serving of beer per week.

9. ***How many drinks of liquor or mixed drinks do you usually have per week?***
(1 serving of liquor = 1.5-ounce shot-glass, or one mixed drink)
Enter the average number of drinks per week. Enter "00" if less than one drink of liquor

per week.

10. ***Which of the following best describes your current smoking status?***
Click on the appropriate response.
If never smoked, the computer will skip to question 14 and continue with the questionnaire.

11. ***On the average of the entire time you smoked…..***

    11a. ***How many cigarettes did you smoke per day?***
    Enter the average number of cigarettes you smoked. Enter "00" if less than one cigarette per day.

    11b. ***Did you inhale the cigarette smoke?***
    Click on the most appropriate choice.

    11c. ***In the morning, how much time after you wake up did you smoke y our first cigarette?***
    Enter number of minutes between the time you wake up and the time you have your first cigarette. Enter "00" if less than one minute.

12. ***Have you smoked cigarettes during the last 30 days?***
Choose "yes" or "no".
If no, computer will skip to question 14.
If yes, computer will continue with question 13

13. ***On average, about how many cigarettes a day do you smoke?***
Provide the number of cigarettes smoked per day.
The participant should record 00 if the average number of cigarettes per day is less than one. Make sure participants record the number of *cigarettes* per day. If a participant answers in number of packs per day, recalculate into number of cigarettes per day (1 pack = 20 cigarettes).

14. ***During the past year about how many hours per week were you in close contact with people when they were smoking? (e.g., in your home, in a car, at work or other close quarters)***
Provide number of hours per week.
The goal of the question is to obtain information on passive exposure to *cigarette* smoke (excluding cigars, pipes, etc.) in any type of close quarters during the past 12 months. Record the number of hours in a *typical* week; do not include isolated or atypical situations, such as holiday gatherings or short-term house guests who smoke. If participants do not remember the exact amount of time, ask them to give their best estimate. Record 00 if participant was exposed to less than 1 hour of cigarette smoke per week.

15. ***Did anyone smoke in your residence in the past 12 months? (This includes you.)***
Choices are "yes", "no", or "don't know".
If response is no, the computer will skip to question 16.

    15a. ***On average, how often did someone smoke in your residence in the past 12 months?***

Click on the most appropriate choice.

15b. ***On average, how many cigarettes per day were smoked in the residence by each smoker in the past 12 months?***
Enter the number of cigarettes per day smoked by each of up to three smokers. If participant is a smoker, smoker 1 should be the participant.

15c. ***On average, how many cigars per day were smoked in the residence by each smoker in the past 12 months?***
Enter the number of cigarettes per day smoked by each of up to two smokers. If participant is a smoker, smoker 1 should be the participant.

16. ***As an adult, have you ever lived with a regular cigarette smoker (not including yourself) who smoked in your home?***
Choices are "yes," "no," or "don't know."
If yes, enter the total number of years you lived with them when they were smoking.

17. ***As an adult, have you ever spent time on a regular basis, when you were not at home, indoors where there were people smoking cigarettes (for example, at work)?***
Choices are "yes," "no," or "don't know."
If yes, enter the total number of years you spent time indoors (away from home) with people who were smoking.

Questions #18 and 19 ask about the participant's family finances. It begins with:

The following questions have to do with family finances. We know from other research that financial strain is common and very important to consider in understanding people's health. The following questions will be used to help give us a picture of the various financial situations experienced by persons participating in the MESA study. Any information you provide is strictly confidential and will be used for research purposes only.

18. ***Below is a list of income groups. Please tell me which group best represents your total combined family income for the past 12 months. This includes the total income, before taxes, earned in the past year by all family members living with you. Please include money from jobs; net income from business, farm, or rent; pensions, dividends, welfare, social security payments, and any other money received by you or any other family member living with you.***
Click on the most appropriate choice.

19a. ***Including yourself, how many people are supported by the income listed in the previous question?***
Enter the number of people supported by the income you indicated.

19b. ***Including yourself, how many of these are:***
- ***Children under 18?***
- ***Adults 65 and over?***

Enter the number of children supported by your income in the first box
Enter the number of adults 65 and over supported by your income in the second box.
Enter 00 if no one in that age category is supported by the given income.

At this point, women have completed the questionnaire. Men will continue on to complete the Erectile Dysfunction (ED) form.

MESA staff will review the questionnaire for completeness, clarify any question that were not answered, and complete the questionnaire by filling out the box "For MESA Field Center Use Only:"

- If the form was self-administered, check for completeness.
- Mark if form was self-administered or interviewer-administered.
- Record Interviewer or Reviewer ID.
- Record Data Entry ID.

Interview is complete.

3.4.8 Erectile Dysfunction (ED)

I. PURPOSE

The ED form is used to study whether early vascular disease and environmental exposures may be risk factors for erectile dysfunction (ED) and to understand the relationship between ED and other diseases.

II. METHODS

General instructions:

This questionnaire will seamlessly follow the Personal History form for men, and will appear to be a continuation of that form. It will not be separately selected from the Clinic Check-off screen. It will never appear for female participants.

Specific instructions:

This form consists of a single question:

Many men experience problems with sexual intercourse. How would you describe your ability to get and keep an erection adequate for satisfactory intercourse? Would you say that you are…..

Choices are "Always or almost always able", "Usually able", "Sometimes able", "Never able", and "Don't know".
Click on the most appropriate choice.

Form is now complete

3.4.9 Physical Activity

I. PURPOSE

The MESA Typical Week Physical Activity Survey (TWPAS) is designed to identify the time and frequency spent in various physical activities during a typical week in the past month. The rationale for the selected time frame of a typical week in the past month is the intention to capture typical activity patterns in one's daily life.

The survey has 20 question items in categories of household chores, lawn/yard/garden/farm, care of children/adults, transportation, walking (not at work), dancing and sport activities, conditioning activities, leisure activities, occupational activities and volunteer activities.

II. METHODS

1. General Instructions

1.1 This survey is a self-administered questionnaire. Provide the participant with a laptop computer with the Physical Activity Survey screen already open and give brief instructions for completion. Ask the participant to try to answer all questions. Remind him/her to request assistance from a staff member if anything is unclear. Most participants should be able to complete the survey on their own.

1.2 Instruct participant to give the laptop back to the interviewer when the form is completed. (Instruction will also be displayed on screen).

1.3 If the participant expresses or appears to have difficulty reading or comprehending the questions, or if he or she has difficulty using the computer, offer to help and make arrangements for an interviewer administered version in the appropriate language. If interviewer administered, questions should be read to the participant verbatim as they appear on the form to ensure standardization. In addition, any introductory and transitional wording should be read verbatim.

1.4 For the majority of the questions, response choices are "Yes" or "No". Record the response given by the participant. If "Yes", ask for the number of days per week, amount of time in hours and/or minutes per day the participant spent doing the particular activity.

2. Specific Instructions

Items to be completed by the interviewer:

- The ***Date*** will be pre-filled. Please verify that the date the form was completed is correct. For example, April 1st , 2010 would be entered as 04/01/2010.
- ***ID#*** & **Acrostic** will be pre-filled. Please verify that the ID and acrostic are a match to the participant.

- Instruct the participant to read each question and its instructions carefully then enter his or her response.
- Show the participant how to click on choices or enter responses.
- Explain that the computer will help by automatically skipping the questions that don't need to be answered because of a prior response.
- Explain that not checking a number is interpreted the same as 0 (e.g. for 15 minutes only the hours would remain unchecked while the minutes would be recorded as 15).
- Explain that if the participant would like to change an answer to one of the questions, that the question response should first be checked "No" (which will reset the days, hours, and minutes all to unchecked). Then if the participant would like to record a different amount of time indicate "Yes" and then proceed with responding with the corrected number of

days, hours, and minutes.
- If he/she is unsure about an exact answer (e.g., for number of days per week of an activity), tell him/her to give a best estimate.

Begin the questionnaire by reading the following instructions to the participant:

Think about the types of activities you did in a typical week in the past month. Please indicate whether you did or did not perform each of the following activities in a typical week. For each item that you respond 'yes,' you will be asked for the number of days in a typical week you did these activities and the average amount of time per day in hours and minutes.

Define intensity levels for the participant:
Most of the survey questions ask about light, moderate, and heavy intensity activities.
Light intensity *refers to activities that require little effort and are easy to do.*
Moderate intensity *refers to an effort that is harder than light intensity but is not an all-out effort.*
Vigorous intensity *is a very hard activity and requires all-out effort.*

Show the participant using the following example: Begin this with, "***Let me show you an example of how we will fill out the survey."***

To orient him/her to the past month, you will identify that period for the participant. In the text box above, if we assume, for instance, that the current date is September 15, the past month would start on August 15.

Using the example below and review each step with the participant. Give him/her time to consider each step and to ask questions. Explain that, if the participant continued usual physical activities while on vacation (or during some other atypical period of time), he/she should report them as usual for a typical week. However, if usual activities were stopped during a vacation, or if the participant took up other activities *only during that period*¸ then he/she should not record them as typical activities.

Questions people might ask about completing the TWPAS, and some sample responses:

- What if every week in the past month was different?
  Think about the week that was most typical of your activity patterns for that activity in other times of the year and fill in the circle for the number of days and hours per day and or minutes per day.

- What if the length of time is different each day?
  Think about the average in all the days that reflects your typical time for the activity in a typical week.

- I was on vacation in the past month when I went on a 2-week bicycle trip. Should I include this in the estimates?
  In this case, think about a typical week in the past couple of months that reflects your usual activity patterns. We are trying to identify the activity patterns you do on a usual basis, so if your vacation was not typical, do not include it.

Once you have reviewed the sample question with the participant and explained the difference between typical and atypical activities, ask if he/she has any questions.

It is possible that a person will spend more time doing activities on one day or another (e.g., weekends). If this is the case, ask him/her to estimate the usual time during each event in a typical week, averaging in the longer and shorter days.

For example, if the participant engages in an activity for 30 minutes/day 5 days/week and 1.5 hours/day 1 day/week, ask him/her to add about 15 minutes extra to each day (45 minutes/day 5 days a week), to take into account a single day that has a prolonged bout of activity compared to the usual.

3. Item-by-Item Clarification

Household chores

"In a typical week in the past month, did you do… (light effort household chores)." The examples should be read to the participant for clarification.
If "no", record no and you will be skipped ahead to the next question.
If "yes", then ask, "***About how many days per week?"*** *record* the number of days.
Then ask, ***"About how many hours or minutes per day?*** Record the number of hours and/or minutes e.g. the participant response 1hr and 15 mins, record 1 under hours and 15 under minutes. These series of questions should be applied to similar types of questions in the questionnaire.

1. Light effort Household Chores: These activities are light intensity, routine, usually daily activities that people do during the care and maintenance of a household. Examples include cooking and cleaning after cooking, straightening up the house, grocery and household shopping and putting things away, changing the bed, doing the laundry, ironing. Housecleaning in a structured, organized way should not be included here, as that would involve more moderate intensity chores.

2. Moderate or Heavy Effort Household Chores: These activities are more structured and might not occur on a daily basis. Examples include heavy cleaning (washing windows, moving furniture to clean), vacuuming, scrubbing the floors or walls, mopping—either standing up or on hands and knees—repairing home appliances or lawn and garden tools, washing the car.

Lawn/Yard/Garden/Farm

"In a typical week in the past month, did you do… (moderate effort lawn/yard etc.)"

3. Moderate Effort Lawn/Yard/Garden/Farm etc. activities: These activities refer to outside chores involved in caring for a house, farm, or ranch. They may involve yard work, cleaning out the garage, raking the leaves, sweeping the porch or sidewalk, or other moderate effort chores. Encourage the participant to think of activities done in a typical week in the past month. This category may include seasonal activities; if so, the activities reported should be typical of the past month.

4. Heavy Effort Lawn/Yard/Garden/Farm etc. activities: These activities require heavy effort and may be seasonal. Examples include digging dirt, shoveling snow or using a snow blower, chipping ice, tilling a garden, chopping and hauling wood, and removing trees.

Care of Children/Adults

"In a typical week in the past month, did you do… (light effort child/adult care)"

5. Light Effort Children/Adult Care: These activities require physical movement by the respondent and include bathing, feeding, changing diapers, playing with a child, or other similar activities. Do not count time sitting with a child (as in babysitting) without active engagement in physical activities. Include only the time spent involved in physical activities.

6. Moderate Effort Children/Adult Care: These are intentional activities that require moderate effort to complete and may include activities of lifting and carrying dependent others, pushing a wheelchair or stroller. Include only the time spent moving.

Walking (not at work)

"In a typical week in the past month, did you do… (walking to get to places, etc.)"

7. Walking to get places. In general, walking is underreported in the time estimates. This would include walking for transportation, walking to and from work, walking to the store or from the car into the store and back, walking to get the mail, etc.

8. Walking for exercise, pleasure, social reasons, walking during work breaks, or walking the dog. These are classified as intentional walking and are different in intention then those in item #7. The walking may be for exercise or part of a daily routine that is done with family members, animals, or for personal reasons. Walking for transportation should be included in item 7.

Dancing/Sport Activities

"In a typical week in the past month, did you do… (dancing in church/team sport, etc.)"

9. Dancing in church, ceremonies, or for pleasure. Remind the participant to think of a typical week in the past month to estimate usual dancing behaviors. Some may dance only occasionally—a few times a year. This would not be included as a "yes" response to this category unless it was typical of the past month. Ceremonial or religious dancing would need to be done regularly enough to represent a typical week in the past month.

10. Team sport. The purpose of including team sports activities is to group exercise activities that are done with others. These are probably seasonal activities that are done in leagues or other organized settings. Remind the participant to think of a typical week in the past month and to stay within that framework when responding.

11. Dual sports. These activities involve mostly racket sports or other one-on-one sports activities. They could include fencing, ping-pong, or other activities done with another person.

12. Individual activities that maybe classified as sports. These may be sports activities, such as golf and bowling, or more individual relaxation/meditation activities, such as yoga or Tai Chi. Remind respondents to think of days and time spent during a typical week in the past month only.

Conditioning Activities.

"In a typical week in the past month, did you do… (moderate effort conditioning, etc.)"

13. Moderate Effort Conditioning Activities. Conditioning activities are those that can be done alone or with others. They are different from sports, because the intention is to gain

an element of fitness rather than have a contest or win a game. Moderate effort activities are not for competition, nor are they all-out effort. Intensity of exercise should be moderate enough that respondents should be able to talk with others while they are performing the activities. Examples are low impact aerobics, recreational (slow) bicycling, rowing on a rowing machine or in a lake, swimming in a pool or lake, or using weight lifting or conditioning machines at a health club.

14. Heavy Effort Conditioning Activities. These are very intense activities done for maximum fitness levels and include high impact aerobics (e.g., Tai-bo, kick boxing), competitive or maximum effort running, bicycling, swimming, and work on health club machines. Exercise at this intensity would be very hard and the respondent would have difficulty carrying on a conversation during the performance.

Leisure Activities.

"In a typical week in the past month, did you do…(these leisure activities, watching TV)"

15. Watching TV. This is a sedentary, leisure-time pursuit. Do not include the time watching TV while doing other things. The question is to be used as a marker of sitting or reclining and watching TV as a single pursuit.

Occupational or Volunteer Activities.

If participant indicated that they are employed or volunteering (personal History form), Questions 16-19 will be available for response.
If the participant has not indicated they are employed or volunteering, questions 16-19 will be skipped and the participant will be asked to continue with question 20.

"At work (or volunteering), did you do…."

16. Light Effort/Sitting Activities. For most respondents, this will be the most hours in the work day.

17. Light Effort/Standing Activities. These are likely intermittent activities that would be done in a clerical setting (e.g., office work related to filing, using a copy machine) or sustained activities done in a labor setting (e.g., check-out clerk in a store, assembly line worker assembling parts, medical field examining patients). Teaching in a classroom falls into this category.

18. Moderate Effort/Standing or Walking Activities: For some occupations (office work, clerical, professional), these may be more intermittent, as in walking down the hall, walking between office buildings, and delivering items. For labor settings, this could relate to jobs such as delivery person (overnight express delivery, food delivery, mail delivery) or jobs that require mostly walking and standing (nurse, custodian, physical education teacher, coach, firefighter, police officer, physical therapist).

19. Heavy Effort/Manual Labor: These occupations require manual effort that involves substantial movement and labor. Types of activities may include digging ditches, ranch or farm labor, delivering furniture, loading and unloading trucks, seasonal farm work.

Walking Pace

20. ***When you walk outside of your home, what is your usual pace?***

Ask respondent to estimate the usual pace he/she walks most of the time. Consider all walking activities (e.g., at work, on the way to work, for exercise, in walking with children or others, or when running errands). Fill in appropriate circle.

Offer the following guidelines:

- Slow or Casual strolling pace = 2 mph = 30 minutes per mile
- Average or normal pace = 2-3 mph = 20-30 minutes per mile
- Fairly brisk pace = 4-5 mph = 12-15 minutes per mile (very fast or almost a slow jog)
- Brisk or striding pace = More than 5 mph = 10 minutes per mile (race-walking)

After completion of the interview, the interviewer should check to make sure all questions were answered then indicate whether the survey was completed as self-administered or interviewer-administered at the end of the survey.

3.4.10 Health and Life

I. PURPOSE

This questionnaire includes several instruments designed to measure psychosocial characteristics that may be important in understanding the causes of cardiovascular disease. These psychosocial factors may themselves lead to increased risk of cardiovascular disease or may interact with other traditional risk factors, such as diet or sedentary lifestyle. The areas assessed as part of this questionnaire include neighborhood characteristics, depression, stress, and social support.

II. MATERIALS/EQUIPMENT

This is a self-administered form. Provide the participant with the form and a pencil and give brief instructions for completion.

III. DEFINITIONS

The terms used in the questionnaire should require no explanation, because they are used in the way they tend to be used by most people in everyday life.

IV. METHODS

1. General Instructions

1.1 It is important that the participant have some private time in a quiet area to complete the form. The participant should be led to a quiet location with a table, handed the form and a pencil for completion and told to answer each question by darkening the circle of the appropriate response. Review the top section of the form with the participant before starting. Emphasize that there are no right or wrong answers and that we are interested in their feelings and opinions about things. Also emphasize that they should not spend too much time on any one question. Show them that additional instructions are provided at the beginning of each section.

1.2 The participant should be asked to try to respond to all questions, unless instructed to skip the question. Remind them to request assistance from a staff member if anything is unclear. Most participants should be able to complete the questionnaire on their own. However, if the participant expresses or appears to have difficulty reading or comprehending the questions, offer to help and make arrangements for an interviewer administered version in the appropriate language.

1.3 Important points for interviewers and participants to consider:

- If the topic should arise, remind participants that all information is strictly

confidential and will only be used for research purposes. Explain that things about people's lives, including the stressful situations they go though, may be important to their health. Knowing about these things may help us understand the causes of heart disease better. Also emphasize that it is important to get complete data so that the study results will be valid. However, if a participant is upset by the questions or does not want to answer, he or she should feel free to skip the question or section. Refusal to answer the questions will not jeopardize his/her participation in the study.

- The measurement of these dimensions is complex. Generally they are measured using scales or collections of questions that attempt to get at the same underlying concept in different ways. For this reason some of the questions may seem repetitive. If questions on this should arise, acknowledge that some questions may seem similar, but ask participants to respond to each one separately as best they can.

- The terms used should be understood by most people. If the participant asks about the meaning of any item or tries to qualify a statement, please re-read the statement (or question) to them. Obtain a copy of a translated form if necessary. Do not attempt to explain the question or provide synonyms (unless specified in the specific instructions below), because this may create problems for some of the scales.

2. Specific Instructions

2.1 Questions 1A-1Y ask about neighborhood characteristics. The instruction on the form reads "For each of the statements below, please indicate whether you agree or disagree by selecting the best response. In answerin these questions, please think of your neighborhood a sthe area within about a 20 minute walk (or about a mile) from your home." Possible answers are "Strongly Agree", "Agree", "Neutral (neither agree or disagree)", "Disagree", or "Strongly Disagree". Answers are selected by filling in the appropriate circle.

Question 1:

A. People in this neighborhood are willing to help their neighbors.
B. People in this neighborhood don't get along.
C. People in this neighborhood can be trusted.
D. People in this neighborhood do not share the same values.
E. I feel safe walking in this neighborhood, day or night.
F. Violence is a problem in my neighborhood.
G. My neighborhood is safe from crime.
H. A large selection of fresh fruits/vegetables is available in my neighborhood
I. A large selection of low fat foods is available in my neighborhood
J. The fresh fruits and vegetables in my neighborhood are of high quality
K. There are many opportunities to purchase fast foods in my neighborhood
L. Food stores in my neighborhood sell high-fiber bread such as whole-wheat

bread.

M. Food stores in my neighborhood sell low-salt products such as salt-free or low-salt soup.
N. My neighborhood offers many opportunities to be physically active.
O. Local sports clubs and other facilities in my neighborhood offer many opportunities to get exercise
P. It is pleasant to walk in my neighborhood
Q. The trees in my neighborhood provide enough shade
R. In my neighborhood it is easy to walk places
S. I often see other people walking in my neighborhood
T. I often see other people exercise in my neighborhood, for example jogging, bicycling, or playing sports
U. There is a lot of trash and litter on the street in my neighborhood
V. There is a lot of noise in my neighborhood
W. In my neighborhood the buildings and homes are well maintained
X. The buildings and houses in my neighborhood are interesting
Y. My neighborhood is attractive

2.2 Questions 2A–2T correspond to the CES Depression scale. The instruction on the form reads, **"Below is a list of the ways you might have felt or behaved. Please tell me how often you felt this way during the past week. Please indicate the number of days you felt this way last week."** Possible answers are "rarely or none of the time (less than 1 day)," "some or a little bit of the time (1–2 days)," "a moderate amount of the time (3–4 days)," or "most of the time (5–7 days)." Answers are selected by filling in the appropriate circle.

Question 7:

A. I was bothered by things that don't usually bother me
B. I did not feel like eating; my appetite was poor
C. I felt that I could not shake off the blues, even with help from my family and friends.
D. I felt that I was just as good as other people
E. I had trouble keeping my mind on what I was doing
F. I felt depressed
G. I felt that everything I did was an effort
H. I felt hopeful about the future
I. I thought my life had been a failure
J. I felt fearful
K. My sleep was restless
L. I was happy
M. I talked less than usual
N. I felt lonely
O. People were unfriendly
P. I enjoyed life
Q. I had crying spells

R. I felt sad
S. I felt that people dislike me
T. I could not "get going"

2.3 Question 3 asks. **"Are you currently married or living with a partner"?** This question requires a "yes" or "no" answer. The participant should answer "yes" if s/he is married *or* living with a partner. If the participant is married or has a partner *but is currently not living with that person, then "no" should be marked.*

2.4 Questions 4A-4D are designed to measure social support. The instruction on the form reads, **"For each question, please choose the best option."** Possible answers for 4A and 4B are "A Lot", "Some", "A little" or "Not at All". Possible answers for 4C and 4D are "Often", "Sometimes", "Rarely" or "Never". Answers are selected by filling in the appropriate circle.

A. How much can you rely on friends and family for help if you have a serious problem?
B. How much can you open up to them if you need to talk about your worries?
C. How often do members of your family or friends make too many demands on you?
D. How often do they let you down when you are counting on them?

2.5 Questions 5A-5D are intended to assess perceived stress. The instruction on the form reads, **"The following questions ask you about your feelings and thoughts during the last month. In each case, please tell me how often you felt this way DURING THE PAST MONTH"** Possible answers are "Never", "Almost Never", "Sometimes", "Fairly Often", and "Very Often". Answers are selected by filling in the appropriate circle.

A. In the last month, how often have you felt that you were unable to control the important things in your life?
B. In the last month, how often have you felt confident about your ability to handle your personal problems?
C. In the last month, how often have you felt that things were going your way?
D. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

2.6 In the box labeled, "For MESA Field Center Use Only," select whether the form was self-administered or interviewer-administered. Record reviewer (if form self-administered) or interviewer (if form interviewer-administered

3.4.11 Food Frequency Questionnaire

I. PURPOSE

1. Background: Diet & CVD

Since the initiation of MESA, it has become clear that multiple dietary factors, singly and collectively, influence the development of cardiovascular disease. Dietary data collected at the baseline MESA examination have contributed to the body of evidence linking dietary factors, particular dietary patterns, with markers of the early development of cardiovascular disease, such as inflammatory markers and physical markers of atherosclerosis. Cross-sectional associations between dietary intake and cardiovascular disease risk factors (novel and traditional) have been subsequently confirmed by data showing associations between prospectively dietary intake (baseline) and incident cardiovascular disease and diabetes events ascertained during follow-up of the MESA cohort. Findings from MESA largely mirror those of other large cohort studies. These examples of internal and external consistency speak to the validity of the baseline dietary assessment. Our charge as we begin another MESA examination is to assure similar quality and provide a robust platform for continuing diet-disease investigations long into the future of MESA.

2. Background: Structure of the MESA food frequency questionnaire (FFQ)

At the baseline examination, usual dietary intake over the previous year was assessed with a modified Block-style 120-item FFQ. Consumption frequency and serving size of each food or beverage was assessed. Following the Block design, serving sizes were quantified as small, medium, or large, with corresponding gram weights imputed according to National Health and Nutrition Examination survey data (Block G 1986). Nutrients were calculated for each FFQ line item according to a weighted recipe using the Nutrition Data Systems for Research (NDS-R) database (Nutrition Coordinating Center, Minneapolis, MN). Additional programming was performed to specify type of milk on cereals, to define cereal type (whole or refined grain), to specify most common type of fat used in refried beans, to incorporate information from responses to questions regarding low-fat choices, and to create food groups. Procedures will be consistently followed for exam 5 data, using a contemporary version of the NDS-R database.

The FFQ used in MESA is based on an FFQ originally designed for the Insulin Resistance and Atherosclerosis Study (IRAS), which was validated in a sample of Non-Hispanic Whites, African-Americans, and Hispanics (Mayer-Davis EJ 1999). In order to maximize accuracy across all four race/ethnic groups represented in MESA, modifications were made to accommodate the unique cuisine of MESA Chinese-Americans.

The same FFQ will be used to assess diet at MESA exam 5, with the addition of a few questions to better address contemporary research questions. Added questions will, 1) differentiate red meat, poultry, and fish intakes; 2) distinguish plain milk from milk-containing coffee beverages; 3) distinguish regular soda from sweetened mineral water and non-alcoholic beer and distinguish diet soda from unsweetened mineral water; and 4) quantify plain chocolate intake.

3. Rationale: Re-assessment of dietary habits

Re-assessment of dietary intake at exam 5 is critical for several reasons. First, two measures of dietary intake better characterize long-term consumption patterns. Second,

two measures of dietary intake improve the precision of our estimates of dietary factors; thus, better positioning MESA for events analyses, which will exponentially increase as the cohort ages. Third, MESA is also involved in initiatives to identify the influence of genetic variation, and the interaction between the environment and this genetic variation, in terms of chronic diseases. More precise measures of environmental exposures, like diet, allow for more rigorous investigation of these gene-by-environment interactions. Thirdly, MESA comprises a multiethnic group of participants, some of whom are recent immigrants to the United States and likely moving through a cultural transition. Thus, a second measure of diet provides an opportunity to characterize the dietary changes that have transpired since the baseline exam. Lastly, MESA food frequency questionnaire (FFQ) can be expanded to address contemporary research questions. To this end, a few clarifying questions have been added, as described above, while maintaining the overall structure and content of the FFQ.

II. METHODS

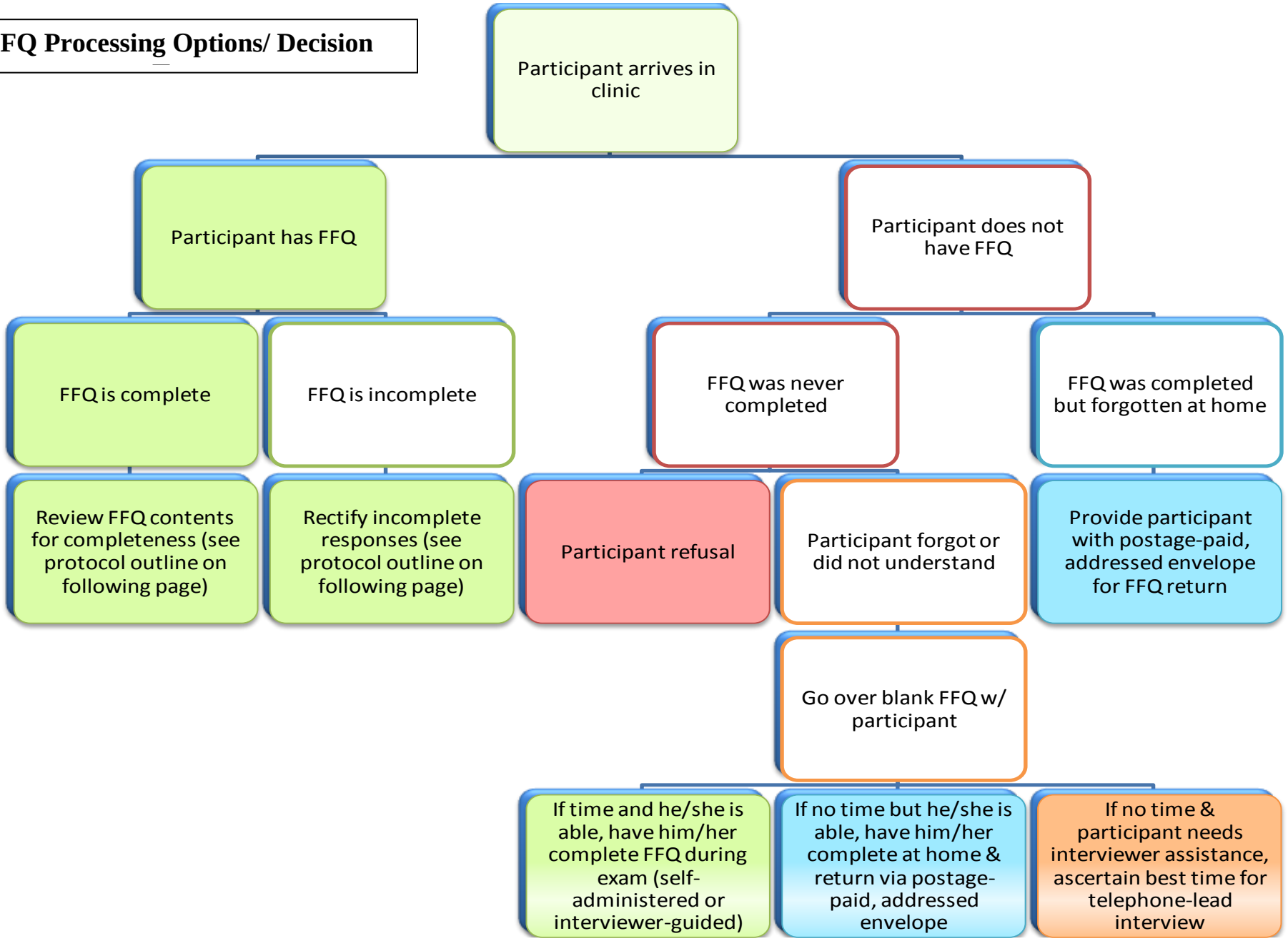
1. Methods: Mode of FFQ administration = self-administered at home prior to scheduled exam

Given time constraints and the estimated length of the in-clinic examination, the FFQ will be sent to MESA participants prior to their scheduled examination date along with other MESA documents reminding them of their examination time, clinic location, and other pertinent information.

The goal of the MESA FFQ is to obtain information about *usual* dietary practices of the participants in the previous year, and to do so in a consistent manner across all field centers and in a manner reasonably consistent with procedures followed at baseline. While in-home self-administration was not the primary mode of FFQ completion at the baseline exam in all MESA field centers, it was the primary protocol at some field centers. Baseline FFQ procedures varied enough at the baseline examination to preclude an exact replication of original procedures. Thus, our emphasis for examination 5 diet data collection is protocol uniformity across field centers, with the collection of data itself being the foremost goal. Ideally, for a hypothetical participant, the same results would be obtained no matter who is administering the questionnaire, how it is administered (self or interviewer), when it is administered, or where it is administered.

To achieve the desired level of consistency, a fairly strict protocol was designed for staff review of FFQs with participants during the in-clinic examination (outlined below). Approximately 10 minutes have been allocated to the task of review. While we recognize that in any multi-center study, differences in the review of questionnaires will exist among staff, among centers, and over time, these differences can seriously bias results of statistical analyses. Thus, standardization of these procedures is critical to the data quality and the overall research goals of MESA. We acknowledge that there is likely to be some degree of variation among participants in the in-home portion; however, this variation is not expected to be greater than that observed when participants complete FFQs in-clinic during an exam.

FFQ Processing Options/ Decision



2. Review of FFQ with Participant

1. Ask participant if he/she has any questions related to the FFQ
    a. Note any pertinent comments the participant provides on the last page of the FFQ (page 19), under "for office use only."

2. Check each page of the FFQ for completeness
    a. PAGES 3 – 14:
        - Be sure that both a frequency option and serving size option is marked for each food/beverage item.
        - Be sure that only one frequency option and one serving size option is marked for each food/beverage item.
    b. PAGE 4: COLD CEREAL
        - Check consistency of responses between question 16 and question 16a, "If you eat cold cereal, what is the name of the cold cereal that you eat most often?" is answered.
            - If the participant responded in question 16 with "rare or never", question 16a should be left blank.
                - If response to 16 was "rare or never" but a cereal brand was given, reconcile the discrepancy with the participant.
            - If question 16 was *not* answered "rare or never," ask participant to tell you the name of the cold cereal he/she most commonly consumes. ***Write this answer in the blank.***
            - If multiple cereals are listed, ask participant which of the cereals is most commonly eaten. ***Circle that cereal, but leave all cereals listed, as written by participant.***
    c. PAGE 5: YOGURT
        - Check consistency of responses to questions 30 and 31 with question 31a, "If you eat yogurt (plain or unflavored) how often is it low-fat or fat-free?" is answered.
        - If the question was left unanswered, check responses to questions 30 and 31.
            - If participant responded that his/her frequency of consumption was more than "rare or never," this question should be answered.
            - If participant responded that his/her frequency of yogurt consumption was "rare or never," this question should be left blank (no bubble filled).
    d. QUESTIONS 58/58a, 63/63a, 65/65a, 75/75a, 77/77a and 79/79a: MOST COMMON MEAT/POULTRY/SEAFOOD INGREDIANT
        - Check consistency of responses.
            - If the participant responded in the primary question 'X' with "rare or never", the secondary question 'Xa' should be left blank.
            - If participant response to primary question 'X' with a frequency that was more than "rare or never," this question should be answered.
    e. PAGES 15 and 16:
        - Be sure that one bubble is marked for each item
        - If an item is left unanswered, ask participant whether he/she inadvertently skipped that item or if he/she does not eat that item. If he/she does not eat that item, mark the bubble under the column "I do not eat the food."
    f. PAGE 17:
        - Question 1: one bubble should be filled, but no more than one bubble
        - Questions 2-4: be sure that each question is answered; up to two bubbles may be filled under each question, but no more than two.

g. PAGE 18:
   - Questions 1 and 2 should each have one response (filled bubbled)
   - Question 4 "Is there any other food that you eat at least once a week that you have not seen listed in the previous pages?" should be answered with a yes or no. If unanswered, ask participant to respond (it is likely his/her answer is "NO," but please clarify).
     - If foods are reported here, please be sure that they are not foods that belong to any one of the FFQ line items. Some foods can be attributed to specific questions (see section 5. 'Other Foods'-FFQ Line Item Matches, below).

h. Question 5 "Is there anything else that you would like to tell us about your eating habits?" should be answered with a yes or no. If unanswered, ask participant to respond (it is likely his/her answer is "NO," but please clarify).

3. <u>Thank participant for completing the questionnaire.</u>

4. <u>Complete final "clinic use only" section of FFQ (PAGE 19)</u>

1. Comments section: please report anything *<u>extremely</u>* unusual. We anticipate that there may be some idiosyncrasies, but overall, these are not expected to detract from the general quality of the FFQ data. Please report only those details that you think could influence the accuracy of the data provided or individuals that may represent outliers.
2. Question "Is review by Diet Data Center required for coding food items?": mark "yes" only if participant reported foods in Question 5 on page 18.
3. Please report how the form was completed. As described in above sections, we strive for consistency within and across field centers. In order to assess how administration of the FFQ influences data quality we must first know how/where the FFQ was completed. Please make certain that this section is marked!!

5 <u>"Other Foods"-FFQ Line Item Matches</u>

For items eaten *at least once a week* and not listed explicitly on the questionnaire, coding rules were developed based on nutrient content of the item in question.

Be sure to add the newly reported food accurately. Do not "double count" items mistakenly reported twice. Add new items to any existing items in the same line (do not simply replace the original answer with the new food). Remember that the total amount consumed is equal to frequency times portion size. In general, if needed, to "add" a newly reported food to a line item, you may assume that "small" is about ½ the amount of medium, and "large" is about 1 ½ times the amount of medium. Thus, you can use any combination of frequency and portion size to approximate the correct total intake. For example, 2/week, small, is the same as 1/week, medium.

<u>Fruits</u>

- Fruits added to cereals should be coded in the fruits section, if the quantity is ¼ cup or more; otherwise, do not code.
- For snack fruit bars, fruit roll-ups, etc., code as dried fruit.
- Code Gatorade with other juice.
- Code 5-Alive as orange juice.
- Code ackee (or akee, achee) as other fruit.

<u>Vegetables/Side Dishes</u>

- Green salad codes (automatically) only for lettuce and a few slices of tomato. If a salad is

usually topped with other vegetables (¼ cup or more), code these items in their respective lines. The serving size will usually be "small" for items added to salads.

- Code V-8 juice as tomato juice.
- Code carrot juice as carrots at twice the reported frequency.
- Code chard as spinach.
- Code artichoke as other vegetables
- Do not code garlic.
- Two rice cakes are coded as 1 small serving plain rice.
- Code a medium serving of tabouli as one small serving of *each* of the following: pasta/couscous; other vegetables; "butter, margarine, or oil on vegetables, rice, or potatoes."
- Code babaganoush as other vegetable.

Meats, Poultry, Fish, Mixed Dishes

- Code red or green chili as a condiment. Code ½ the reported frequency to account for the difference in portion size for chili as a condiment compared to a bowl of chili as a main dish.
- Code pasta Alfredo as pasta with cream sauce or cheese (no meat).
- Code pesto sauce or olive oil on pasta as "butter, margarine or oil on vegetables, rice or potatoes."
- Code pizza rolls as pizza.
- Code menudo as beef.
- Code pickled herring as tuna at ½ the reported frequency.
- Code caviar as shellfish (not fried).
- Code frozen entrees or dinners by their component items (e.g., code each item in a chicken, rice, and green beans frozen dinner separately). Ask the DAC for assistance if needed.
- Code jerky (beef, chicken or fish) as bacon.
- Code barbacoa as sausage at twice the reported frequency.
- Code chicken and dumplings as "meat, chicken, or turkey stew, pot pie or empanada."
- Record veggie burger in the comments section on page 17 and fill in bubble 3 (Diet Center review needed).

Breads and Snacks

- Code chips made with safflower or canola oil as regular chips.
- Code blue corn chips as regular corn chips.
- Code low-salt chips, including low-salt blue corn chips, as regular corn chips.
- Code low-salt popcorn as regular popcorn.
- Code low-sodium crackers as regular crackers.
- Code matzo balls as biscuits, scones, etc.
- Code potato bread as white bread.
- Code unsalted butter or margarine on bread as regular butter or margarine.
- Code trail mix as half "salty snacks" and half "nuts and seeds."

Breakfast Items

- Code Eggbeaters or other low-fat egg substitute at ½ the reported frequency.
- Do not code egg whites only.
- Code cooked bulgur, kasha, oat bran, etc., as cooked cereals.

- Code French toast as one egg and one serving bread.
- Code wheat germ as cold cereal.
- If the participant does not use milk on cereal, decrease milk as a beverage by half the frequency of reported cold cereal use.

Sweets

- Code sugar-free "spreadable fruit" as "other fruit" (or add to the specific line item for that fruit if possible) at ½ the frequency.
- Code sweet dim sum as cake doughnuts.
- Code graham crackers as cookies.
- Code NutraSweet fudgsicles or other specialty dairy desserts as frozen yogurt/ice milk.
- Code granola bars, breakfast bars, Power bars as cookies, cake. If bar is a meal replacement bar, code as "Instant Breakfast, Ensure, Slimfast."
- Code "dietetic" cookies and cakes as such at ½ the servings reported.
- Do not code sugar-free Jell-O.
- Do not code calorie-free candies, mints, gum, etc.
- Code syrup with other candy, jelly, honey, etc.
- Code Weight Watchers Mousse Dessert as frozen yogurt/ice milk.
- Code Weight Watchers Cookies as regular cookies at ½ the reported frequency.
- Code Cracker Jack as cookies/cakes/dessert.
- Code non-fat cookies at ½ the reported frequency.
- Code sugar-free pudding at ½ the reported frequency.
- Code light syrup at ½ the reported frequency.
- Code diabetic ice cream as frozen yogurt/ice milk
- Code non-fat ice cream as frozen yogurt/ice milk.

Dairy Products

- Code fat-free, sugar-free yogurt as plain yogurt

Beverages

- Code Crystal Light or other calorie-free, unfortified beverage mix as diet soda.
- Code Malta as lemonade, sweetened mineral water
- Code Mocha Mix in coffee as non-dairy creamer.
- Code evaporated milk in coffee at twice the reported frequency.
- Record other milk substitutes (like LactAid, soy milk), specifying brand and fat content, in the comments section on page 17 and fill in bubble 3 (Diet Center review needed).
- Code lactose-free milk as milk (whole, 2%,1%, skim).
- Code Optifast as Ensure/Instant Breakfast.
- Code evaporated milk as twice the frequency of milk.
- Code flavored coffee mixes as regular or decaffeinated coffee at twice the frequency reported; plus sugar in coffee (large) and non-dairy creamer (large).
- Code Yoohoo as chocolate (one medium serving for one bottle).
- Code light beer as regular beer
- Code wine cooler as wine.

Fats

- Code diet soft margarine as soft margarine.
- Code vegetable shortening as stick margarine.
- Code unsalted butter or margarine as regular butter or margarine.

- Do not code Molly McButter or other calorie-free butter flavoring, fat-free margerine, fat-free mayonnaise, or fat-free sour cream.

Miscellaneous

- If a participant reports luncheon meat, remember to probe for some type of bread.
- Code matzo ball soup as other soups.
- Code SlimFast Cup-A-Soup as other soups.
- Do not code mustard, soy sauce, pica, or calorie-free seasonings.
- Code amaranth as cooked cereal.

4. Study Contacts: MESA Diet Coordinating Center (DCC)

The MESA DCC, in association with the University of Washington Coordinating Center, will lead staff training prior to exam 5, oversee all aspects of dietary data collection, and field all questions related to the administration and processing of FFQs. The field center staff are asked to contact the MESA DCC Principal Investigator (Dr. Nettleton) should questions arise related to any aspect of the dietary assessment portion of exam 5.

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3.5 Clinic Examinations

3.5.1 Anthropometry

I. PURPOSE

Anthropometry was obtained in MESA Exam 1 (the baseline exam) as well as at follow-up exams 2-4. In the prior exams anthropometry procedures were limited to measurement of height, weight, hip circumference, and waist circumference. For Exam 5, a bioelectric impedance procedure (measurement of lean body mass, fat mass, and body water) is added. Overall, the purpose of the anthropometry procedures are the same as for Exams 1-4 (to acquire general measures of body size, shape, and obesity) and the resulting data will be useful in virtually every analysis and manuscript produced that makes use of the Exam 5 data.

II. MATERIALS AND EQUIPMENT

- Stadiometer (Accu-Hite Measure Device with level bubble) (height ruler with triangle level is used at some centers)
- Detecto Platform Balance Scale in lbs/kg
- Gulick II 150 cm anthropometric tape
- Full length mirror
- Valhalla Scientific BCS-2 Body Composition scale (and accompanying printer)
- Lysol Disinfectant Spray (BCS-2 manufacturer recommended).
- Four 50-pound weights (certified prior to first MESA visit) to calibrate scales

III. METHODS

Methods for completing the height, weight, waist circumference, and hip circumference procedures Exam 5 are identical to Exams 1-4. Some important points are reiterated here.

General Instructions:

For all measurements, participants should wear light clothing but no shoes (thin socks or "pillow slippers" are OK, but will need to be removed for bioelectric impedance measurement). Have participants completely empty their pockets and remove excessive amounts of jewelry that could affect the weight measurement. Provide lockers with locks for valuables.

Pregnant women should not be measured, regardless of gestational stage (check exclusion criteria for pregnancy). The Clinic Coordinator should ascertain pregnancy status, both for measurements and for subsequent coronary calcification measurement.

Take a single measurement at each body site and record on the anthropometry form using specific rounding rules for each procedure. Record any modifications in measurement techniques (e.g. height decreased from a hunched posture or weight that exceeds the capacity of the scale).

Specific Instructions:

1. Completing the Boxed section at the top of the form

   Make sure to verify participant ID and acrostic and verify/record Technician ID# and date of

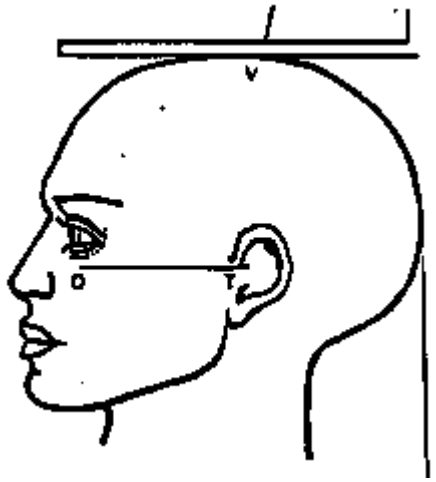
the procedure at the top of the form.

2. Standing Body Height ~ procedure is the same as for Exams 1-4.

2.1 Equipment
- Stadiometer (Accu-Hite Measure Device with level bubble) (height ruler with triangle level used at some centers is adequate)

2.2 Before measuring height, make sure the floor is level, the wall is at a 90 degree angle to the floor, the wall is straight, and the Stadiometer is mounted perpendicular to the floor.

2.3 For accurate measurement of height, the participant must be standing in a vertical plane. To achieve this position, have the participant stand erect on the floor or horizontal platform, with back against the vertical Stadiometer, heels against the wall, and feet *or* knees together—whichever come together first. Have the participant look straight ahead, with head in the Frankfort horizontal plane (Figure 1, below).



The Frankfort Plane includes the lower margin of the bony orbit (the bony socket containing the eye) and the most forward point in the supratragal notch (the notch just above the anterior cartilaginous projections of the external ear)—also referred to as the upper margin of the external auditory meatus (the hole in the ear).

Figure 1. Frankfort Plane for Measuring Body Height

2.4 Place the headboard over the crown of the head, with the headboard forming a right angle to the scale. The headboard should touch the scalp lightly.

2.5 Ask the participant to step out from under the headboard. *Record the participant's height to the nearest 0.1 centimeter in Box 1a of the Anthropometry Form.*

2.6 If you are unable to measure the actual height of the participant because the headboard does not rest directly over the scalp, *estimate height to the nearest 0.1 cm, record in Box 1a of the Anthropometry Form and answer "yes" to the question, "Was there a modification in protocol?"*

2.7 Record the results, to the nearest tenth (0.1) of a cm, in Box 1a on the Anthropometry Form.

2.8 If any modification was made to obtain height, *record "yes" to the question, "**Was there a modification in protocol?"***

3. Body Weight ~ procedure is the same as for Exams 1-4.

3.1 Equipment
- Detecto Platform Balance Scale in lbs/kg

3.2 Always balance the scale so that the indicator is at zero when no weight is on the scale. The scale should be on a firm, level surface (not on a carpet, for example). Instruct the participant to stand in the middle of the platform of the balance scale, with head erect and eyes looking straight ahead. Adjust the weight on the indicator until it is balanced. *Record the results, to the nearest 0.5lbs, in Box 2a.*

3.3 If the participant is too obese to stand securely on the scale's platform when looking straight ahead, he/she may stand sideways on the scale to take the weight measurement; facing to the side rather than the front will provide the participant a wider base and more stability.

3.4 If a participant has a prosthetic limb or breast prosthesis, measure weight with the prosthesis *on.*

3.5 If a participant is frail or unsteady, measure weight while participant is lightly steadied by you or an assistant.

3.6 If a participant is unable to stand on the scale for a weight measurement, do not attempt a weight measurement.

3.7 If any modification were made to obtain weight, *record "yes" to the question, "**Was there a modification in protocol?"***

4. Girth Measurements ~ procedure is the same as for Exam 1.

4.1 Equipment
- Gulick II 150 cm anthropometric tape
- Full length mirror

4.2 Technique
- Do *not* take abdominal and hip girth measurements over loose clothing. It is ok if taken over light well-fitted clothes.

4.3 Abdominal Girth

Apply a Gulick II anthropometric tape horizontally at the level of the umbilicus and instruct the participant to breathe normally. Move to the participant's right side to take the measurement; do not take this measurement from the front. Be sure to keep the tape horizontal while making the measurement; use the wall-mounted mirror to assure horizontal placement on all sides.

Round abdominal girth measurement to the nearest 0.1cm and record in Box 3a.
If the circumference exceeds 150 cm, record "yes" for the question, ***"Was there a modification in protocol?"***

4.4 Hip Girth

Take the hip girth measurement from the participant's right side; do not take this measurement from the front. Instruct the participant to stand with his/her feet together. Measure hip girth at the maximum circumference of the buttocks. Check to see that the tape is level in front and back.

Round hip girth measurement to the nearest 0.1cm and record in Box 3b.
If the circumference exceeds 150 cm, record "yes" for the question, ***"Was there a modification in protocol?"***

5. Body Composition procedure (new to Exam 5).

5.1 Equipment

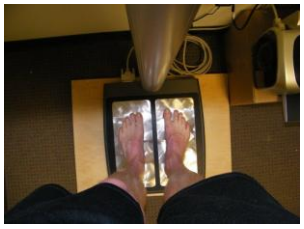
- Valhalla BCS-2 Body Composition Scale (& accompanying printer)

5.2 Exclusions

- Do not attempt a measurement if the participant has a pacemaker or other medical device.
- Do not attempt a measurement of the participant is pregnant.
- Do not attempt a measurement if the participant has a prosthetic limb.
- BCS-2 scale has a capacity rating of 700 lbs (317 kgs), so exclusion based on participant weight is not expected.

5.3 Disinfect the scale prior to each participant's use. Manufacturer recommends use of Lysol Disinfectant Spray (and a dry wipe). The scale should be on a firm, level surface (not on a carpet, for example). Please make sure the BCS-2 scale and printer are plugged in (there is no other on/off switch) and that the printer has paper.

5.4 Participant must have bare feet and hands for this procedure. Remove socks or light slippers.



5.5 Instruct the participant to stand in the middle of the platform of the balance scale, with

head erect and eyes looking straight ahead. Once the participant is on the scale, wait for weight to calibrate. Height will say "ON" while calibrating and weight lights will oscillate.

5.6 Using the device touchpad, select centimeter (cm) setting, enter height rounded to the nearest cm from form box 1a, and select enter. Indicate participant gender (separate buttons for indicating male and female). Enter current age of participant rounded to the nearest year and select enter.

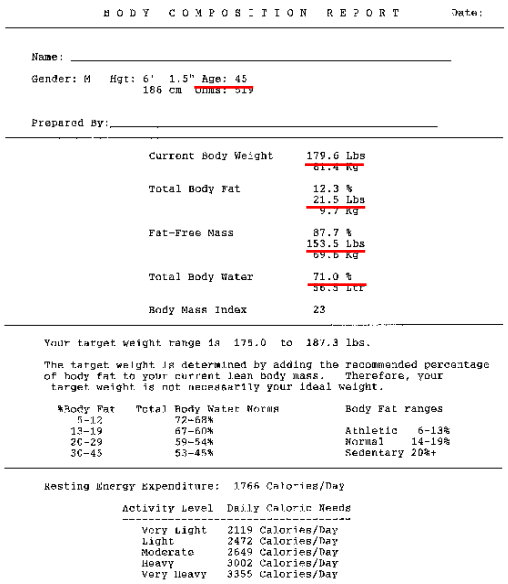
5.7 When the word "grip" appears on the display, ask the participant to please grip the metal handles on each side of the BCS device. Thumbs should rest comfortably on the top metal handle and fingers should be in firm contact with the lower metal handle.

5.8 Participant must remain still with hands in contact with handles and feet in contact with metal surface of the base until Body Weight and Body Fat % results are displayed AND results are sent to the printer (should be just a few seconds; see next step).

5.9 Make sure the printer is turned on and has paper in the hopper. Using the device touchpad, select plain and select enter in order to print results. Results must be printed in order to collect important information (ohms, fat mass, fat free mass, and total body water) not reported via the display.

5.10 After results are successfully printed, participant may step off of the device and put their socks or slippers back on.

5.11 From the printout, record ohms, current body weight (rounded to nearest 0.1 kg), total body fat (rounded to nearest 0.1 kg), fat-free mass (rounded to nearest 0.1 kg), and total body water (rounded to nearest 0.1 liter) in anthropometry form section 4 questions a-e.



BODY COMPOSITION REPORT Date:

Name: _____

Gender: M Hgt: 6' 1.5" Age: 45
186 cm Ohms: 519

Prepared By:_____

Current Body Weight	179.6 Lbs 81.4 Kg
Total Body Fat	12.3 % 21.5 Lbs 9.7 Kg
Fat-Free Mass	87.7 % 153.5 Lbs 69.6 Kg
Total Body Water	71.0 % 36.3 Ltr
Body Mass Index	23

Your target weight range is 175.0 to 187.3 lbs.

The target weight is determined by adding the recommended percentage of body fat to your current lean body mass. Therefore, your target weight is not necessarily your ideal weight.

%Body Fat	Total Body Water Norms	Body Fat ranges
6-12	72-68%	
13-19	67-60%	Athletic 6-13%
20-29	59-54%	Normal 14-19%
30-45	53-45%	Sedentary 20%+

Resting Energy Expenditure: 1766 Calories/Day

Activity Level	Daily Caloric Needs
Very Light	2119 Calories/Day
Light	2472 Calories/Day
Moderate	2649 Calories/Day
Heavy	3002 Calories/Day
Very Heavy	3355 Calories/Day

6. Comments/Modifications to the Protocol

If you have comments or if there have been modifications to the protocol as described above, answer "yes" to question 5 on the Anthropometry Form and record comments in the space provided. If there are no comments or modifications, answer "no" to question 5.

7. Quality Control ~ Calibration Check of Scales and Equipment Check

7.1 Equipment:

- Four 50-pound weights (certified prior to first MESA visit) to calibrate scale
- Gulick II anthropometric tapes

7.2 Check scales for accuracy on a monthly basis.

7.21 Place two weights on the balance beam scale and record the numeric value obtained in the "Light Poise" column of the "Scale Calibration Checklist." Add two more weights and record the numeric value obtained in the "Heavy Poise" column.

7.22 The balance beam scale values should be within ±1.0 pound of the expected weight. If either value exceeds this limit, the scale must be calibrated by the manufacturer or by the appropriate institution personnel.

7.23 When the balance beam scale is not in use, keep it balanced at 300 pounds. This

keeps the tension off the internal spring mechanism.

7.24 Place two weights on the BCS-2 body composition scale, record height = 1 cm, and record the displayed body weight value in the "Light Poise" column of the "BCS-2 Calibration Checklist". Add two more weights and record the displayed body weight value in the "Heavy Poise" column.

7.25 The BCS-2 scale values should be within ±1.0 pound of the expected weight. If either value exceeds this limit, the Coordinating Center must be notified and the scale must be calibrated following instructions provided by the manufacturer.

7.3 Examine anthropometry tape measures on a weekly basis for sign of wear.

3.5.2 Seated Blood Pressure

I. PURPOSE

Blood pressure (BP) level is a major risk factor for coronary heart disease, congestive heart failure, and stroke. Seated Blood Pressure was obtained in all previous MESA exams to provide longitudinal measures. The purpose of a specific measurement protocol, or training and certifications of technicians, and of ongoing quality control is to minimize variability due to known exogenous factors and to reduce imprecision and biases in measurement. Seated Blood Pressure was obtained in all previous MESA exams to provide longitudinal measures. The Dinamap® automated device will continue to be used for consistency and to reduce the potential for observer biases.

In addition, measurements of lean body mass and fat mass will be measured by bioelectrical impedance. The purpose is to provide a more accurate and direct measure of body fat than can be provided by calculation of the body mass index.

Pulse oximetry will also be measured at this time.

II. MATERIALS AND EQUIPMENT

- Dinamap® automated blood pressure device (Dinamap Monitor Pro 100®, which includes printer paper, power cable, and power converter.)
- Blood pressure cuffs in a variety of sizes (Dura-cuf Adult Assortment Pack® [#2699]).
- Measuring tape (for arm circumference).
- Watch or stop watch (to time five-minute rest and resting heart rate).
- Copy of Critikon® chart for choosing correct BP cuff size (see Table 2).
- Information sheet on interpretation of BP from JNC VI (see Table 1).
- Valhalla Model BCS-2 Body Composition

III. DEFINITIONS

1. Sphygmomanometry: Measurement of blood pressure.

2. Oscillometric device: Method for measuring blood pressure that relies on the oscillation or fluctuation in arterial pressure generated by the cardiac cycle and transmitted to an inflated blood pressure cuff overlying an artery. This method differs from the auscultatory method, which relies on audible changes over an artery during deflation of an inflated cuff.

IV. CLASSIFICATION OF THE PARTICIPANT'S BLOOD PRESSURE WITHIN THE JNC VI CATEGORIES AND CRITERIA FOR ALERTS AND REFERRALS

This classification and the criteria for alerts have not changed from Exam 1. However, they are important and are reiterated here.

The 1997 Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI) defines categories of blood pressure and recommends follow-up according to the following criteria:

Table 1. Classification of BP in Adults Aged 18 Years or Older*.

BP Category	SBP (mm Hg)		DPB (mm Hg)	Action
Optimal	<120	and	<80	Recheck in 2 years
Normal	<130	and	<85	Recheck in 2 years
High-normal	130–139	or	85–89	Recheck in 1 year
Hypertension*				
Stage 1	140–159	or	90–99	Refer within 2 months
Stage 2	160–179	or	100–109	Refer within 1 month
Stage 3	≥180	or	≥110	Refer within 1 week or immediately

* When recommendation for follow-up of DBP and SBP are different, the shorter recommended time for recheck and referral should take precedence. This classification applies only to participants not taking antihypertensive drugs.

** Diagnosis of hypertension must be based on two or more readings taken at each of two or more visits following an initial screening.

SBP= systolic blood pressure. DBP= diastolic blood pressure.

1. Alert levels requiring **immediate referral** (send participant directly to a physician or hospital) for MESA participants are:

   - **Systolic BP >210 mm Hg**
   - **Diastolic BP >120 mm Hg**

2. Alert levels requiring *urgent referral* (*within one week*) are:

   - *Systolic BP 180–210 mm Hg*
   - *Diastolic BP 110–120 mm Hg*

3. Alert levels requiring follow-up within two months time, and, therefore, we recommend physician notification for systolic or diastolic BP above these levels.

   - BP >140/90 mm Hg

4. JNC VI states that blood pressure classifications and referral recommendations are based on the average of two or more readings on two or more occasions. In MESA we intend to use the average of the 2nd and 3rd blood pressure readings (see below) in order to reduce the impact of reactivity (higher first reading) on the estimate of the value of the underlying blood pressure. Thus, in deciding whether a participant meets criteria for an alert level, the average of the 2nd and 3rd readings should be used. This will require on-the-spot arithmetical manipulation of the systolic and diastolic values. A hand calculator may be useful. The data forms include fields for these averaged values and for any actions taken.

V. METHODS

1. Preparation

   1.1 Record the date of the procedure and the Dinamap® number on the Seated Blood Pressure screen during the five-minute rest period.

1.2 Before the BP measurement procedure, explain to the participant what to expect and how long the procedure will take. The following script is suggested:

This part of the exam involves taking your resting blood pressure. It will take about 10 minutes. We would like you to sit with both feet on the floor and your arm supported on the table. We will have you sit quietly for five minutes. Then we will take your blood pressure three times, one minute apart, using an automated device. We will give you your blood pressure readings and some material to help you interpret them at the end.

2. Cuff Size Selection

2.1 Use the proper cuff size to avoid under- or over-estimation of the correct blood pressure. Selection of the proper sized cuff is based on the guideline that the length of the inflatable bladder in the cuff should be at least 40% of the arm circumference. Measurement of the bladder length in the Critikon® cuffs confirms that the chart in Table 3 conforms to this guideline. A copy of this chart should be available during the BP measurement procedure for easy reference. *Selection of cuff size should be based on the Critikon® chart in Table 2, and only Critikon® cuffs should be used.* If the participant's arm size falls in a range in which there is overlap of two Critikon® cuff sizes, use the *larger* cuff.

2.2 Measure the right arm circumference as follows:

- Ask the participant to bare the upper arm.
- Ask the participant to sit or stand holding forearm horizontal, i.e., parallel to the floor.
- Measure arm length from the acromion (bony extremity of the shoulder girdle) to the olecranon (tip of the elbow) using a metric tape.
- Mark the midpoint on the dorsal (back) surface of the arm.
- Ask participant to relax arm along side of the body.
- Draw the measuring tape snugly around the arm at the midpoint mark, keeping the tape horizontal. Only pull the tape snug enough so that the first red-bead marker can be seen. Tape should not indent the skin. If you can see both bead, the tape is too tight. *Record the arm circumference measured to the closest (0.1) cm in Field 1 on the Seated Blood Pressure Form.*
- Use the criteria in Table 2, below, to determine cuff size. *Check the cuff size used in Field 2 on the Blood Pressure Form by filling in the appropriate circle.*

Table 2. Cuff Size Indicated by Measured Arm Circumference

Arm Circumference*	Cuff Name**	Bladder Length (cm)
12-19	Child	8
19.1-25	Small Adult	10
25.1-33	Adult	13
33.1-40	Large Adult	17
40.1-50	Thigh	

* These circumferences are printed on the corresponding cuff for verification.
** Critikon Dura-cuf® nomenclature is also printed on the cuff.

3. Setting up the Dinamap® BP Machine

3.1 Load the printer paper by opening the flap on the side of the device. There is a diagram showing how to thread the paper on the inside of the door. There is a gray plastic wheel to the left of the roller. Just to the right of the gray wheel is a *gray plastic lever.* Gently flip this lever up. This releases the roller so that you can use the gray plastic wheel to turn the roller to thread the paper. Flip the gray lever down when finished.

3.2 To turn on the Dinamap® device, push the "Off/On" button on the front control panel (lower left).

3.3 After five seconds an initial message will appear on the LCD screen. It will consist of a WARNING and the instruction, "PUSH A FRONT PANEL KEY TO START."

3.4 In the main menu select PRINT using the gray toggle knob. In the next menu, select AUTO and then push the toggle knob. This will program the device to print the blood pressure measurements.

3.5 Do not touch the monitor again until you have completed steps 4–6, below, and you are ready to proceed with blood pressure measurement.

4. Positioning the Participant

4.1 The workstation should be free of excessive noise or distractions.

4.2 The participant should be seated and relaxed in a comfortable chair, to ensure that:

- He or she is sitting up (not slouched).
- Both feet are on the floor (legs/ankles not crossed).
- Right forearm is supported resting on the table.

4.3 The participant should not talk, eat, or drink during the procedure.

4.4 Ideally, the Dinamap output will not be visible to the participant during the measurement, as this may cause anxiety.

5. Application of the Blood Pressure Cuff

5.1 Place the appropriate cuff around the upper right arm so that the mid-height of the cuff is at heart level. Palpate the patient's brachial artery and place cuff so that the artery is aligned with the cuff arrow marked "artery."

5.2 Place the lower edge of the cuff, with its tubing connections, two centimeters above the natural crease across the inner aspect of the elbow.

5.3 Wrap the cuff snugly around the arm, with the palm of the participant's hand turned upward.

5.4 Secure the wrapped cuff firmly by applying pressure to the locking fabric fastener over the area where it is applied to the cuff.

5.5 Do not wrap the cuff too tightly around the arm. You should be able to insert the first

joint of two fingers under the cuff. The cuff should be snug but not tight.

5.6 Be sure all air is squeezed out of the cuff before each inflation.

6. Rest Period

6.1 The participant should rest for five minutes (timed using a watch or stop watch) prior to the heart rate and blood pressure measurement.

6.2 When the five-minute rest period is over, but before the first blood pressure measurement is started, enter the Dinamap number in the field at the top of the screen.

7. Blood Pressure Measurement

7.1 To begin the blood pressure procedure, access the Main Menu on the Dinamap® by pushing the "Start/Stop" button at the lower right of the monitor. (Please note that the gray knob located at the upper right of the monitor allows you to change selections in the monitor screen, in a manner similar to a computer mouse or pointing device. Rotate the knob in order to move from one item to another in the monitor screen, and push it to select the desired option.)

Use the knob to select the SET BP option from the menu and then press the knob (equivalent to clicking a mouse) to implement the selection. The next menu appears automatically.

Use the knob to select AUTO BP and then press the knob.

7.2 Immediately after you select AUTO BP the monitor will start the first blood pressure measurement. However, during this first inflation, select the window that has appeared to the right of AUTO BP and push the knob, so that there is a black number against a clear background in the window. Rotate the knob to select "2." This will select two minutes as the interval between sequential blood pressure measurements. Push the knob again (colors in window will reverse) to implement the selection. (The device will retain this setting, even after it is turned off, so you will not have to repeat this step again.)

7.3 Palpate the radial pulse during inflation. The radial pulse should not be palpable at peak inflation pressure. If the participant's radial pressure remains palpable when the device begins to deflate, the device will complete its deflation procedure and then should automatically reset itself for a higher inflation pressure and repeat the measurement. In the unlikely event that this does not occur, manually reset the inflation pressure:

7.31 Rotate the knob until the window to the right of TGT PRESSURE is highlighted, push the knob and rotate it again until it reaches 210, and push it again to select. Repeat the blood pressure measurement.

7.32 It is not necessary to repeat or prolong the five-minute rest period if this happens, but explain the change in the procedure to the participant (e.g., "I think we need to use a higher inflation pressure—I'm just going to reset the machine").

7.33 If a higher maximal inflation pressure is needed, reset this parameter at 260 mm

Hg, and, if necessary, at 300 mm Hg. Check carefully to be sure that the cuff is properly positioned on the participant's arm with the arrow at the brachial artery.

7.4 When the radial pulse is obliterated at maximal inflation, the first blood pressure measurement will be obtained. The device will automatically obtain the 2nd and 3rd measurements, at two-minute intervals.

7.5 *Record the three sequential blood pressure readings and pulse rate in Fields 3, 4, and 5 on the Seated Blood Pressure Form as soon as they are displayed on the Dinamap.*

7.6 After the 3rd measurement is obtained, return to the main menu and select TREND and then PRINT ALL. When printout is obtained and verified, proceed to TREND and then CLEAR. When the monitor requests confirmation, select YES. Keep the printout in the participant chart in case needed for confirmation of values.

7.7 In order to keep the machine from continuing with further automatic blood pressure measurements go to the main menu, select SET BP and then MANUAL. There is no need to turn off the machine if another participant is ready. If for any reason the machine automatically starts an unnecessary inflation, push the "Start/Stop" button at the lower right hand corner of the monitor and then select MANUAL, as explained above. Remove the blood pressure cuff from the participant's arm and thank the participant for his/her time.

8. Pulse Oximetry

8.1 Place oximeter on participant's finger prior to the beginning of the blood pressure measurements.

8.2 After the blood pressure measurements have been completed and recorded on the screen, record the oximetry value in the Pulse Oximetry field.

8.3 If participant was using supplemental oxygen during the test, click the Yes button for item #7 and record the flow rate in the field provided

9. BP Measurement Instructions for Participants With Short, Thick Arms

9.1 Occasionally there will be a participant whose upper arm is too thick and short for the thigh cuff or on whom the thigh cuff pops open on inflation. The alternative procedure in this case is to obtain the resting blood pressure in the right *forearm.*

9.2 Measure the forearm circumference at the midpoint between the olecranon (elbow) and the ulnar stylus (wrist bone on pinkie side). Select the proper size cuff based on the forearm measurement. The blood pressure procedure is otherwise the same.

9.3 You must document on the Seated Blood Pressure Form that you have measured the *forearm blood pressure.*

10. Reporting Blood Pressure Results to Participants

10.1 Once the three blood pressure measurements have been entered, the computer will

calculate the average of the last two pressure readings and display it below the third reading.

10.2 The technician may verbally provide the participant with the blood pressure reading (the average of the last two pressures), *if asked,* after the procedure has been completed.

10.3 Alternatively, if the blood pressure is normal (<140/90), the technician may say that it is normal, particularly if asked.

10.4 If the blood pressure is not normal (>140/90) but not at an alert level (>210 mm Hg), the technician should exercise the standard option of not discussing the interpretation or stating that it does appear to be high (or "somewhat elevated") but that, again, it will be discussed later.

10.5 If an alert level is identified, the technician should calmly notify the clinic coordinator when the procedure has been completed. (If symptoms of severe hypertension are present, the technician should notify the clinic coordinator immediately.)

10.6 Any additional comments or notes may be entered in the Comments box at the bottom of the screen.

VI. QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES FOR DINAMAP PRO 100®

1. Once a week each device should be used simultaneously with a paired device to simultaneously measure the blood pressure in each arm of a non-smoker under the age of 50, in whom there is no reason to suspect that the blood pressure in the two arms should differ. Repeat the measurement three times.

2. If the paired blood pressure measurements agree within 4 mm Hg or less, for both systolic and diastolic BP, the devices are considered to be in calibration.

3. Investigate any systematic divergence, even if less than 4 mm Hg (e.g., by switching arms and/or pairing the devices with a third device).

4. If the two devices differ by more than 4 mm Hg, calibration must be done*. It should be recognized that, if the cuff deflation rate is 2 mm Hg/sec and the heart rate is 60 bpm, divergences of 2–4 mm Hg would be expected, even if the device is in perfect calibration.

VII. CERTIFICATION FOR RESTING BLOOD PRESSURE MEASUREMENT IN MESA

Certification requires five documented, correctly performed blood pressure measurement, following the MESA certification form.

3.5.2A Resting Oxygen Saturation

I. PURPOSE

The rationale for measurement oxygen saturation is to measure cardiopulmonary function.

II. MATERIALS AND EQUIPMENT

Pulse Oximeter (manufacturers….)
Instruction of the manufacturers

III. METHODS

Preparation

1. Resting oxygen saturation will be measured while the participant is resting for blood pressure measurement.
2. Explain the procedure to the patient
3. Verify that the probe is clean, dry and in good condition before applying it to the participant
4. Ask the participant to remove her nail varnish or acrylic nails that may impair the effective transmission of light (have some nail varnish remover on hand just in case).

Positioning the Participant

1. The workstation should be free of excessive noise or distractions
2. The participant should be seated and relaxed in a comfortable chair
3. The participant should not talk, eat, or drink during the procedure
4. Pulse oximeter should be placed on the subject's finger
5. Verify that the probe is well positioned

Reading the pulse oxymeter

Record the apparent median value obtained while observing the monitor over a one-minute observation period

For participants using oxygen

If the subject is using oxygen, the pulse oximeter should be placed on the subject's finger first. Next, the subject's oxygen should be discontinued while monitoring the oximeter for a period of five minutes. If the pulse oximeter reading falls to 82% or less, oxygen will be replaced and a reading of 82% will be recorded as the subject's oximetry. The apparent median value obtained while observing the monitor over a one-minute observation period should be recorded.

Quality Assurance/Quality Control Procedures for Oxymeter

1. Clean sensor/probe as per manufacturers' guidelines in between each patient use.
2. Calibration as per manufactures instructions
3. Daily quality control check on self
4. Carry spare alkaline batteries
5. To avoid any leakage from the batteries, remove them if the pulse oximeter is not in regular use
6. Report immediately to the Clinic Coordinator if the pulse oximeter appears to be malfunctioning

Certification requires five documented, correctly performed pulse oxymetry measurements, following the MESA certification form

Ankle-brachial index (ABI)

I. BACKGROUND AND RATIONALE

Lower extremity eripheral arterial disease (PAD) will be assessed with the ankle brachial index (ABI). The ABI is a reproducible and valid measure of lower extremity PAD. A normal ABI is from 1.00 to 1.40, with progressively lower values below 1.00 corresponding to more severe PAD. Many persons in the group with an ABI >1.40 will also have PAD. These higher ABIs are uncommon and reflect medial arterial calcification and partial or complete incompressibility of blood vessels, and primarily occur in persons with diabetes.

II. EQUIPMENT AND SUPPLIES

- Two Nicolet Dopplers, Elite 100R, EN 50R with rechargeable batteries.
- Two full tubes of ultrasound transmission gel.
- Two aneroid sphygmomanometers, DS66 trigger type, #5098-30.
- Eight Welch Allyn adult arm blood pressure (BP) cuffs, 2 piece, 1 tube bladder, #5082-43 (4
- of these 8 will be for the ankle).
- Four large adult arm BP cuffs, 2 piece, 1 tube bladder, #5082-44.
- Four thigh size BP cuffs, 2 piece, 1 tube bladder, #5082-77.
- Tissue or wash cloth to remove ultrasound contact gel.
- Non-toxic dry erase marker
- T-Spray (Pharmaceutical Innovations, Inc.) and/or Clorox Disinfecting Wipes (both contain the same disinfectants)
- Tegaderm (3M)
- Polylined towels (sterile drapes - multiple internet sources)

III. DEFINITIONS

1. PAD, peripheral vascular disease, peripheral atherosclerosis, lower extremity arterial disease, and peripheral arterial obstructive disease are synonyms. PAD does not refer to venous disease, small-artery obstructive disease, vasospastic disease, cold sensitivity, or capillary disease. For this protocol, PAD does not refer to carotid artery disease, or to other peripheral non-coronary atherosclerotic disease.
2. The ABI is a ratio of the ankle to arm systolic blood pressure (SBP), and is computed separately for each leg. Specifics of the ABI computation for MESA Exam 5 are given below.

IV. METHODS

1. Preparation

1.1 The ABI should always proceed the blood draw. If for some reason it does not, see precautions below.

1.2 Explain the procedure to the participant and allow him/her to ask questions.

1.3 Conduct the examination in a quiet, warm, and comfortable room. If the room is cool, a blanket may be used to cover the participant (including arms, hands, and feet), except

while the actual measurements are being made. Have the participant lie supine on a comfortable horizontal examination table. The head and heels must be at the same level, and therefore the table must be long enough so that for each participant, the entire head and both feet must be on the table, not overhanging. Because having the feet even slightly lower than the rest of the body will produce an invalid ABI measurement, an oversized examination table must be available at the field center for tall study participants.

1.4 Arms below the shoulder and legs below the knee should be bare.

1.5 Inspect all four blood pressure (BP) cuffs before placement and use only cuffs that are clean and dry. Do not place blood pressure cuffs over any lesion that could be a potential source of contamination. Do a visual exam and use a protective, non-penetrable covering over any such lesions. See below for specific instructions.

1.6 Have the participant rest quietly for at least 5 minutes before beginning the measurement procedure. The participant may be sitting or supine while resting.

1.7 Prepare Ankle Brachial Index Computer Screen

1. Select the participant ID from the displayed list of checked in participants.
2. Select "Ankle Brachial Index" from the list of available forms.
3. Tech ID will be pre-entered with the ID of the tech who is logged in. This may be edited if necessary.
4. The form will be displayed with the ID number and acrostic already filled in.

1.8 While the participant is resting, place an appropriate BP cuff around both arms, based on arm circumference at midpoint. The general rule is that the cuff width must be at least 40% of the arm circumference. The 3 cuff sizes should be employed as follows:

- Adult cuff for arm circumference of < 32 cm
- Large adult cuff for arm circumference of 32-42 cm
- Thigh cuff for arm circumference of ≥ 43 cm

The widths of the bladder for "Adult", "Large Adult" and "Thigh" cuffs are 12, 16 and 20 cm, respectively. Please note that on the Welch Allen cuffs, there are numbers next to the names of the cuffs (e.g. "Adult 11" and "Large Adult 12"). These numbers do **not** correspond to the width of the cuff and should **not** be used to determine which cuff is placed on the arm.

1.9 Place an adult cuff size on each ankle. Place the cuff so that the tube is facing the torso, not the toes, and the lower portion rests 3 cm proximal to the greatest protuberance of the medial malleolus (ankle bone).
Once all four cuffs are in place and the 5 minutes of resting are complete, you may begin the measurements as described below.

Before you begin the procedure, instruct the participant to remain relaxed and to refrain from helping you (e.g. lifting the arm to facilitate placement of the cuff). Once you begin the procedure, explain the steps to the participant as you proceed.

2. Arterial Systolic Blood Pressure (SBP) Measurement

2.1 *This step is optional, but will likely be necessary in some participants*: By palpation, locate the brachial artery on both arms (antecubital fossa), and the dorsalis pedis (dorsum of the foot and often in direct line with the 2nd toe) and posterior tibial (medial ankle just behind the medial malleolus) arteries on both legs. Mark the location of each artery with a marker. Sometimes an arm or ankle pulse will not be palpable but can be found with the Doppler.

2.2 Using the procedure below, measure SBPs in the following order (same as on the computer screen):

1. Right brachial artery
2. Right dorsalis pedis artery
3. Right posterior tibial artery
4. Left posterior tibial artery
5. Left dorsalis pedis artery
6. Left brachial artery

2.2.1 Place an appropriate amount of ultrasound conducting gel over the end of the Doppler.

On occasion, there may be skin lesions on the arms, legs or at the insonation site that are of concern for performing the measurement of the SBP. In these instances, we recommend the following procedures be followed in an effort to reduce the potential for contact with blood borne pathogens.

1. For major open lesions, omit the BP measurement in the affected extremity. For minor lesions that are a potential source of contamination (lacerations, abrasions, rash, etc) at the site for placement of the blood pressure cuff, either arm or leg, wrap the affected area with a polylined towel (i.e. sterile drape). Then wrap the blood pressure cuff around this towel and conduct the pressure measurement as described.
2. For lesions with contamination potential at an insonation site, do not perform the Doppler measurement at the affected site.
3. For the specific circumstance when the lesion is a venous puncture due to a blood draw performed before the ABI measurement, apply a Tegaderm dressing over the puncture site and perform the measurement as described. In this situation, the Doppler probe should be cleaned with T-Spray or a Clorox Disinfecting Wipe both <u>before and after</u> insonation.

2.2.2 After palpating the location of the pulse, turn on the Doppler and place the probe over the artery. With this large probe, careful angulation is not necessary. Place the probe in line with the artery and move it from side to side until the strongest pulse is heard. Don't press too hard on the artery with the probe. Rest your hand comfortably so that the probe is secured in place once a strong pulse is heard. Explain the procedure to the participant and ask if the participant has any questions before the measurements begin. If applicable, suggest to the participant to rest comfortably and to try to be quiet and still.

In a small percentage of participants, you may not be able to find the posterior tibial or dorsalis pedis pulse. If you are having trouble, be patient and continue to search for at least three minutes. If you are still unable to locate a pulse, enter a systolic pressure of "000" for that artery.

2.2.3 Inflate the cuff until the pulse is no longer audible. **Inflate to 20 mm Hg above the level at which the pulse sound disappears**. (If the pulse cannot be obliterated, you may raise pressure to a maximum of 300 mm Hg. If not obliterated at that point, record "300"). Deflate the cuff slowly allowing the pressure to drop at a rate of **2 mm Hg per second**. Record the pressure at which the first sustained (more than one beat) pulse reappears. This is the SBP at this location. Deflate the cuff completely.

2.2.4 *Enter the measurement in the appropriate field on the ABI computer screen immediately*. . If a given measurement was not done, click the "Not Done" bubble and a list of reasons will appear on the screen. Check the appropriate reason. If "Other" is selected, be sure to enter the specific reason in the box provided. Then repeat the process to obtain a pressure measurement at each of the remaining sites.

2.2.5 If the signal remains faint as more pressure is released or if the probe moves off the artery, deflate the cuff completely, and then repeat the measurement.

2.2.6 Click save on the computer screen when all measurements have been performed and the results entered into the computer screen,

After completing the ABI measurements, thoroughly clean the Doppler probe with T-Spray or a Clorox Disinfecting Wipe. Please note that the Doppler must be completely clean and dry between participants.

Important note: record "000" for arteries where the pressure is not detectable, and record "300" for arteries that are not compressible (the highest pressure attempted). For arteries that could not be assessed, record "999", click the "not done" bubble, and select the appropriate choice for "reason not done". If the 'other" box is chosen, please specify the reason.

Calculation of the ABI A computer program will calculate the ABI from your measurements. For your information, the procedure is given below.
The ABI denominator - There is only one ABI denominator per participant for both the left and right ABIs. This denominator is the higher arm SBP.
The right ABI numerator is defined as the higher of 1) the right posterior tibial SBP or 2) the right dorsalis pedis SBP.
The left ABI numerator is defined as the higher of 1) the left posterior tibial SBP or 2) the left dorsalis pedis SBP.
The right ABI is the right ABI numerator divided by the ABI denominator.
The left ABI is the left ABI numerator divided by the ABI denominator.

3.5.4 Phlebotomy & Spot Urine Collection

I. PURPOSE

The purpose of the Phlebotomy and Spot Urine forms is to record information related to the blood draw and spot urine collection to facilitate the tracking of samples and to inform the central laboratory of samples collected and any issues that may affect processing

II. MATERIALS/EQUIPMENT

See Section 3.7 for specific materials.

III. METHODS

General instructions

1.1 There are six possible blood draw configurations depending on the studies in which participants are enrolled.

1.2 Data entry software will create the appropriate Phlebotomy form automatically.

1.3 The process of labeling of tubes and cryovials for a given participant will be dictated by the specific version of the Phlebotomy form for that person.

1.4 Copies of the Phlebotomy form and the Processing form are included with the shipment of samples to the Central Laboratory.

1.5 The full lab section (3.7) includes the details about the blood draw and processing.

Specific Instructions:

1 Spot Urine Form

1.1 The spot urine form is an electronic data entry screen. Indicate whether or not the urine collection was done.

1.2 If urine was not collected, select the reason why not

1.3 Indicate whether or not the participant is selected as a QC subject.

2 Phlebotomy Form

2.1 Print the participant's phlebotomy form immediately after he or she has signed the informed consent and the responses have been recorded in the Consent screen. The informed consent responses are necessary to print the correct version of the Phlebotomy Form for that participant.

2.2 Give the Phlebotomy form to the phlebotomist or lab tech as soon as possible to inform him or her how to complete the setup of the sample tubes and cryovials, or if the prepared MESA setup is all that is needed.

2.3 After the blood draw has been completed, retrieve the completed Phlebotomy form and enter the information in the appropriate fields on the electronic data entry screen.

3.5.5 Electrocardiogram

Table of Contents

I. INTRODUCTION

The MESA Central ECG Reading Center (CERC) is located at Wake Forest University Health Sciences, Department of Public Health Sciences, EPICARE Section. The CERC main contacts are:

Elsayed Z. Soliman, MD, MSc, MS, Principal Investigator
Phone: (336) 716-8632
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esoliman@wfubmc.edu

Susan Hensley, BS, Computer ECG Technician
Phone: (336) 716-9616
Fax: (336) 716-0834
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Lisa Keasler, AAS, Assistant Project Manager
Phone: (336) 716-0387
Fax: (336) 716-0834
lkeasler@wfubmc.edu

Charles Campbell, AAS, BS, Data Manager
Phone: (336) 716-3915
Fax: (336) 716-0834
chcampbe@wfubmc.edu

Contact Susan Hensley or Lisa Keasler with questions and/or comments pertaining to study ECG acquisition and transmission.

II. BACKGROUND AND PURPOSE

The ECG recordings in MESA will serve to establish the distribution of subclinical disease findings and the development of new disease (including silent myocardial infarction, left ventricular hypertrophy, ischemia, prolonged QT interval, reduced heart rate variability (HRV), and arrhythmias) as well as the development of subclinical ECG findings that are determined to be associated with a poor prognosis. Like the other non-invasive cardiovascular function measures to be employed in MESA, the ECG recordings will be used both to detect new (incident) cardiac disease and to develop predictive equations for future morbidity and mortality based on newly tested ECG findings. The MESA ECG reading will include classification of ECG abnormalities using Minnesota code and Novacode as well as providing continuous measures of the ECG waveforms.

III. FIELD CENTER PROCEDURES

The field center procedures include ECG acquisition and transmission to the CERC, and local ECG reading by the clinic physician. The ECG transmission will be detailed under data management procedures (III.3).

- At each MESA examination 3 digital ECGs will be recorded electronically for each participant using a GE MAC 1200 electrocardiograph.
- Each MESA site has two electrocardiographs, the software of which has been configured for correct transmission of signals over analog phone line by modem to the CERC.

- At each MESA visit, the clinic ECG technicians will record the scheduled ECGs with the participant *fasting.* That is, the ECG must be recorded after an overnight fast (and after this history is checked in the clinic) *and before any snack* (or at a later date the possibility of an oral glucose tolerance test) is given.
- Each participant will have *three immediately sequential ECGs* recorded at each visit.
- The ECGs stored in the MAC1200 will be transmitted to the CERC at least twice weekly.
- ECGs will be processed and monthly reports with results will be sent to the MESA Coordinating Center (CC).

III.1. ECG ACQUISITION PROCEDURES

III.1.1 Electrocardiograph

The electrocardiograph to be used for ECG recording and transmission for MESA is the GE/Marquette MAC 1200 portable electrocardiograph. Each clinic is provided two of these machines, configured specifically for MESA study ECG acquisition and transmission.

- The MAC1200 is to be used for resting ECG recording.
- It is not intended for use as a vital signs physiological monitor.
- Only EPICARE certified technicians may acquire MESA study ECGs.
- The MAC1200 is a portable device and can easily be moved from one location to another.
- The MAC1200 has a liquid crystal display (LCD) that shows three leads at a time.
- The MAC1200s used in MESA have a customized menu specific to the MESA study.
- Appendix A includes the instructional charts that outline the SETUP for MESA MAC1200 ECG machines. All ECG technicians for MESA should become familiar with the CERC manual and the GE MAC1200 Operator's Manual,

III.1.2. Supplies

Table 1 summarizes the equipment and supplies needed for recording and transmitting ECGs. Always order in advance.

Table 1

Equipment	Supplies
• GEMSIT MAC1200 Electrocardiograph with its 10 lead acquisition module and attached modem • HEARTSQUARE • Telephone jack cable • Scissors • Felt tip non-toxic washable markers • The CERC contact list • Reference guides for "Patient Data Entry" (Table 2) • Reference guide for "Transmission of ECG" (Appendix B) • GEMSIT MAC1200 operation manual	• MAC1200 ECG paper • GEMSIT disposable silver chloride electrodes • Alcohol swabs and gauze pads • Cotton surgical tape • Examining table disposable paper

III.1.3 Preparation for ECG recording

Three ECGs will be recorded after 12 hours of overnight fasting and *before* any snack or juice is given to the participant at the clinic.

- Participant should be relaxed and comfortable in supine or semi-recumbent position.
- Examination table/bed should be adequate to comfortably accommodate the participant. Supply drape for exposed upper torso. An additional covering may be needed to prevent the participant from becoming chilled.
- Make sure ankles and wrists are accessible for electrode application.
- ECG electrode placement should always be performed with the technician standing to the participant's left side.
- Reference guide for "Participant Data Entry" instructions should be available to insure accuracy.
- Supplies needed for ECG acquisition should be assembled and arranged efficiently.

III.1.4 Location of the ECG electrodes

III.1.4.1 Location of limb electrodes (Figure 1)

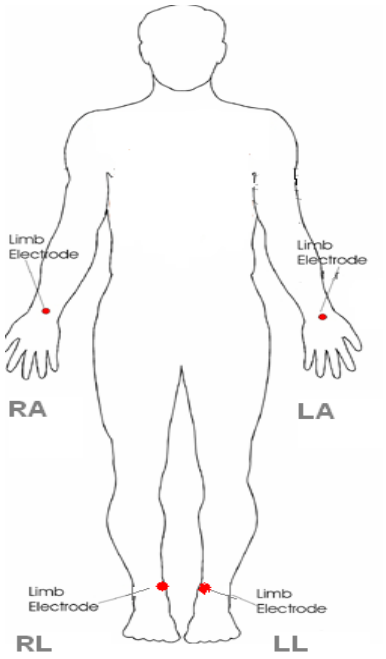
RIGHT LEG (RL) AND LEFT LEG (LL):

- The right leg electrode serves as a ground connection, and problems there will influence all other leads.
- On the inner side of the right leg (RL), above the ankle, rub briskly an area about 1-2 inches in diameter with an alcohol swab using firm, circular motions
- Mark the position to place the electrode later.
- Repeat this procedure for the left leg (LL).
- In amputees, the leg lead electrode may be placed higher up on the torso.

RIGHT ARM (RA) AND LEFT ARM (LA):

- Rub the inner side of the right arm (RA) above the wrist similar to what you did with the right and left legs.
- Mark the position to place the electrode later.
- Repeat the process for the left arm (LA).
- In amputees, the arm electrode may be placed on the shoulder, below the clavicle.

FIGURE 1



III.1.4.2 Location of chest electrodes

V1 AND V2:

- First locate the sternal angle about the width of your 3 middle fingers below the sternal notch (Figure 2).
- Feel the sternal angle between the index and middle fingers of your right hand, keeping the fingers wide apart and moving your fingers firmly up and down. While feeling the sternal angle, move your fingers to the left side of the sternum and feel the 2nd rib between your fingers where it joins the sternal angle.
- Move your middle finger to the interspace below the second rib and with your index finger

locate the interspace below the next rib (3rd) and again below the next (4th) rib. This is the 4th intercostal space. Mark an X at this level at the midsternal line. X is the reference level for V1 and V2. Mark their locations at the right and left sternal border (Figures 2 and 3).

FIGURE 2

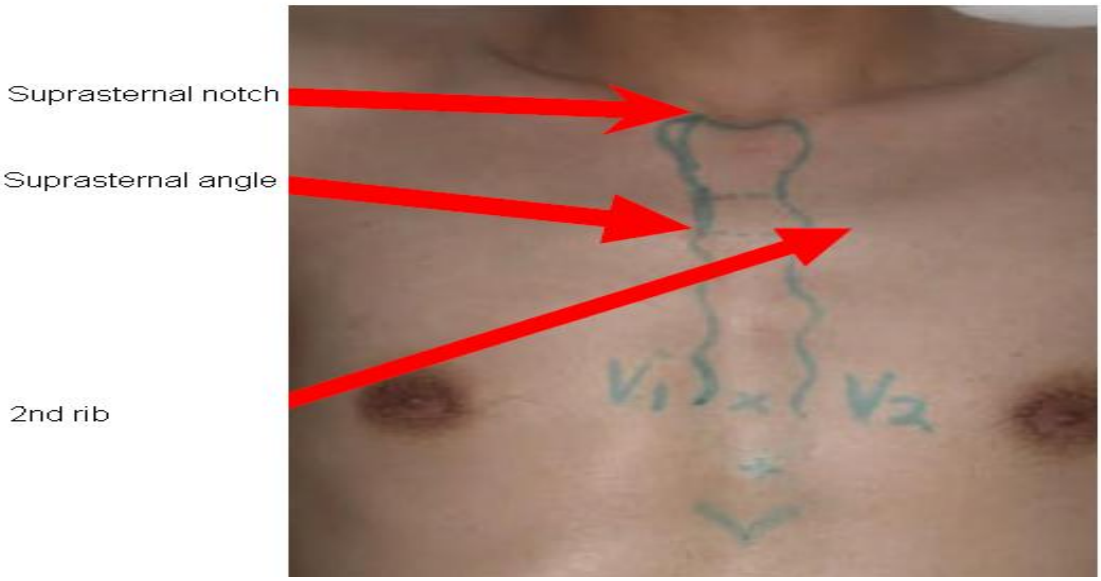
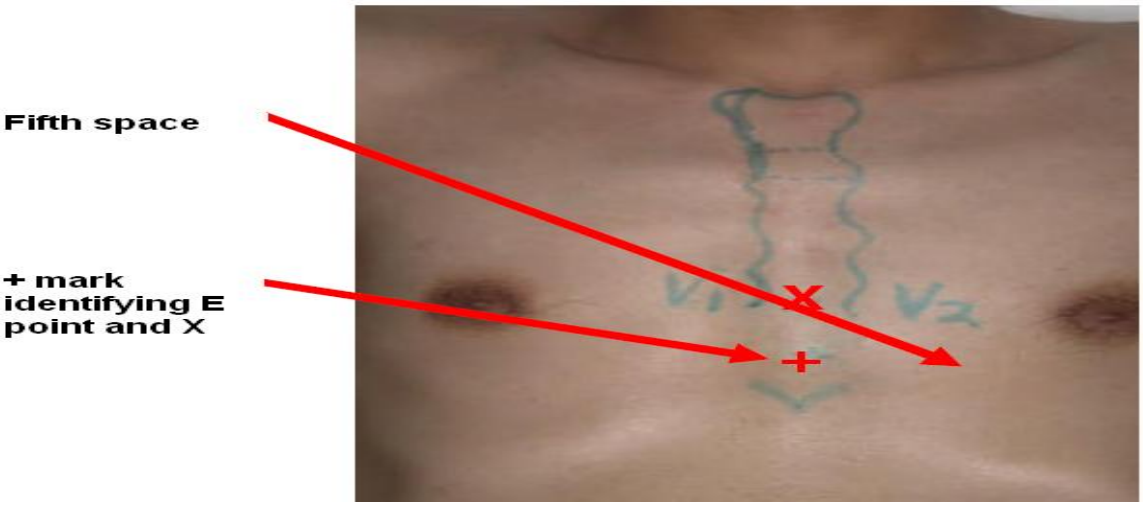


FIGURE 3



REFERENCE POINT "E" FOR LOCATING V4, V5, AND V6

- From the location of V2, palpate with the middle finger of your right hand the intercostal space and follow it laterally outside the sternal border and at a slight angle down. Feel the 5th rib between your index and middle fingers and feel the 5th intercostal space with your index finger.

- At the level of the 5th intercostal space, mark a + at the midsternal line below your x mark for V1-V2 level. This + is the reference level "E" for V4, V5, and V6 (Figure3).
- In overweight persons and in women with tender breast tissue, it is often difficult to locate the 5th intercostal space. In such a case, mark the + for E point 1 ¼ in (3 cm) below your reference level X for V1 and V2 (in smaller adults, 1 inch. (2.5 cm) is enough).

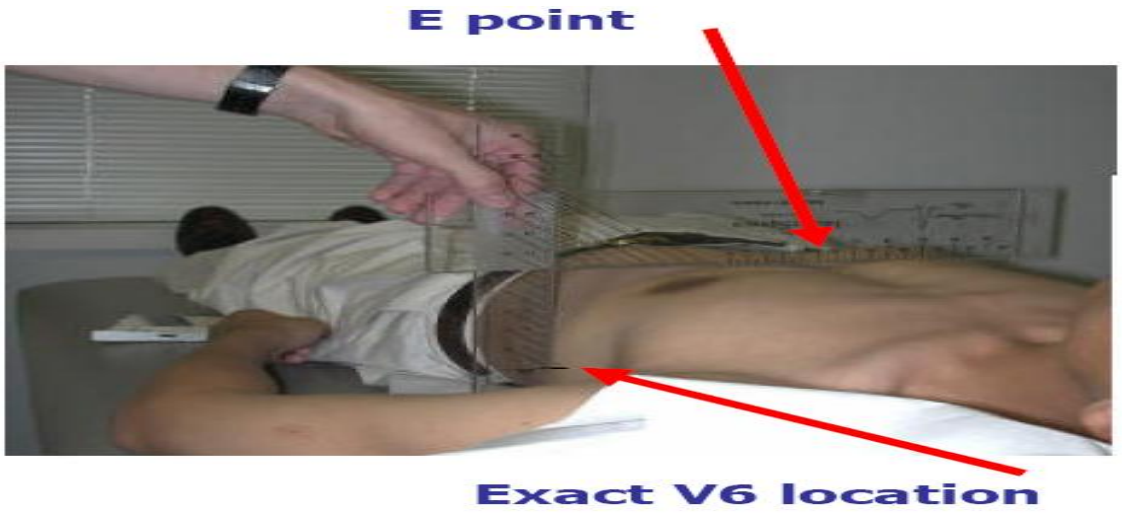
APPROXIMATE LOCATION OF V6

- Move the left elbow laterally without moving it anteriorly or posteriorly, while observing the anterior and posterior axillary folds. The left elbow must be supported properly.
- Follow a line exactly in the vertical midplane of the thorax (mid-axillary line - Figure 4) down where the line meets the horizontal plane of e point. Using your marker, make a vertical one inch long line there as an approximate location of V6.

EXACT LOCATION OF V6

- Exact location of V6 is determined by using the HeartSquare.
- Place the HeartSquare horizontally with the wider arm (E arm) at level e point (Figure 4).
- Slide the V6 arm of the HeartSquare towards the midaxillary line until the arrow points to the mark at the midaxillary line. Mark the exact location of V6 at the level of the arrow on the V6 arm.

FIGURE 4



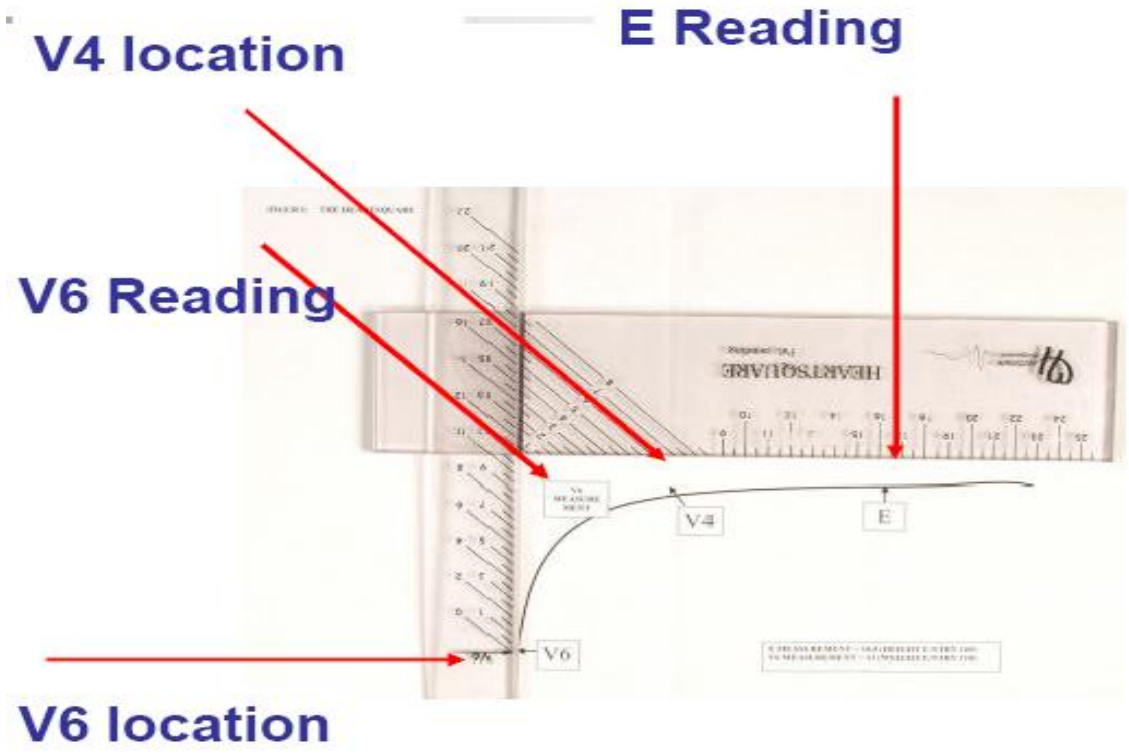
EXACT LOCATION OF V4

- While keeping the HeartSquare in the horizontal position with the arrow on the V6 arm pointing toward the V6 position, observe the reading at E point. (Figure 5)
- Use this e reading on the centimeter scale on the V6 arm, and follow this same E reading along the 45 degree lines towards the torso to locate the exact position of V4.
- Now that you have located V6 and V4, secure the V6 arm with your thumb to prevent it

from sliding. Note the V6 reading which is the distance from the arrow on the V6 arm to where this arm intersects the E arm at right angles. You may then remove the HeartSquare.

- Enter the E and V6 measurements as three digits. Figure 5 shows that the E entry is 160 and the V6 entry is 120 for the readings of 16.0 cm and 12.0 cm, respectively. Enter the 160 for E in the height field of your Mac 1200 and 120 for the V6 measurement in the weight field (DO NOT ENTER THE HEIGHT AND WEIGHT OF THE PARTICIPANT).

FIGURE 5



LOCATIONS OF V3 AND V5

- Mark V3 exactly halfway between V2 and V4.
- Mark V5 exactly halfway between V4 and V6. (Figure 6)

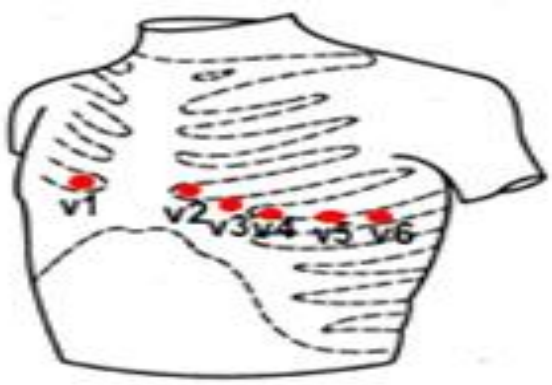


FIGURE 6

III.1.4.3 Attaching the electrodes:

- After you have marked electrodes positions and rubbed them with Alcohol swabs, you may apply the electrodes.
- Do not place electrodes directly over bone.
- Attach lead wires in the same, correct order every time to establish routine and to eliminate lead swaps.
- Position the MULTI-LINK on the participant's abdomen.
- Grasp each lead at the MULTI-LINK attachment point.
- Follow lead wire to the electrode attachment end.
- Attach wire to electrode, making sure clip is not in contact with electrode adhesive.
- Make sure lead wires have some slack and are hanging loosely.
- You may secure the lead wire to the skin by applying paper tape 1-inch below the clip, especially if the ECG shows baseline noise despite careful preparation.

III.1.5. ECG recording

Three ECGs will be recorded for each participant.

- The first ECG
  - MAC1200 prompt: NEW PATIENT, Enter **"YES"**
  - Enter information according to "Participant data entry to the MAC1200" Table 2.
  - First Name Field: Enter "Exam 5- ECG 2"

- Second ECG:
  - MAC1200 prompt: NEW PATIENT: Enter **"NO"**
  - All other information remains the same. ECG may be acquired.

- Third ECG:
  - MAC1200 prompt: NEW PATIENT: Enter **"NO"**
  - All other information remains the same. ECG may be acquired.

Table 2 Participant Data Entry into the MAC1200 for the MESA Study

Category Listed on Mac1200	***Entry to Machine by ECG Technician***
NEW PATIENT	YES
LAST NAME	1st 4 letters of last name + 1st 2 letters of first name + gender (m or f)
FIRST NAME	Enter "Exam 5-ECG 2"
PARTICIPANT ID	Enter the 7-digit ID number
SECONDARY ID	Enter Same as Participant ID
PACEMAKER	Select YES or NO
GENDER	Select Male or Female
HEIGHT	Enter E Measurement of HeartSquare (e.g., if E=16.0, enter 160) **DO NOT ENTER HEIGHT**
WEIGHT	Enter V6 Measurement of HeartSquare (e.g., if V6=12.0, enter 120) **DO NOT ENTER WEIGHT**
RACE	Choose Other and highlight defined race codes (defined on the MAC1200)
REFERRING PHYSICIAN	No action required
TECHNICIAN	Choose Other and select technician
LOCATION	No action required

[NOTE: Keep these instructions with the MAC1200 for quick reference as needed by the ECG technician.]

III.2. Local ECG reading

III.2.1 Baseline Exclusions

Local reading of ECGs was done at the baseline ECG to confirm atrial fibrillation (AF), atrial flutter, or the presence of a *pacemaker*. The presence of any of these abnormalities confirmed by a local clinic physician excluded such potential participants from the study and further testing (carotid ultrasound, coronary calcium score, or cardiac MRI) was not done.

III.2.2. "Alert" ECGs

Because there are no available diagnostic statements from the CERC except as monthly measurement reports to the Coordinating Center and because the diagnostic statements printed on the ECG by the MAC 1200 are not always correct, the local clinic reading of the ECGs is essential for safety purposes

Below is a list of alerts that *do not require* immediate physician review. There are many other possible alerts that will also not require physician review, but these are the most common. It is expected that the field centers will add to this list as they gain experience with the types of alerts typically seen at their sites.

- 1st degree AV block
- Axis deviation

- Early repolarization
- Intraventricular conduction defect
- Low voltage
- Occasional PAC
- Occasional PVC
- Sinus bradycardia
- Sinus arrhythmia

Below is a list of alerts that *require immediate* physician review. Follow the specific directions given for each alert. Do not unnecessarily alarm the participant or venture a diagnosis. There are other alerts that will require immediate physician review.

- Atrial fibrillation.
- Atrial flutter.
  These are not an emergency in a person without symptoms; but these ECGs should be reviewed by a physician in the clinic, and, if the ECG diagnosis is confirmed, the participant and his/her physician must be notified. The clinic physician should also determine the urgency of advising the participant's physician and if the participant requires urgent care. Participants with atrial fibrillation at the baseline visit are not eligible for MESA.

- Pacemaker.
  No urgent or semi-urgent notification is required. This finding can simply be included in the results letter mailed to the participant and his/her physician. However, participants with pacemakers at the baseline visit are not eligible for MESA.

- WPW
- Idioventricular rhythm
- Ventricular tachycardia
  These are not an emergency in a person without symptoms; but these ECGs should be reviewed by a physician in the clinic, and, if the ECG diagnosis is confirmed, the participant and his/her physician must be notified. The clinic physician should also determine the urgency of advising the participant's physician and if the participant requires urgent care.
- Complete heart block
- Left bundle branch block
- Acute pericarditis.
- Injury, infarct, or ischemia characterized as acute or marked.
  All of these are potential emergencies. The ECG should be reviewed by a physician in the clinic. If the findings are confirmed, the physician should make a judgment about whether urgent transportation for further care is required. The participant and his/her physician must be notified immediately.

If an alert is not detected by the MAC 1200, fill in the "no" bubble. If an alert is detected, and if it is reviewed and confirmed by physician review, fill in the "YES confirmed" bubble; if the alert is reviewed but not confirmed, fill in the "YES not confirmed" bubble.

III.3. Data management procedure

III.3.1 Communications setup for transmission

As previously mentioned, internal set up of the machines must be done according to the instructions established by CERC. Correct internal set up should enable the clinics to send the ECGs via a modem and a phone lines. Adding 9 (or other number) to get an outside line and/or adding an access code for long distance are taken into consideration. [NOTE: Contact the CERC any time with questions and/or comments]

III.3.2 Before transmitting ECGs to the CERC

- Ensure that all previously transmitted ECGs are deleted only after confirmation of receipt.
- Check to ensure that all IDs are valid. You can correct any variable from your participant data information by doing the following: While holding the "Shift" key down, press the Store/Retrieve key, move the cursor to the ID in question, highlight "Change" and then proceed to make corrections as needed

III.3.3 Transmitting ECGs to the CERC

- Secure the modem cable into the 9-pin connector found on the right side of the MAC1200 and the 25-pin connector found on the rear of the modem.
- Plug one end of the phone cable into the connector marked "LINE" on the rear of the modem and the other end into any "**analog**" (fax) phone line**.**
- Start at the 12-lead screen. While holding the "Shift" key down, press the "Store/Retrieve" key. Press the down arrow 3 times and then hold the shift key and the down arrow together to get to the desired ECG to be transmitted. The screen will show black squares on the right and left sides of the ECG selected for transmission.
- To skip an ECG press the down arrow without using the shift key.
- Repeat this procedure until all ECGs that are to be transmitted have been selected.
- Once selections are made, press the "Enter" key. This will return you to the top of the screen.
- Use the right arrow to highlight "Send" and press the "Enter" key.
- Another screen will appear which states "to start transmission, press enter". Once transmission is complete, press the "Start/Stop" key, located on the far bottom right of the keyboard, to return to the 12-lead screen.
- Confirmation of receipt of transmitted ECGs could be made by logging into the EPICARE website using a user name and password specific to each clinic (*Technicians will be communicated with web-access information and their username/password)*. Allow 24 hours between transmission and confirmation of receipt through the website and deletion of ECGs form the ECG machine to allow system backup at EPICARE. **No ECGs are to be deleted from the electrocardiograph until receipt is received from EPICARE**

III.3.4 Directory management

Keep your directory correct and current by doing the following:

- BEFORE TRANSMISSION: Delete all unwanted ECGs like those with flat lines, poor quality or duplicates. Correct any errors in participant data entry like ID numbers, or HEARTSQUARE measurements

- AFTER TRANSMISSION: Delete transmitted ECGs ONLY after confirming that EPICARE has successfully received the ECGs.

VI. READING CENTER TECHNICAL DETAILS

Set-up of the machines is ONLY allowed to be done at the CERC or with assistance of one of the CERC staff or an authorized study personnel if it has to be done at the clinic. It may be necessary to re-program the machine after the start of the study if a malfunction occurs, or the battery has been allowed to become dead. The machine set-up and programming instruction are listed in Appendix A.

VI.1. Data processing

All MESA scheduled electronically transmitted ECGs (three per participant per scheduled visit) will be received at the CERC by a GE- MUSE ECG management server.
The digital ECGs are stored in an electronic database at the MESA CERC, in a Marquette measurement matrix, by participant ID. This database will remain unaltered. Additionally, a second and third database will be created after technician editing of correct onset and offset of the waveforms. These two databases are then transformed into Minnesota Code and Novacode categories by the EPICARE ECG coding program. These codes will be transmitted to the MESA CC. Continuous measurements of wave durations and QT interval can be used by the CC to test for trends in editing. A diagram of the data flow is outlined in Appendix C

VI.2 Data reporting

The format and route of data transfer will be determined by agreement between the Coordinating Center (CC) and the CERC. Monthly reports will be sent from the CERC to the CC. All electronic ECGs from receipt at the CERC to transmission of data to the CC will be within 30 days

V. QUALITY CONTROL ISSUES AND PROCEDURES

V.1 Quality grades

The ECG reading center evaluates and ranks the ECG quality through an automated system with visual confirmation of the results if needed. There are 4 grades; from 1 to 3 (which are automatically assigned by the GE-MUSE) and 5 which is manually decided by EPICARE staff for poorest quality- No grade 4. The best grade is 1 and the worst is 5. Generally, grades 1 and 2 are difficult to separate visually and they are considered good. Grades 3 is given to ECGs that have correctable problems i.e. the ECG problems could be adjusted for on reading them. Grade 5 ECG are given for the ECGs that there have major problems which make it impossible to read them

V.2 Certification/Recertification procedures

- All ECG technicians **must go through the certification** process before they are allowed to acquire study ECGs.
- Each technician must acquire and successfully transmit 2 good quality ECG sets (3 ECGs each).
- The 2 ECG sets should be performed on 2 different volunteers or on 1 volunteer provided that there is at least 30 minutes between each ECG set**.**
- Recertification process (required every two years) is the same as the certification process.

- The participant data entry should be done according to the instructions in table 3 after pressing the "pat info" key on the MAC 1200 keyboard

Table 3 Entry into the MAC1200 for certification of technicians **ONLY**

Category	**Entry**
New Patient	YES
Last name	Enter technician's last name
First name	Enter technician's first name
Date of birth	Enter volunteer's birth date (MM/DD/YY)
Participant ID	Enter 99999999 (Press "Shift" key to enter numbers)
Secondary ID	Enter 99999999 (Press Shift key to enter numbers)
Pacemaker	YES or NO
Gender	M or F
Height	E Measurement of HeartSquare (e.g., if E=16.0, enter 160)
Weight	V6 Measurement of HeartSquare (e.g., if V6=12.0, enter 120)
Race	Choose "Other" and choose defined race codes
Referring physician	No action required. Pre-programmed data
Technician	Choose "Other" and select technician's last name
Location	No action required. Pre-programmed data

V.3. Examples of common ECG quality problems and possible solutions

- EXCESSIVE BASELINE DRIFT (Figure7): This occurs if the participant is moving around or there is tension on the lead wires. Ask the participant to lie still for a few seconds. Drift in excess of 1 mm between baseline points (QRS onset) of any two successive complexes is a sign of significant drift.
- EXCESSIVE MUSCLE NOISE (Figure 8: The participant is either tense due to lack of body support or may be cold. Use a wide bed and blanket to cover the participant.
- BASELINE DRIFT DUE TO TANGLED WIRES (Figure 9): Ensure that the wires are not pulling. Be sure to establish a good electrode connection. Lay a towel across the wires, if necessary. Adjusting the angle of the clip at the electrode often helps. You may need to tape down the chest leads; use only hypoallergenic medical tape to prevent allergic reactions. Use a U loop (not a cross loop) with the electrode wires, i.e., the wire should not cross but remain open like a U; never crossover wires
- LOOSE ELECTRODE CONNECTION (Figure 10): Loose electrode connection may cause a wavy baseline in some ECG leads. Check each electrode to ensure that it is secure.
- SIXTY HZ NOISE (Figure 11): Periodic 60 HZ noise is sometimes visible in the record. This may be caused by AC interference from a nearby machine. Make a visual check of this before recording the ECG. Unplug any unnecessary surrounding electric equipment *Note:* Jewelry does not cause 60 HZ noise.
- MISSING LEADS AND LEAD REVERSAL (Figures 12-14): To minimize the chances of having lead reversal and missing leads, always make sure that there are no flat lines in the ECG recording and/or mainly positive QRS in aVR lead. Also, always have a second look at the connections before recording

Figure (7) Excessive baseline drift due to sudden movement of the participant

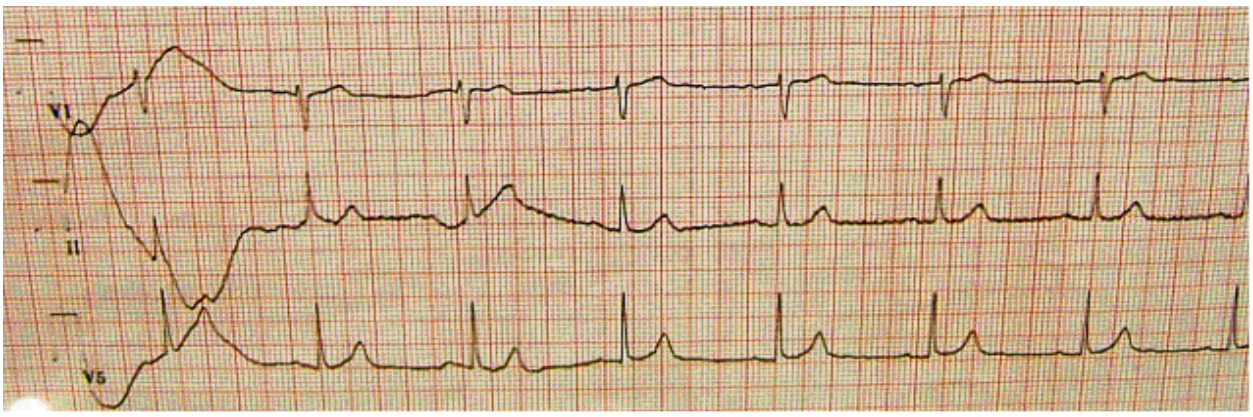


Figure (8) Excessive muscle noise

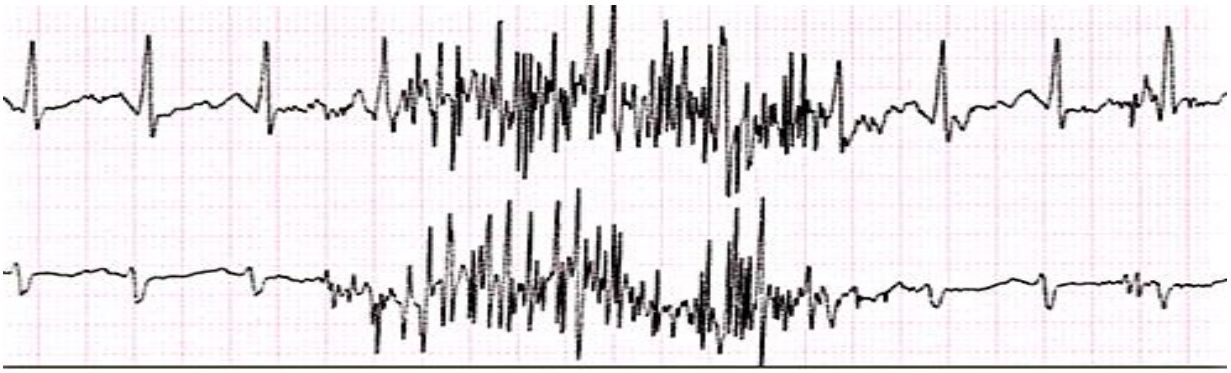


Figure (9) Baseline drift due to tangled wires

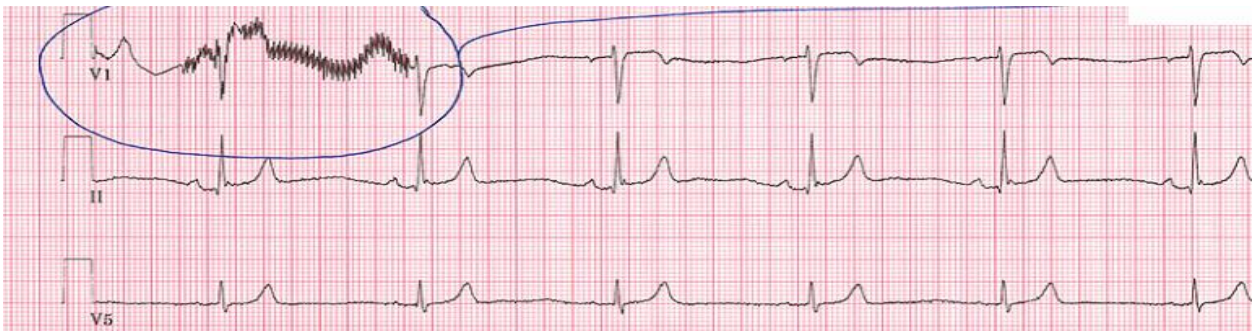


Figure (10) Wavy V1 baseline due to loose electrode

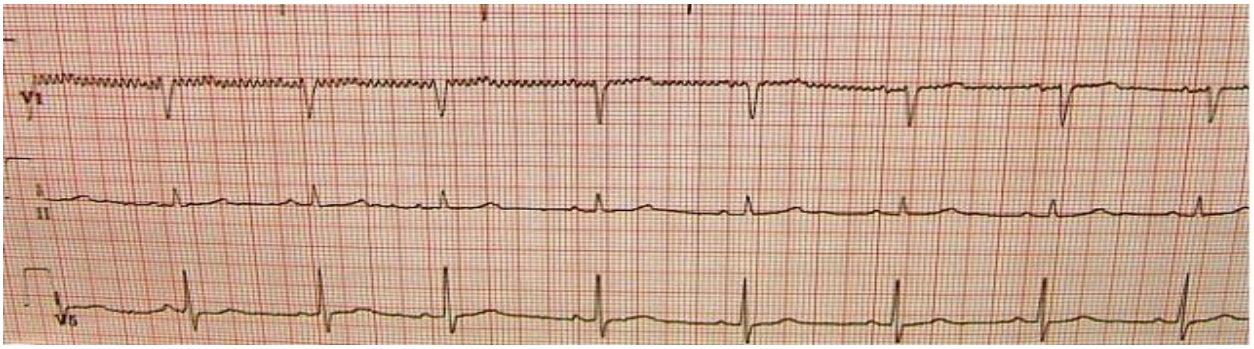


Figure (11) Sixty Hz electrical interference

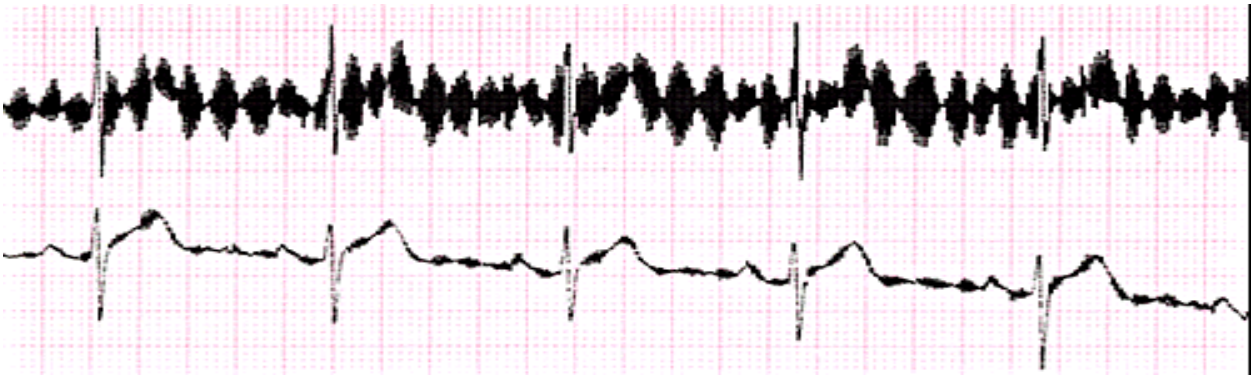


Figure (12) Flat line due to missing V1 lead

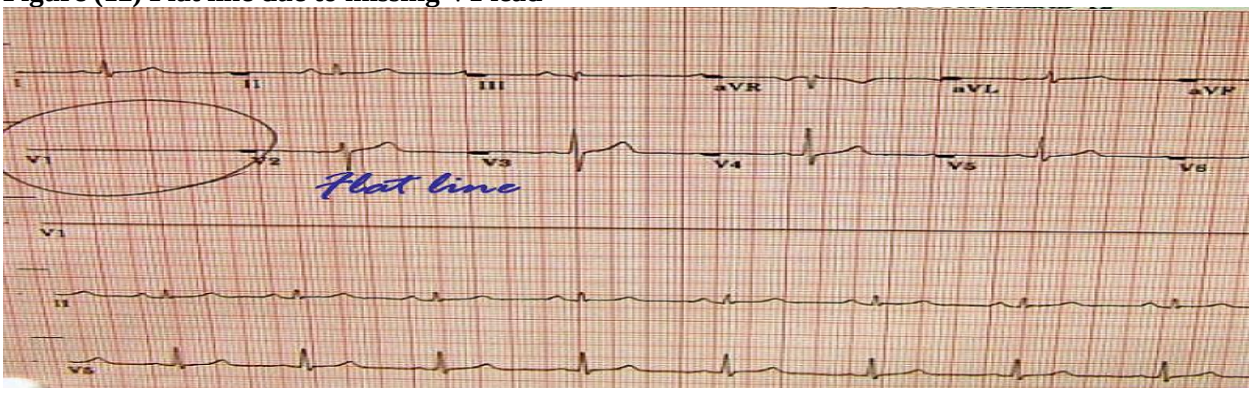


Figure (13) Lead reversal denoted by positive aVR (upper panel) **compared to the normal** (lower panel)

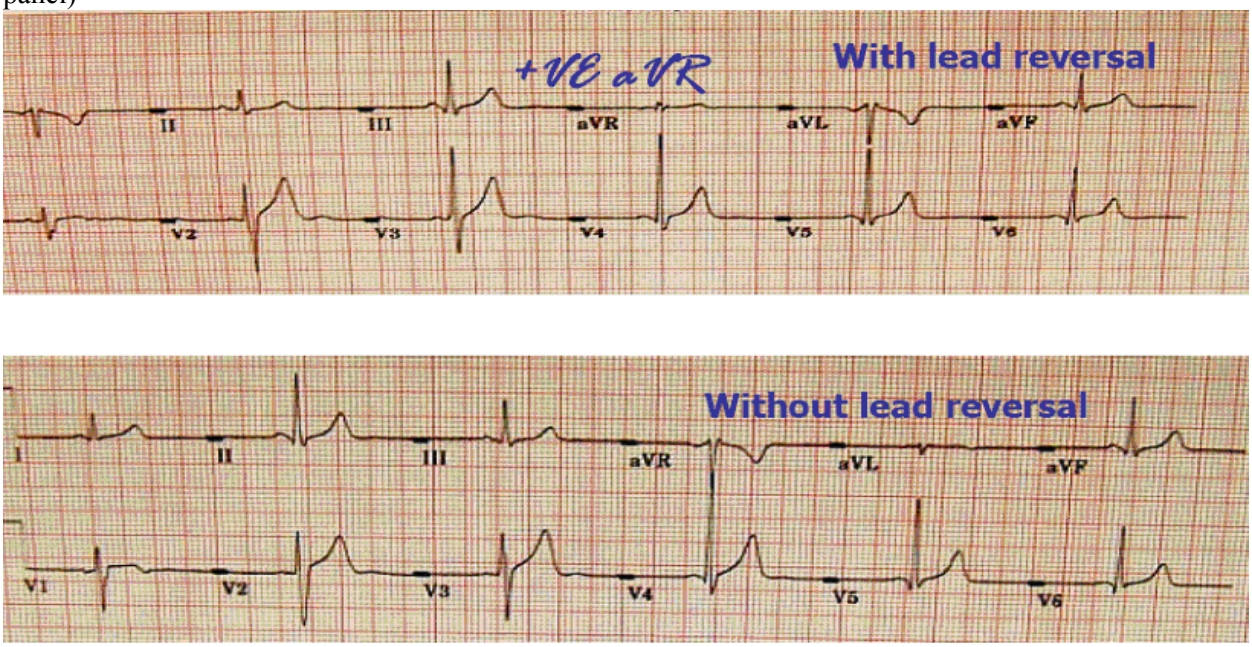
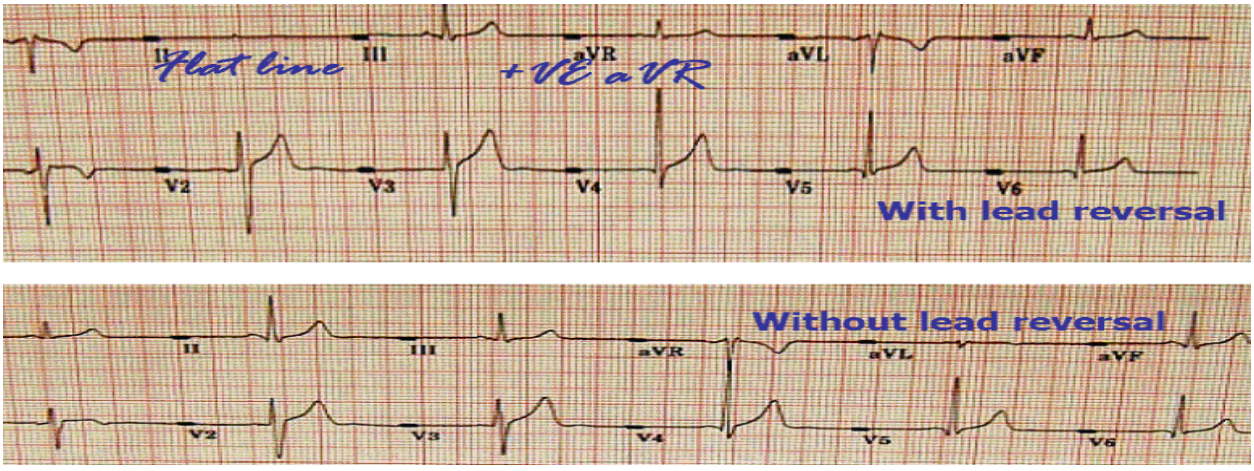


Figure (14 Lead reversal denoted by flat line in one of the limb leads (upper panel) **compared to the normal** (lower panel)



Appendix A
MAC 1200 PROGRAMMING AND SETUP

In order to setup a MAC1200 for the MESA study, turn the ECG machine ON. After the self-test completes, the ECG machine will be at the 12-lead screen (3 flat lines). Press the "Setup" key. Press "Enter" to select either 12-lead setup, system setup, communication setup or participant data setup. To make a selection, use the four arrow keys to highlight any selection and press "Enter".

12-Lead Setup

CATEGORY	SELECTION
REPORT SEQUENCE	[STANDARD]
RHYTHM LEADS	[II]
GAIN	[10]
REPORT FORMAT	[4x2.5R1]
DETAILED RESULTS	[NO]
MUSCLE FILTER	[NO]
MUSCLE FILTER FREQUENCY	[40 Hz]
AC FILTER	[YES]
MANUAL COPY TO	[HOST]
NO. OF COPIES	[1]
DELETE ECG AFTER TRANSMISSION	[NO]
AUTOSAVE ECG	[YES]
USE SCREENING CRITERIA	[NO]
SUPPRESS NORMAL STATEMENTS	[NO]
SUPPRESS ABNORMAL STATEMENTS	[NO]
INTERPRETATION	[YES]
PRINT INTERPRETATION	[YES]
OVERRIDE FUNCTION	[YES]

When finished, press the STOP key

Press the Down Arrow key to highlight System Setup, and press ENTER.

System Setup

CATEGORY	SELECTION
ORDERING PHYSICIAN	Name of the local clinic coordinator
REFERRING PHYSICIAN	Enter MESA 2, then your field clinic name and field clinic number. [For example, MESA 2 WFU 30].
TECHNICIAN	Choose OTHERS, press ENTER. Press ENTER until the cursor is under the LAST NAME; type the technician's LAST NAME then press ENTER. Type the technician's FIRST NAME then press ENTER. Press the Stop key.
INSTITUTION NAME	Type MESA 2 plus name of institution
CART NUMBER	Enter your **field clinic number (**see list below). [For example, WFU would enter 30.] Use this number for all MESA 2 ECG machines at your location, eg: If WFU has 2 ECG machines for MESA 2, then both machines have the same CART #, the CART # assigned to the WFU field clinic.
SITE NUMBER	Enter **175** This is EPICARE's Study Number for MESA 2.
LOCATION NUMBER	Enter 1 for one of your ECG machines and 2 for the other ECG machine.
DATE (mm/dd/yyyy)	Enter the correct date using the mm/dd/yyyy format.
TIME (hh:mm)	Enter the correct time in the hh:mm format.
LEAD FAIL BEEP	[NO]
HIGH HR BEEP	[NO]
LEAD LABELS	[AAMI]
PACE ENHANCEMENT	[NO]
BASELINE ROLL FILTER	[0.08]
DATE	[MM/DD/YYYY]
TIME	[24]
UNITS	[Cm, Kg]
MAINS	[60 Hz]
LCD LIGHT OFF AFTER	[5 MINS]
LOW BATTERY BEEP	[0 sec]
DEFAULT MODE	[12 LEAD]
LANGUAGE	[ENGLISH]
ENABLE PASSWORD	[NO]
TEST DATA	[NO]

RESTORE DEFAULTS	[NO]
PRINT SETUP LISTS	[NO]

Field Clinic	**Field Clinic #**	**Transmission Telephone #**
Wake Forest University, NC	**30**	**13367131102**
Columbia University , NY	**40**	**13367131103**
Johns Hopkins University, MD	**50**	**13367131103**
University of Minnesota, MN	**60**	**13367131105**
Northwestern, IL	**70**	**13367131102**
UCLA, CA	**80**	**13367131104**

When finished, press the STOP key
Press the Down Arrow key to highlight Communication, and press ENTER.

Communication Setup

CATEGORY	SELECTION
BAUD RATE (PC)	[9600]
PROTOCOL	[CSI]
MODEM	MultiTech 56k
DIAL MODE	TONE

PHONE NO.	• Enter your field clinic specific **"Transmission Telephone #"** (see list above).
	• If an Access Code is required to dial a long distance number, enter the Access Code and the transmission telephone number at EPICARE, the same way you would dial a long distance number from your institution (using your Access code), For example:
	  • If the Access Code is needed AFTER entering the transmission number, then enter: 13367131102,,,123456789 where 123456789 is the Access code
	  • If the Access Code is needed BEFORE entering the transmission number, then enter 123456789,,,13367131102 where 123456789 is the Access Code
	• **Note:** Access codes are separated from EPICARE transmission telephone number by three commas (,,,). This allows the MAC1200 to pause before another telephone number is dialed.

OUTSIDE LINE	If you need a digit to obtain an outside line, like a "9" or and "8", please enter that digit here. Otherwise, leave it blank.

When finished, press the STOP key

Press the Down Arrow key to highlight Patient Data Setup, and press ENTER.

Participant Data Setup

CATEGORY	SELECTION
NEW PATIENT	[YES]
PACEMAKER	[YES]
GENDER	[YES]
HEIGHT	[YES]
WEIGHT	[YES]
RACE	[YES]
SYSTOLIC BP	[NO]
DIASTOLIC BP	[NO]
ORDERING PHYSICIAN	[NO]
REFERRING PHYSICIAN	[YES]
TECHNICIAN	[YES]
PHONE NO.	[NO]
MEDICATION	[NO]
COMMENTS	[NO]
ID REQUIRED	[YES]
PATIENT ID LENGTH	7
SECONDARY ID	[YES]
SECONDARY ID REQUIRED	[YES]
LAST NAME (Required)	[YES]
FIRST NAME (Required)	[YES]
LOCATION #	[NO]
ROOM #	[NO]
ORDER NUMBER	[NO]
EXTRA QUESTIONS	[Leave Blank]

When finished, press the STOP key. Press the STOP key once again to exit the Setup menu. The Option Code Setup requires NO action.

Appendix B

Transmission of MESA study ECGs to the CERC

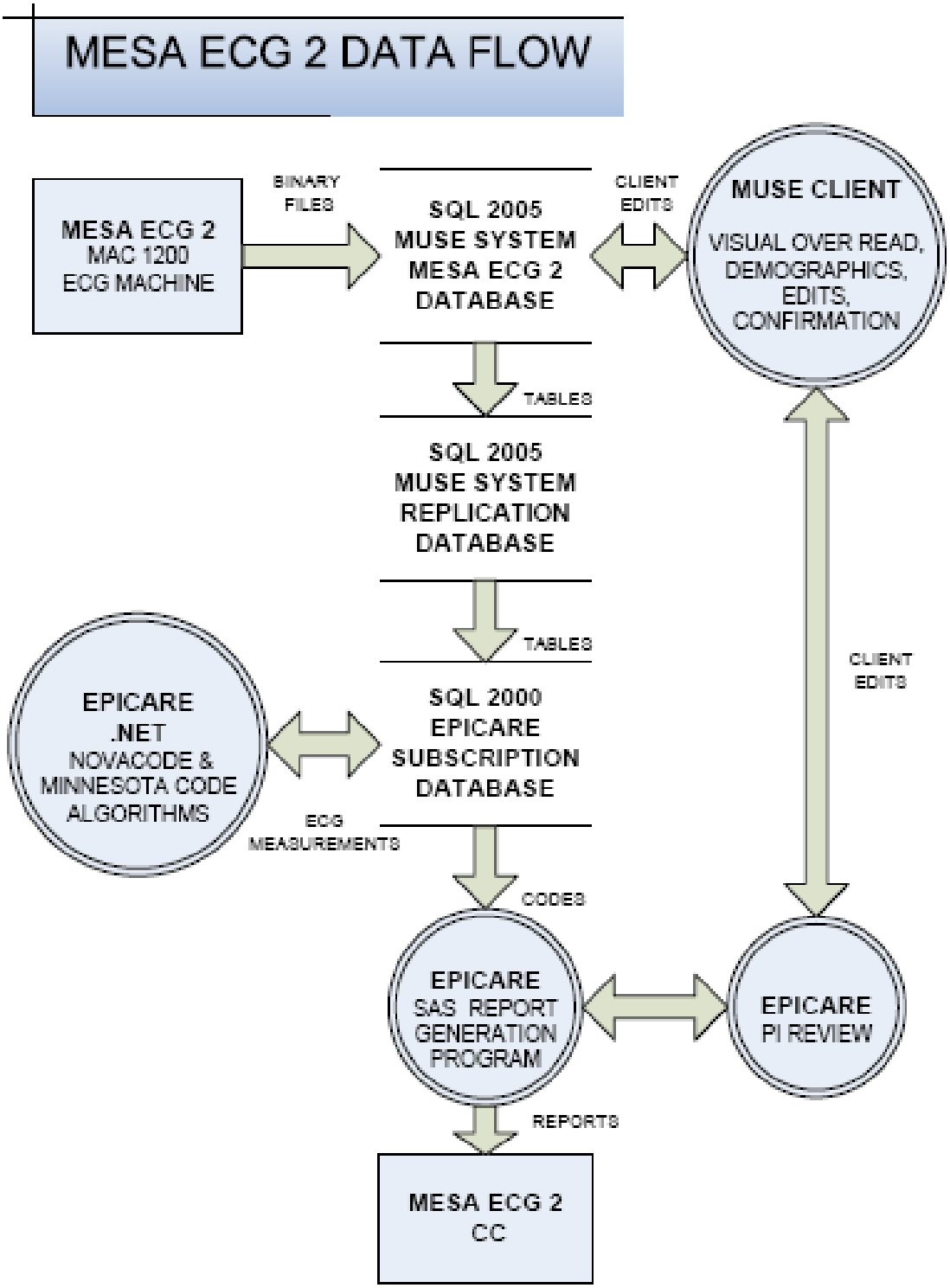
Before transmitting ECGs to the CERC

1. Ensure that all previously transmitted ECGs are deleted only after confirmation of receipt by the CERC.
2. Check to ensure that all IDs are valid.
3. You can correct any variable from your participant data information by doing the following:
    a. While holding the "Shift" key down, press the Store/Retrieve key,
    b. Move the cursor to the ID in question,
    c. Highlight "Change"
    d. Proceed to correct information

Transmitting ECGs to the CERC

1. Plug one end of the phone cable into the connector marked "LINE" on the rear of the modem and the other end into any "analog" (fax) phone line.
2. Start at the 12-lead screen.
3. While holding the "Shift" key down, press the "Store/Retrieve" key.
4. Use the arrow keys to get cursor to the send option below the ECG to be transmitted, then press the "Enter" key. To skip an ECG press the down arrow without using the shift key. Repeat this procedure until all ECGs that are to be transmitted have been selected
5. Press the enter key to start the transmission
6. Once transmission is complete, press the "Start/Stop" key, located on the far bottom right of the keyboard, to return to the 12-lead screen.
7. Confirmation of receipt of transmitted ECGs could be made by phone or by logging into the EPICARE website using a user name and password specific to each clinic (under construction).

Appendix C


MESA ECG 2 DATA FLOW
MESA ECG 2
MAC 1200
ECG MACHINE
BINARY
FILES
SQL 2005
MUSE SYSTEM
MESA ECG 2
DATABASE
CLIENT
EDITS
MUSE CLIENT
VISUAL OVER READ,
DEMOGRAPHICS,
EDITS,
CONFIRMATION
TABLES
SQL 2005
MUSE SYSTEM
REPLICATION
DATABASE
TABLES
CLIENT
EDITS
EPICARE
.NET
NOVACODE &
MINNESOTA CODE
ALGORITHMS
SQL 2000
EPICARE
SUBSCRIPTION
DATABASE
ECG
MEASUREMENTS
CODES
EPICARE
SAS REPORT
GENERATION
PROGRAM
EPICARE
PI REVIEW
REPORTS
MESA ECG 2
CC

3.5.6 MESA – Retina, Retinal Photography

1. Introduction

MESA-Retina is an extension of MESA and seeks to determine the relationship of retinal microvascular disease to the presence of subclinical and clinical CVD in the same cohort. Specifically, MESA-Retina will examine all MESA participants during the MESA Exam 5 beginning April 1, 2010.

1.1 Objective of Protocol

In MESA-Retina, retinal photographs of both eyes of the MESA participants will be obtained. These photographs will be graded at the Ocular Epidemiology Reading Center (OERC) at the University of Wisconsin-Madison for retinal microvascular characteristics, including focal arteriolar narrowing, arterio-venous nicking and retinopathy (e.g., microaneurysms, retinal hemorrhages). In addition, generalized arteriolar narrowing will be quantified using a computer-based measure of retinal vascular caliber. Other significant retinal conditions will also be noted, such as retinopathy or vascular occlusions in people with and without diabetes.

1.2 Background

A microvascular etiology has been suggested to play an important role in the pathogenesis of cardiovascular disease. The retinal vasculature, which can be visualized non-invasively, can potentially be used to evaluate the role of the microcirculation. Abnormalities of the retinal vasculature (such as generalized retinal arteriolar narrowing, arterio-venous nicking and retinopathy) have been shown to reflect microvascular damage from hypertension and arteriolosclerosis. These changes may be markers for related vascular pathology in the coronary and cerebral circulations, and may predict future clinical cardiovascular events. A quantitative way of assessing one of the microvascular changes - generalized arteriolar narrowing in the retina - was recently developed and used in the Atherosclerosis Risk in Communities (ARIC) study population. In the ARIC study, an independent association between generalized arteriolar narrowing in the retina and MRI-detected cerebral infarct[5] and clinical stroke was observed. Generalized arteriolar narrowing in the retina was measured from computer-scanned images of retinal photographs and summarized by a retinal AVR.

2. **Equipment and Material**

2.1 **Equipment**

A Canon CR6-45NM fundus camera equipped with a digital Canon D-60 camera back will be used for this project. A laptop computer, supported by an articulating arm, will contain image acquisition and archive software EyeQSL, provided by Digital Healthcare Inc. The fundus camera and computer are mounted on a motorized instrument table to allow maximum subject comfort and optimum camera alignment. Both photographer and subjects have pneumatically adjustable stools with a backrest.

2.2 **Supplies**

An inventory of supplies for each of 6 study centers, assuming an average of 1,100 subjects per center (10% over-estimate), follows:

(a) 1 box Long-grain Red Cross sterile lens cotton batting, (4 oz/box)
(b) 1 bottle Lens cleaning alcohol, (100% alcohol, 8 oz/bottle)
(c) 6 boxes Facial tissues, (200/box)
(d) 1 canister Compressed air with plastic delivery tip
(e) 1 each Spare view lamp (#BH3-3277)
(f) 2 each Canon camera fuses (125V, 4 amp)

3. Equipment Set Up

The fundus camera should be placed in a room that can be completely darkened during the photography procedure. This is because no dilating drops will be used and we rely on the natural dilation of the pupil that occurs in the dark to perform the fundus photography. The camera should be placed in the room so that the patient has easy access to the examination stool and so that the photographer has ready access to the room light switch. A small reading lamp located near the camera may be used to help the photographer see to navigate and write during the procedure. This lamp should be turned off during photography to reduce the risk of pupil constriction. Light from the computer screen may need to be adjusted to minimize pupil constriction.

The fundus camera will be set on the motorized instrument table and the laptop computer will be attached to the articulating arm located above the camera's video display monitor. The pneumatic stools are positioned on opposite sides of the fundus camera.

3.1 Daily Set-up Procedure

The camera dust cover and lens cap should be removed at the beginning of the day and the lens inspected and cleaned (see section 3.2) as necessary. Dust is the greatest enemy, producing the majority of artifacts on the photographs. When the camera is not in use, the lens cap should be in place and the special dust cover must remain on the camera.

3.2 Camera Lens and Body Care

Before each photograph, the camera lens must be inspected and, if dirty, cleaned with the brush and air bulb to remove debris. Should more extensive cleaning of the lens be required, the lens can be fogged with your breath or moistened with absolute alcohol and then cotton should be used in a circular polishing motion until no dirt or oily film is visible on the lens when it is viewed from the front with the alignment lens removed and the view lamp on and turned up to its near maximum intensity (see page 28 in the Canon Non-Mydriatic Retinal Camera CR6-45NM Operation Manual). The body of the camera should be kept clean and free of dirt with a soft cloth and water or a common spray cleaner. The foreheadrest may be cleaned with alcohol.

3.3 Instrument Table and Stools

The instrument table and stools can be kept clean by wiping with a common spray cleaner and a soft cloth. Occasionally the castors on the table and stools may squeak requiring a drop of light oil. The electric motor on the table requires no lubrication. The motor is protected by fuses, which may need replacing should excessive current blow them out. The power rating of the fuse will be indicated near the instrument table fuse holder.

3.4 Flash, View and Split Lamp Concerns

The Canon cameras are equipped with new lamps at the beginning of the study. It is not anticipated that the flash, view or split lamps will fail during the study. The view and split lamp should last approximately one to two years and are easily replaced as needed. The flash lamp has a life of at least 5,000 flashes, enough to complete the study. Since the view lamps are relatively inexpensive bulbs, one spare should be ordered from Canon USA, Inc. and kept at the field center. Clinic staff in the field can replace the view lamp. Remember to keep all oil from your fingers off these lamps during replacement. The flash and split lamp should be replaced by a qualified technician. Both lamps can be ordered from Canon USA, Inc. when needed for overnight delivery.

As the flash lamp ages, the light output can diminish, producing progressively darker photographs. This can temporarily be over-ridden by an adjustment of the transformer output, though ultimately the lamp should be replaced. The decision to replace the lamp, due to dark photos, will be made with the Photography Consultant following routine review of the digital photographs. The flash lamp requires careful handling during installation (the burnt out lamp may be hot, and the new lamp must be properly aligned.

Only field center staff trained to do this should attempt lamp replacement.

3.5 Camera Malfunctions or Errors

Since the camera requires virtually no other maintenance, any malfunction will need to be investigated first by the examiners at each center and, when necessary, via telephone with the OERC staff. Trouble-shooting tests can be performed in consultation with the OERC staff to diagnose any malfunction. Because the photographer can see the digital images immediately after they are taken, they will be alerted to problems not evident until the processed films are reviewed with conventional film-based fundus cameras. This includes problems with transformer power settings, problems with a dirty objective lens; improperly focused images or missed images caused by a patient's blink or other software/hardware malfunctions.

Some camera malfunctions or photographer errors may not be evident to clinic staff during photography and will not be discovered until the digital images are reviewed at the OERC. This includes unusual image artifacts or problems related to the camera or software. For this reason, prompt transmission of images to the OERC is important and we anticipate that images will be archived and sent to the reading center once each week. Additionally, a telephone should be available in the camera room so that the site photographers and the OERC photography consultants can speak should a malfunction be discovered during photography or should the photographers have a problem or question needing immediate attention. The photography consultants, Anne Goulding or Michael Neider can be reached at 608-410-0628.

Service information can also be obtained directly from Canon USA, Inc., Irvine, California or Itasca, Illinois. Our contacts there are Mark Scheckle, 15955 Alton Parkwa, Irvine California, 92618-3616 (telephone number: 949-753-4193, e-mail: mscheckel@cusa.canon.com) or Tom Penkala, Canon USA, Inc., 100 Park Blvd., Itasca, Illinois, 60143-2693 (telephone number: 630-250-6230, e-mail: tpenkala@cusa.canon.com).

4. **Subject Photography Overview**

Digital images will be captured into a table mounted laptop computer, archived to CDs for the site's use, and uploaded weekly to the secure FTP site at the OERC in Madison, Wisconsin, for analysis. Before the first patient of the day is photographed, the photographer prepares the camera by first turning on the power to the Canon D-60 camera back. The power is then turned on for the Canon CR6-4NM fundus camera and to the Dell laptop computer. The photographer escorts the patient into the photography room, positioning them before the fundus camera, and explains the procedure. The EyeQSL software program is initiated and subject ID and photographer information is entered into the appropriate computer screens and the software is set to acquire images.

Patients will have one eye assigned as the study eye and the other eye assigned as the fellow eye. Photography will be performed of both eyes with the study eye photographed first. All subjects will have two 45-degree photographs taken of each eye. The first photograph will be centered on the optic nerve and this is referred to as Field 1. The second photograph will be centered on the macula and will be referred to a Field 2. A diagram of the location of these two fields can be seen below in Figure 1. The images are stored on the hard drive of the computer as the procedure for the first patient is ended and the camera is prepared for images acquisition of the next subject. At the end of each week, each site archives the images to CD to be kept at the clinical site for backup and also uploads the images to the secure FTP site at the OERC in Madison, Wisconsin.

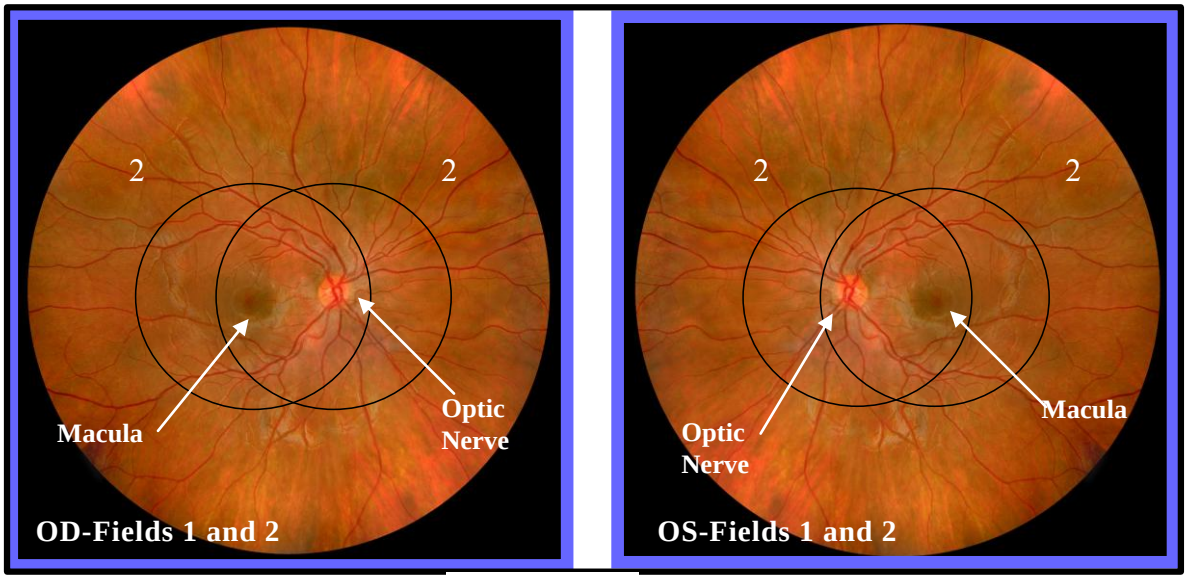


Figure 1

The study eye will be selected based on the subject's ID number. When the sixth digit of the ID number is even, the right eye will be photographed first, and when it is odd; the left eye will be photographed first. If the eye specified by this algorithm is considered too difficult to photograph with adequate photographic quality, only the opposite eye should be photographed, and an explanatory note entered in the MESA Eye Study Non-Myd Photography Shipping Manifest form (Attachment 1 Manifest Form). Conditions falling into this category are (based upon the technician's judgment): eye missing, inability to dilate at least 4 mm, inability to fixate adequately for proper photographic fields, and opacities of the media preventing a reasonably clear view of the retinal vasculature and resulting in unreadable digital images.

4.1 Subject Exclusion

The photographer will attempt photography on subjects with poor visual acuity who may be unable to direct their gaze so that their optic nerve or macula is properly positioned on the camera monitor (as may be the case where both eyes are blind or when the subject is deaf and communication with them is impossible). In these cases, the photographer should get the best field definition possible. If, in the photographer's judgment, no acceptable photograph can be taken of either eye, the subject will be excused from photography.

The photographer should attempt photography on those subjects who are physically disabled, to the point that they can be comfortably positioned at the camera. To facilitate this, the subject may remain in a wheel chair positioned before the motorized camera table lowered to the appropriate height. Care should be taken when lowering the camera table to avoid pressing against the subject's legs. If, in the photographer's estimation, the subject cannot be comfortably positioned, no photography will be performed.

4.2 Pre-examination Procedure

Before attempting photography, the photographer should become very familiar with the camera through a training session and by learning the terminology on pages 4 and 5 of the Canon Non-Mydriatic Retinal Camera CR6-45NM Operation Manual. This Retinal Photography Protocol uses terminology from the Operation Manual and it is recommended that each photographer review the entire manual before performing photography.

The retinal camera should remain covered when not in use. High humidity or temperatures must be avoided. Dusty conditions mean that the camera will need frequent cleaning. The objective lens should be checked and cleaned with the air bulb if necessary before each subject is photographed. A more extensive cleaning is required to remove grease, smudges or stubborn spots from the lens. This cleaning requires removal of the lens "boot" and external alignment lamp ring and should be referred to the chief photographer at each field center.

4.2.1 Subject Explanation

Photography begins with a complete explanation of the procedure by the photographer. A color illustration may be useful to show what the retina looks like. It is important to reassure the subject that no retinal damage is caused by this procedure. The subject should know to expect several flashes. The pictures will include the macula (area of central vision) and it is normal to experience a blue or red tint to vision immediately following the flash. This disappears within five to seven minutes. No dilation drops will be used for this examination, and the eyes will not be touched. The following script or a modified version based on FC participant comfort and understand should be use to explain the retinal photography (suitable for use as written material for deaf or interested subjects):

We will be taking several photographs of the inside of the back of both of your eyes (the retina) so we can study the blood vessels and look for any unusual changes. We will not be touching your eyes or be giving you any eye drops to take the pictures. Instead, you will be asked to sit in a darkened room before a special camera with your chin in a chin rest. We darken the room so that your pupils will dilate and we can align and focus the camera on your retina. While your pupils are dilating, we may ask you some questions about your vision and the health of your eyes. During the aligning

process you will only be aware of some small red lights and a blinking green box and red bars visible in the camera lens. We will ask you to follow the blinking green box as we move it. Just before we take the picture, we will ask you to blink your eyes and then open them real wide. The camera will flash a light from within the camera lens as each picture is taken.

Just after the picture is taken, you may see a blue or red circular spot before the eye photographed. This will disappear within 5-7 minutes and causes no damage to the eye. Please remember that we are only taking pictures (not an x-ray) of a small portion of the back of your eyes and that this picture will not substitute as an eye examination. You will certainly be notified should we notice anything requiring immediate attention. Please continue to see your eye doctor on a regular basis for your complete eye examinations.

4.2.2 Completing the MESA Eye History Form

At some sites, before photographing the subject, the photographer will complete the MESA Eye Vision History Form (Attachment 2), which concerns the subject's ophthalmic history. The form can be completed while the subject becomes sufficiently dilated to be photographed. This will depend upon adequacy of ambient light for the photographer (to be able to read questions and record answers) and upon the time required to answer the questions). For logistical reasons, this form may be completed as a paper form, and later entered into the computer system.

4.2.3 MESA Retina Completion Form

The MESA Retina Completion Form (Attachment 3) records the circumstances of the photographic session, and can only be completed as the session begins. The participant's ID# and acrostic will appear at the top right hand corner of the form. The sixth digit of this number will determine the study eye (right eye if even, left eye if odd); this eye should be photographed first. Before beginning the photography session, the photographer records the site number, photographer ID#, the photography date and the date that the Vision History Questionnaire was completed. If either eye cannot be photographed for a reason gathered during the ophthalmic history (e.g., that eye has been enucleated) or for a reason that emerges during the first part of the session (one eye does not dilate sufficiently well to be photographed), the photographer should record this in the comments section. The flash setting (2 unless re-adjusted by the photographer) and the estimated diameter of the pupil at the time of the first photograph of each eye should be recorded. The photographer is encouraged to comment on anything unusual such as artifacts, pathology or other problems encountered during the session. When the photography session is over and the photographer is reviewing the images in the contact strip, the eye and fields photographed should be marked. If extra images were taken, mark other and record the reason for extra images in the comments section. When the final images for each have been decided upon by the photographer, he/she can record the total number of images taken of each eye. This form will be sent electronically along with the images to the OERC in Madison.

4.2.4 Preparing the Camera

The Canon D-60 digital camera back must be turned on first. This is done using the small dial located on the back left side of the camera body. This must precede turning on

the Canon fundus camera or computer since the camera “looks” for the D-60 camera body during start up. The Canon CR6-45NM video display is activated when the power switch on the side of the main unit is turned on. If no photography or switch operations are performed for 10 minutes, a power saving mode is activated, turning the lamps and display off to prevent unnecessary wear. During this power saving mode a green "ready" lamp blinks on the monitor. Pressing any button below the monitor will reactivate the system.

Notice that the flash power setting (located on the lower right-hand corner of the monitor) blinks when the main unit is switched on. This indicates the system is charging up. Do not attempt to take photographs until the blinking stops, indicating a fully charged flash.

The camera contains an internal clock and the date will automatically change each day. The photographer must manually change the date if this clock should fail or if the camera is left unplugged for a long period of time. The date is displayed on the fundus camera monitor and is changed through the menu located in the “Set 3” screen (see pp. 18-20 in the Canon Non-Mydriatic Retinal Camera CR6-45NM Operation Manual). The date format will read Month-Day-Year. The display form “Disp Form” screen (“Date” sub-screen) is used to adjust the current date.

The Canon D-60 camera body should be attached to the main unit and set with the following settings:

Shutter speed	60
Mode Dial	Manual
AF-WB	Flash
Quality	First Level Large
Red Eye	Off
AEB	0-
ISO	400
Beep	Off
Auto power off	Off
Review	On
Review Time	8 seconds
LCD	Normal

* The camera should remain ON until the end of the day

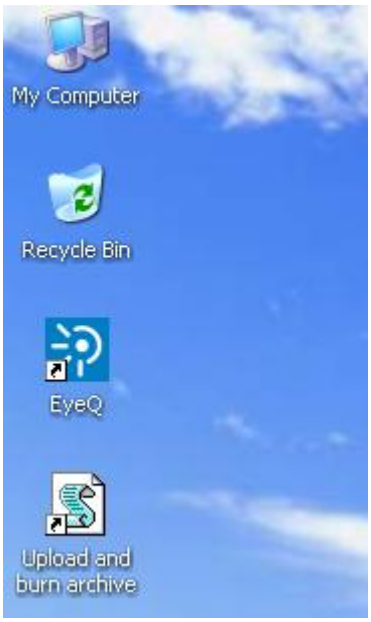
5. **EXAMINATION PROCEDURES**

5.1 Subject Positioning and ID Entry

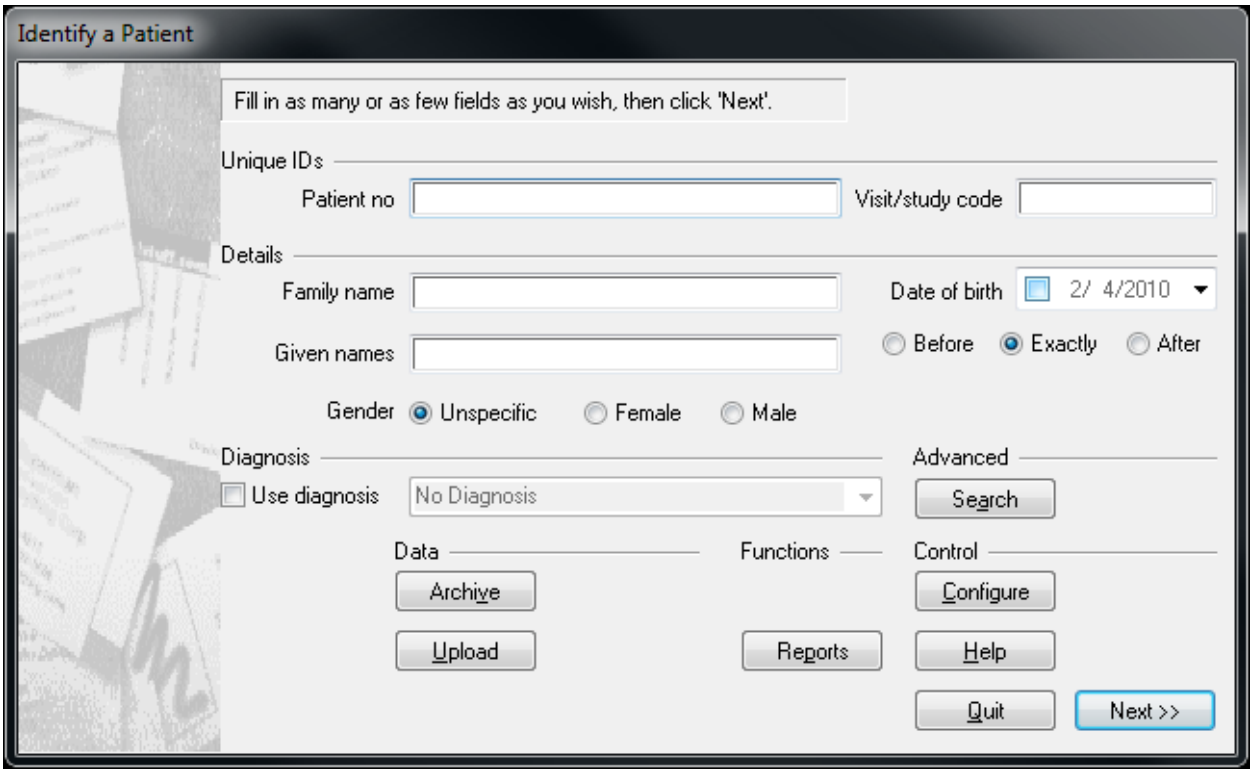
The subject and photographer are seated on the appropriate sides of the retinal camera. The subject is positioned so that he/she is comfortable with chin and forehead in the headrest. Chin height should be adjusted so that the eyes are approximately level with the height adjustment mark on the face rest pole. The room is darkened to the level where a newspaper can barely be read (equal to about 5 lux) and the camera room door is closed. The only light in the room should come from the display monitors. If a lamp is used to aid the examiner during administration of the questionnaire, it must be turned off when photography is performed.

While the subject begins to dilate, the photographer begins the image capture procedure by

double clicking the desktop EyeQ program.



An “Unarchived Visits” screen will appear and will contain images from any patients who have not yet been archived. Select the “Next” button on this screen to open the “Identify a Patient” screen.



The photographer then enters the patient’s unique 7-digit subject ID number in the Patient Number. The subject’s last name and first name are not used to preserve subject anonymity. The

first six letters of the acrostic (name code) and photographer ID# should be filled in the "Family name" and "Given names" boxes, respectively. These fields must be filled in before the photographer is able to begin capturing images. Additional information may be entered into fields as indicated in the study protocol. The "Next" button is clicked until the "Confirm Patient number" box appears, at this point, the patient number is re-entered and the "OK" button is selected. A "Visual Acuity" box will appear, click "Cancel".

The photographer is ready to begin taking photographs when the 4-panel split screen and timer box appear. The timer box displays the time the photograph was taken and also the current photo number.

When the photographer takes a picture, the timer stops and the image will briefly appear on a screen located on the digital camera back. It will then take a few seconds for the image to transfer from the camera to the computer screen. The timer will restart after the image appears on the screen. The photographer will review the image for quality and may take another image if they believe a better quality image can be obtained or they may accept the image by simply taking the next photograph when the patient is ready and the pupil is adequately dilated.

At the time the photographer has completed the patient's photographs, he/she will select the "End" button located on the timer box. The photographs that have been taken will then appear on the computer screen in a "Contact Strip" box. The photographer reviews all of the images in the contact strip and may delete any unwanted images at this time. The photographer may then elect to continue the photography session, if they wish to retake a particular field, or may select "Quit" to end the photography session. At this point, a new subject may begin being photographed by filling in the new patient information and repeating the steps stated above. If no other participants are to be seen, the photographer can "Quit" the program.

5.2 Pupil Size and External Camera Alignment

The camera stage holding knob is unlocked, the alignment switch is turned on and the stage is moved to center the eye to be photographed horizontally and the height adjustment ring is used to position the eye vertically. The pupil should appear on the TV screen coincident with the central circle on the monitor. The camera joystick is moved forwards or backwards until the pupil appears perfectly round. At this point, proper external alignment has been achieved. A pupil larger than the central 4mm circle on the monitor is required for adequate photography. If the eye

does not dilate to at least 4mm after a 5-minute waiting period, the fellow eye should be examined for pupillary dilation as well. If dilation of the fellow eye is larger, the photographer will photograph the fellow eye first. At this point, the pupil size is measured using the alignment circle on the monitor as a gauge. This measurement is estimated to the nearest 1mm and it is recorded on the MESA Retina Completion form.

5.3 **Internal Eye Alignment**

Once proper external pupil alignment is achieved, the alignment switch is pressed to provide a view of the fundus, split focusing lines, corneal reflection dots, and the fixation light. If no split lines are seen, the height or left/right adjustment is improper, the "Split" (split lines) setting is set to “off” (Set 1, Split/FCD), or the diopter compensating slider is pulled out. The split lines may fade in and out if the pupil is too small, the alignment of the camera is not centered on the pupil, or if the eyelashes or lids eclipse the light. If no corneal reflection dots are seen, the forward/backward adjustment is improper. The best photographs are obtained when the eye is well dilated, fixation is on the target; and lids and lashes are held wide open.

5.4 **Alignment, Focus and Proper Fixation of Fundus Photographs**

While viewing the fundus image on the screen, the photographer carefully adjusts the fixation target control button to locate Fields 1 and 2 correctly on the screen. Field 1 is always photographed first.

Any fine adjustment of fixation is made by moving the fixation lever and instructing the subject to look into the lens of the camera at the green target box. In the event that the subject sees no fixation light with the eye being photographed, the photographer must carefully instruct the subject to make micro movements (fine movements up, down, left or right) until the protocol fields are located.

Once the fixation is confirmed, the photographer must constantly adjust and position the camera to maintain the correct position of the corneal reflection dots. It is important that these dots be properly positioned at the three and nine o'clock positions before the picture is taken. This will ensure the correct distance from the eye and will allow capture of a sharp digital image. Focus is done manually by dialing either of the focus knobs located on the side of the fundus camera. Proper focus is obtained by aligning the two horizontal focus bars visible on the fundus camera monitor (see p. 12 of the Canon Non-Mydriatic Retinal Camera CR6-45NM Operation Manual). Good focus is critical for grading photographs.

The photographer will instruct the subject to blink once or twice just before the picture is taken. This blinking will insure a moist (and subsequently clearer) cornea and will safeguard against unwanted blinks at the moment of exposure. Once alignment is satisfactory, the shutter release, located in the tip of the joystick, is depressed and the exposure is made. The quality of the digital image is evaluated by the examiner and ungradable images, which in the opinion of the photographer may be improved, may be retaken. Once the best quality image is obtained, the second field is photographed and the photographer conducts the same image evaluation procedure.

6. PHOTOGRAPHIC CHALLENGES

6.1 Photography Through Small Pupils

The photographer will experience much more difficulty attempting photography through small (less than 4mm) pupils because all of the camera light doesn't enter through the smaller pupil. This usually results in uneven illumination (seen as dark shadows) on the monitor. In this situation, the photographer must make careful camera adjustments to position the shadows as far away from the optic nerve in the Field 1 photo and away from the macula on the photo of Field 2.

A small percentage of subject's eyes will not dilate to the minimum 4mm required for adequate photography. Certain medication may prevent any dilation and the pupil size observed on the monitor may be 2-3mm, inadequate for the photographer to appreciate all retinal landmarks on the viewing monitor. In this case it is recommended that the photographer introduce the small pupil aperture located on the left side of the fundus camera body (see p.10 of the Canon Non-Mydriatic Retinal Camera CR6-45NM Operation Manual). This function adjusts the illumination to concentrate more of the retinal illumination into a smaller area providing more even illumination to the center of the field while sacrificing illumination at the field perimeter. If after introducing the small pupil function only one focus line is visible, it might be necessary to adjust the camera position either vertically or horizontally to bring the second line into view so that accurate focus can be set. However, there may be circumstances that prevent the second line from appearing, even when the camera adjustments are made. In these cases it may be helpful to remove the small pupil function momentarily while searching for the second line and adjusting focus, reintroducing the small pupil function before taking the photograph.

If no retinal landmarks are visible, often the case with <2mm diameter pupils, the photographer should adjust the camera slightly to position the corneal reflection dots slightly above or below their optimum position. This technique allows a portion of the illumination light (which falls on the iris when the pupil is small) to enter the eye. If any retinal landmarks become visible with this technique, a picture should be taken. However, if no retinal landmarks are visible, no picture is taken. The photographer notes the problem on the MESA Retina Completion form.

6.2 Exposure Compensations for Dark or Light Retinas

The normal flash output used in this study will be flash number 2. The flash output can be increased or decreased by first pressing the "DA" button followed by the +/- buttons located under the main video display screen of the fundus camera monitor. Higher numbers increase flash output and produce brighter images while lower numbers decrease flash output and produce darker images. A one number increase or decrease in flash intensity is usually all that is needed. Photography through small pupils, media opacities or of darkly pigmented retinas (Black or Asian) will require increased flash output (to flash 3) to avoid underexposed pictures. Photography of lightly pigmented retinas (blond, albino or Scandinavian) will require decreased flash output (to flash 1) to avoid overexposed pictures. The photographer should check the color saturation of the first image taken of each participant, and adjust the flash if necessary before proceeding.

6.3 Focus with High Myopia or Hyperopia

The diopter compensation slide should be set to the "0" position for most eyes. This is the only setting in which the focus bars are active and allows photography of eyes with refractions between -12 and +15 diopters. In the event that the eye photographed falls outside this range and focus cannot be achieved, as in the case of aphakia or high myopia, the diopter compensation slider must be adjusted for the clearest focus to the "+" or "-" position and the focusing knob is then turned manually to provide the sharpest image as seen on the monitor. This can be facilitated by obtaining a brighter retinal image by increasing the view light intensity. The

normal setting for the view light intensity adjustment is approximately 4.

Standard TV monitor functions can be adjusted for the photographer's viewing comfort (including contrast and brightness) by opening the access door below the TV monitor. These are standard controls similar to those found on a home TV set and only effect viewing; they do not affect final photo quality.

7. SHIPPING MANIFEST FORM

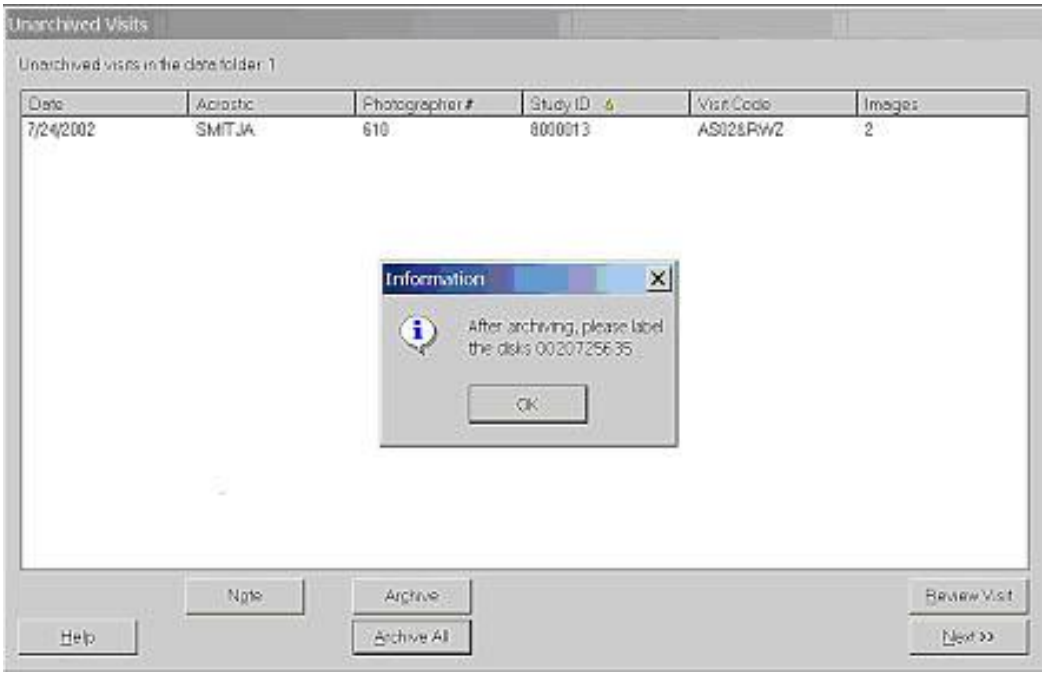
At the end of each week, all MESA Eye Study Retina Completion forms will be given to the person who will archive the images. Whoever creates the CDs for the site should compare the photography forms to the "unarchived" images before archiving to confirm that all images are present. Once the images have been archived, the contents of the CD (patients' ID, acrostic, photo date, eye and fields photographed) should be recorded on the MESA Eye Study Non-Myd Digital Photograph Shipping Manifest (Attachment 1). Shipping manifests will be numbered sequentially per site. The date the images were archived and the 10 digits CD number (assigned by the EyeQSL software during the archiving process) should be recorded on the shipping manifest, as well as the name of the person preparing the shipment and their fax number. If a second person would like to have confirmation of OERC receipt of CDs, their name and fax number should be written beneath the person preparing the shipment. Comments about the archiving process, inconsistencies between the MESA Eye Retina Completion Forms and MESA Shipping Manifest, or other deviations from protocol should be recorded in the comments box on the shipping manifest. See Attachments 4 and 5 for editing participants information and deleting visits.

8. DIGITAL FILE HANDLING

8.1 Archive Procedure

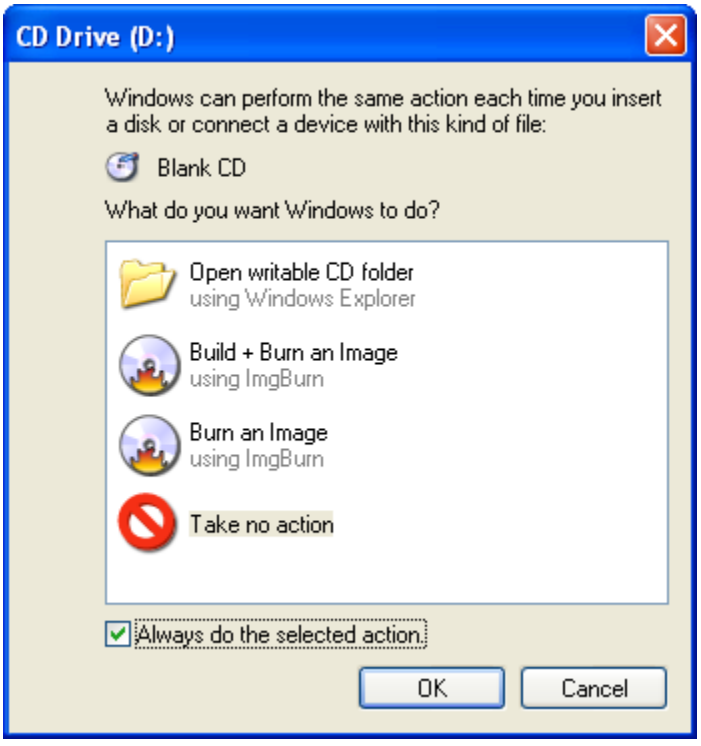
The archiving process will be completed at the end of each week after the final participant has been photographed. The photographer is required to make two archive copies, the first will be sent to the reading center and the second will be kept at the clinic. The photographer proceeds to the "Identify a Patient" screen and select the "Archive" button. All of the visits for the week that have not already been archived will appear on the "Unarchived Visits" screen.

The photographer has the option of selecting the "Archive All" button or selecting or highlighting individual visits and clicking on the "Archive" button separately. Note that only fifteen participants can be archived at a time. If more that fifteen visits are unarchived, select the first fifteen and hit "Archive". Record the information for these visits on the shipping manifest. Repeat this entire archive procedure again in groups of 15 until there are no more visits left to archive.

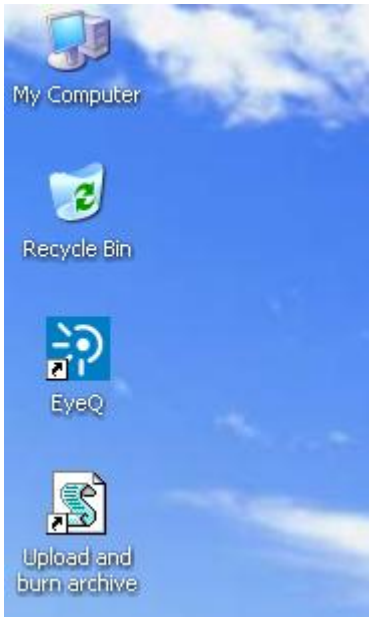


Press "OK" and the computer will begin archiving. This should only take a few seconds. (Note: The CD is labeled later in the archival process, so the photographer can ignore the prompt to label the disc for the moment). When finished, quit out of the EyeQ application.

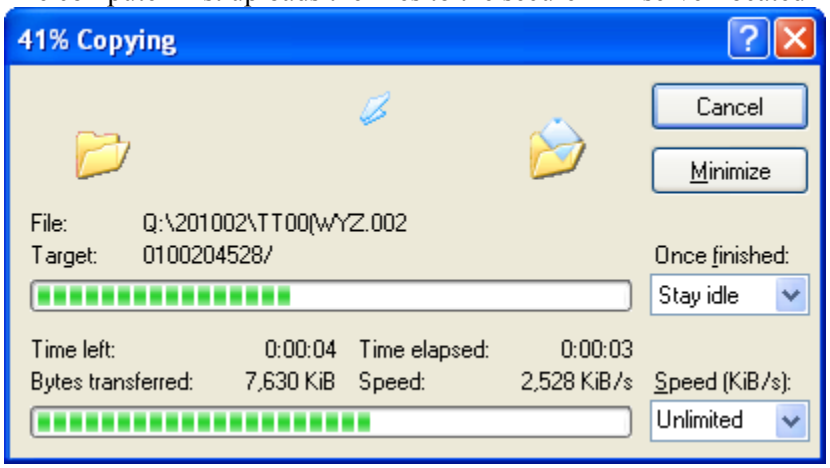
The photographer next inserts a blank CD-R disk. If the following window appears, the "Take no action" and "Always do the selected action" options should be selected to prevent the dialog from appearing again.



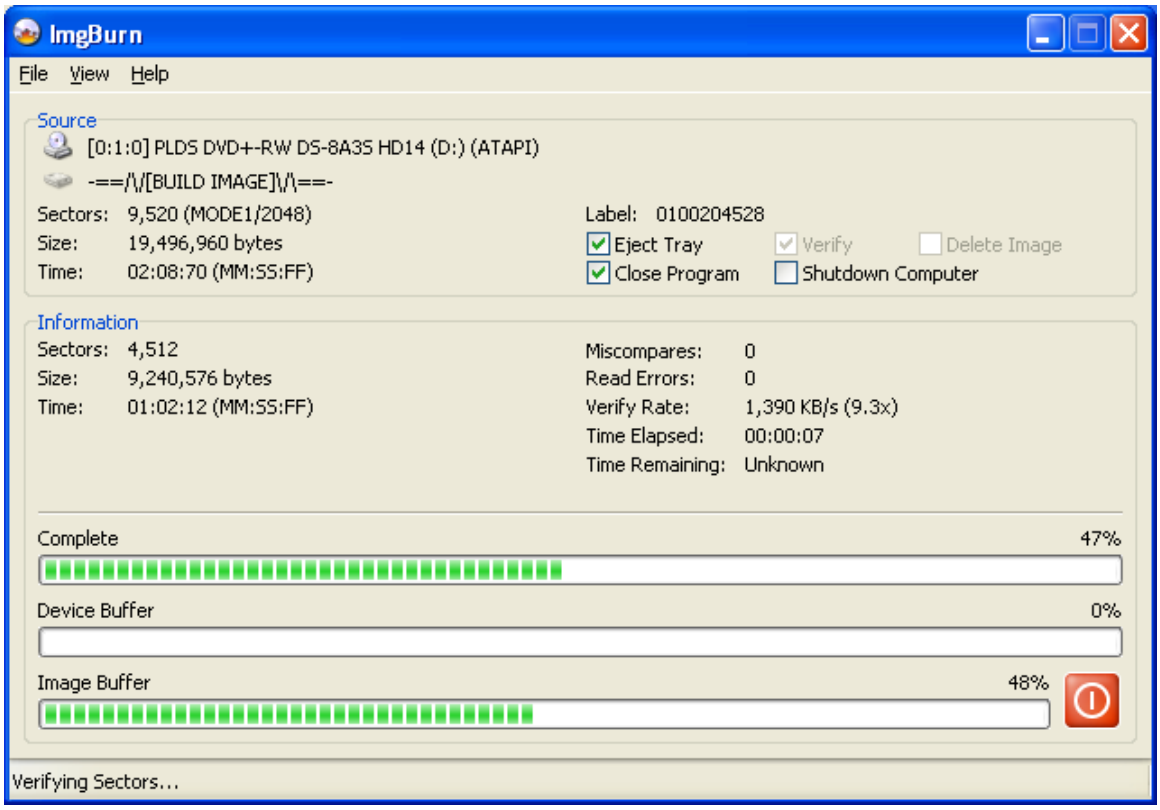
Next, the photographer double-clicks the “Upload and burn achive” icon on the computer.



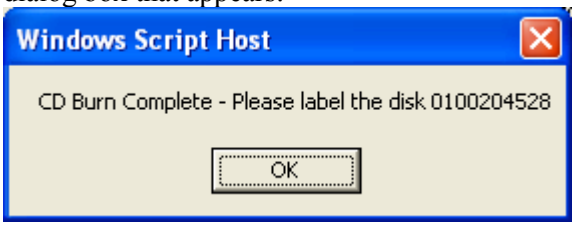
The computer first uploads the files to the secure FTP server located in Wisconsin:



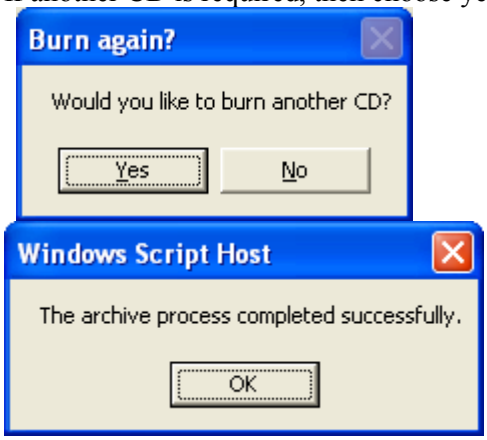
And then burns the files to the CD-R disc:



When the burn process is complete, the disc is automatically ejected from the laptop. The photographer will then label the disk according to the steps in section 8.2 with the label in the dialog box that appears:



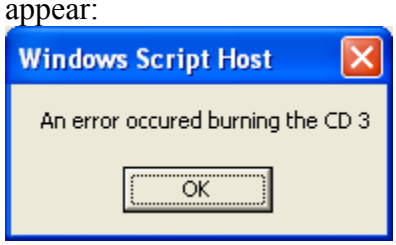
If another CD is required, then choose yes, otherwise the process is complete:



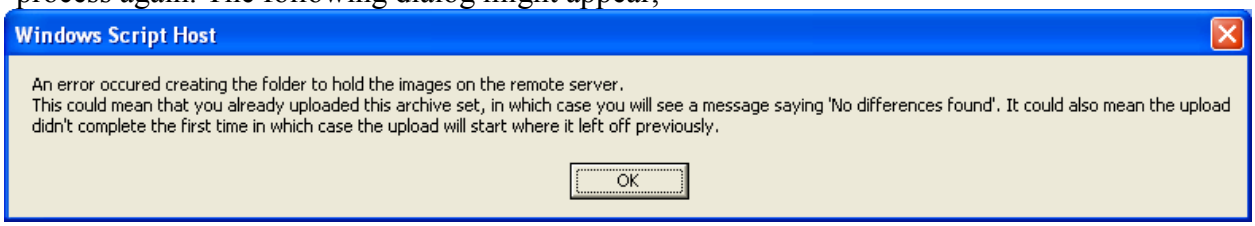
8.1.1 Problems with the archive process

In the unlikely event that the archive process encounters an error, the laptop will indicate what the problem was and how to proceed.

For example, if the CD burning process does not complete, the following dialog will appear:



In this case, the photographer should dispose of the invalid disc and try the archive process again. The following dialog might appear,



indicating that the files had already been successfully transferred to the secure FTP server, and what explaining what further prompts the photographer might see.

8.2 CD labeling

The archived CDs, and its jewel case (clinic copy) will be labeled (Figure 2) using the following format..

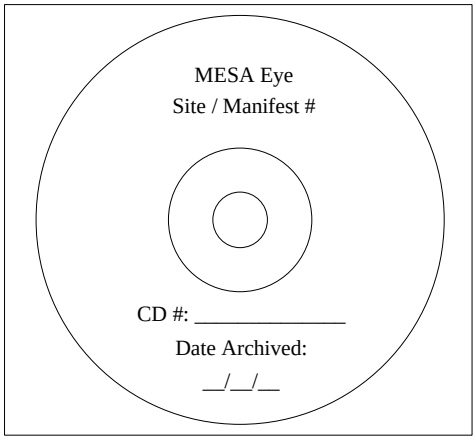


Figure 2

The person creating the CD will complete the CD label, providing the manifest sequence number, date the CD was archived as well as the **10 digit CD number** assigned by the EyeQSL software. The CD will remain at the site for backup and should be placed in a jewel case for storage.

8.3 Photo Transmission

TONY

Once the images have been archived, they must be transmitted to the OERC via the secure FTP site. The Shipping Manifest must also be faxed after the transmission is complete, as The Reading Center will use these forms to verify the receipt of shipments, noting the date the photos are received and the name of the person checking the shipment. After the Reading Center receives each shipment, a copy of the shipping list will be faxed to the site.

9. PHOTOGRAPHER CERTIFICATION

9.1 Obtaining Certification

Each examiner taking fundus photographs will need to become certified before taking photographs for the study. The initial group of photographers will receive didactic and hands-on training provided by the OERC team on February 18-19, 2010. Training will take place during the Central Training for Exam 5 located at the Wachovia Center in Winston-Salem, NC. Following the training, each photographer will return home to setup their photography system, practices taking photographs and prepares photographic sets for submission to Michael Neider for certification. Certification begins with the completion of the Photographer Certification Request Form (Attachment 6). This form will be submitted along with images of 10 eyes (5 right eyes and 5 left eyes, F1 and F2 of each) imaged following the study protocol. A photographer is fully certified after submitting satisfactory quality images of 10 eyes taken on non-study volunteers and the form is signed and sent to the Coordinating Center by the OERC. These photographs must show proper field definition (Fields 1 and 2 of each eye, 20 images total), proper exposure, alignment and focus. Photography certification subjects should be assigned study ID numbers using the digit technician ID number followed by a sequential 3 digit number, starting with 001. The acrostic for all certification subjects will be "CERTIFY", and the photographer will be the technician's ID number. For example, if a technician's ID number was "109", the patient information for their first certification subject would be as follows:

Study ID: 109001 (109002, 109003, etc.)

Acrostic: CERTIFY

Photographer: 109

The photographs must be exported to the secure FTP site. Once certification is complete, the photographer's information and ID number will be entered into the database. The OERC will notify the photographers at each site or will provide helpful feedback requesting the submission of additional images to resolve a problem and complete certification.

9.2 Certification of New Photographers

As additional personnel need training to become certified, a certified photographer at that center will provide complete instruction and copies of the Ocular Epidemiology Photography Protocol and Canon Non-Mydriatic Retinal Camera CR6-45NM Operation Manual. The trainee photographer will practice on volunteers and, when ready, prepare and submit photographs of 10 eyes for consideration for certification.

9.3 Certification Maintenance Requirements

In order to maintain certification, photographers must have complete photograph sets, a minimum of six eyes per month and 75% of their photographs must be gradable. This will be determined from the monthly photo quality report generated by the study coordinator. If a photographer fails to meet either of these requirements, he or she will be contacted and given feedback by a photography consultant. During the following month, the photographer will be allowed to continue to take photographs of study participants only if supervised by a fully compliant certified photographer. If the photographer does not meet these standards of quantity or quality of their photographs for three consecutive months, certification will be revoked. The technician will not be able to take study photographs until he/she has submitted a new set of acceptable certification photographs.

10. QUALITY CONTROL

10.1 Photograph Quality

Photographers will provide the first assessment of photo quality, a big advantage of digital imaging. This "on the spot" review of images allows for the immediate assessment of image quality and the opportunity to retake the images before the patient leaves the camera photography area. Additionally, reading center staff will continuously monitor photographic quality throughout the study. Initially all photographs will be reviewed by reading center staff and feedback will be provided to the photographers in cases that warrant critique. A telephone call, e-mail or letter will be used detailing problems and suggesting improvements. Once the study is underway and the photographers sufficiently trained, data on quality will be generated from the photograph readers' evaluations of all photographs. The Photography Consultant will review a small percentage of the photographs, and feedback will be provided to the photographers in cases that warrant critique. In cases where problems with photo quality persist, the additional training may be arranged at the Ophthalmic Photography Learning Center (OPLC) located in Madison, Wisconsin.

10.2 Photographer Quality Control

Each site will perform repeat photography on one eye of one study participant each week. The selection of which study participant and which photography technician will perform the repeat photography will be determined by the coordinating center so as to optimize inter-tech and intra-tech quality control. The repeat photos will be of field one (optic nerve) of one eye only and will be numbered in such a way that the reading center knows that it is a repeat photograph. All repeat photographs will undergo an A/V grading by the same grader who initially graded the original photograph at the reading center.

11. COMMUNICATION CHANNELS

It is vital that proper and frequently used channels of communication be established for the effective exchange of questions and information between all staff members. Following is a listing of names, addresses, and telephone numbers to facilitate this exchange:

Ocular Epidemiology Reading Center
610 North Walnut Street, 405 WARF
Madison, WI 53726-2336

Lisa Grady Study Coordinator	(608) 263-0285 grady@epi.ophth.wisc.edu
Stacy Meuer Senior Grader	(608) 263-8835 meuers@epi.ophth.wisc.edu
Michael Neider Photography Consultant	(608) 263-9858 neider@rc.ophth.wisc.edu
Anne Goulding Imaging Consultant	(608) 263-9858 goulding@rc.ophth.wisc.edu
Ronald Klein, MD, MPH Principal Investigator	(608) 263-7758 kleinr@epi.ophth.wisc.edu
Barbara EK Klein, MD, MPH Co-Investigator	(608) 263-0276 kleinb@epi.ophth.wisc.edu
Larry Hubbard, MA Co-Investigator	(608) 263-2245 hubbard@rc.ophth.wisc.edu

CANON USA, Inc.

Mark Scheckel Canon USA, Inc. 15955 Alton Parkway Irvine CA 92618-3616	(949) 753-4193 mscheckel@cusa.canon.com
Thomas Penkala Canon USA, Inc. 100 Park Boulevard Itasca, IL 60143	(630) 250-6230 tpenkala@cusa.canon.com

Attachment 1:	MESA-Eye Study Non-Myd Digital Photograph Shipping Manifest (Shipping Manifest)
Attachment 2:	MESA-Eye Vision History Form
Attachment 3:	MESA Retina Completion Form
Attachment 4:	How to Edit Participant Information
Attachment 5:	How to Delete Patient Visits
Attachment 6:	MESA-Eye Study Photographer Certification Request Form

MESA Eye Study
Non-Myd Digital Photograph Shipping Manifest

This form is to be completed whenever images are transmitted to the OERC secure FTP site.
Each Shipping Manifest is to have a unique number and is to be numbered in numerical sequence (e.g., 001, 002, etc.)
Once images have been successfully transmitted fax form to:

Lisa Grady
Ocular Epidemiology Reading Center
(608) 265-0285 **grady@epi.ophth.wisc.edu** **FAX (608) 265-5799**

Person Preparing Shipment:	Site No.:	Manifest No.:

Each manifest is to have a unique number and is to be numbered in numerical sequence (e.g., 001, 002, etc.).
Shipping Manifest must match the transmitted photos.

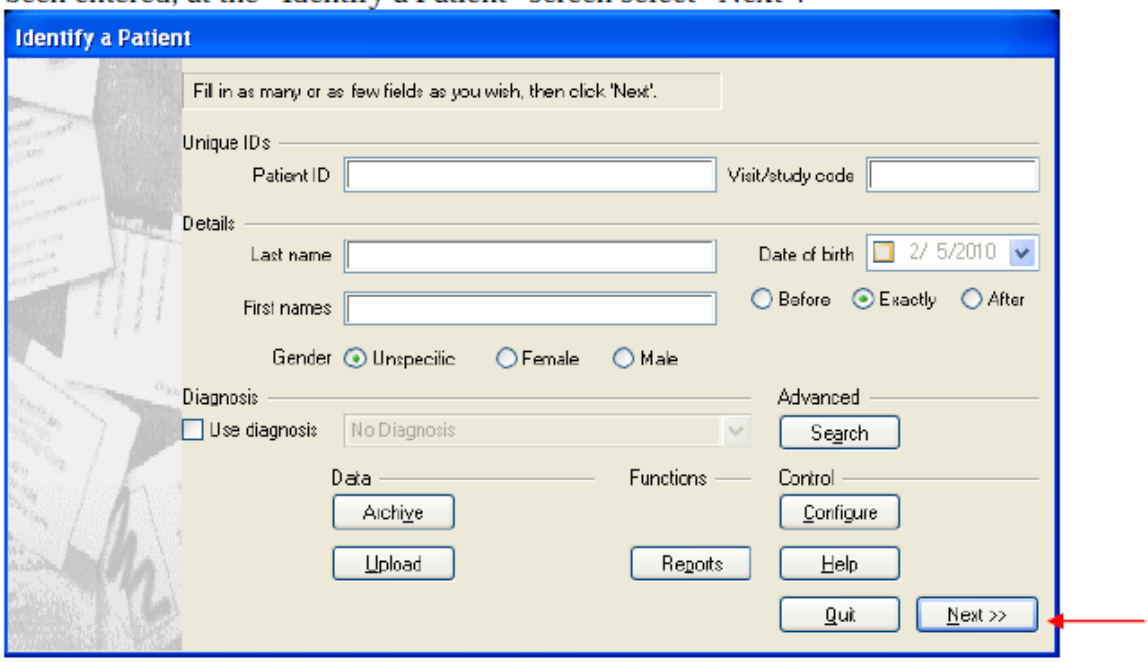
Patient ID#	Acrostic (Name Code)	Photo Date	Eye (Circle)	Fields Present
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2
			OD OS	1 2 1 2

Received By:	Date Archived:	Date Transmitted:

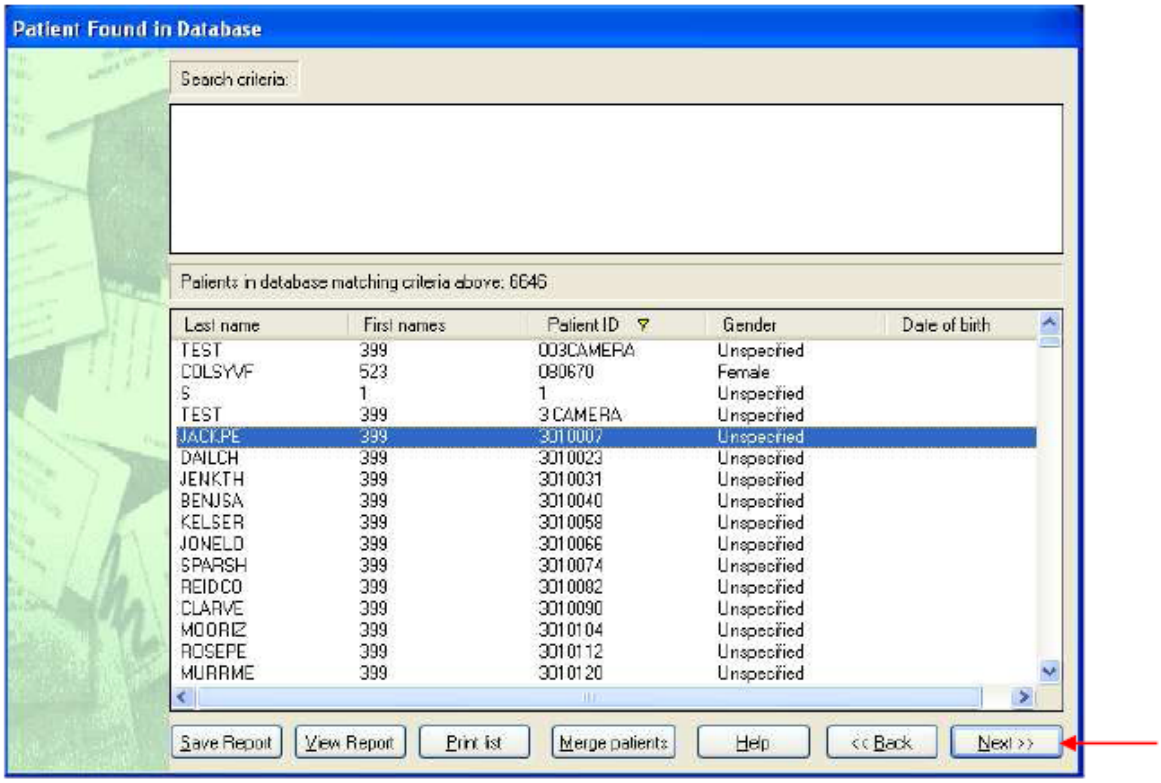
Date Received:	CD Number:
Comments:	FAX No: ATTN: Notification of receipt of photos will be faxed to you.

Procedure to edit participant information

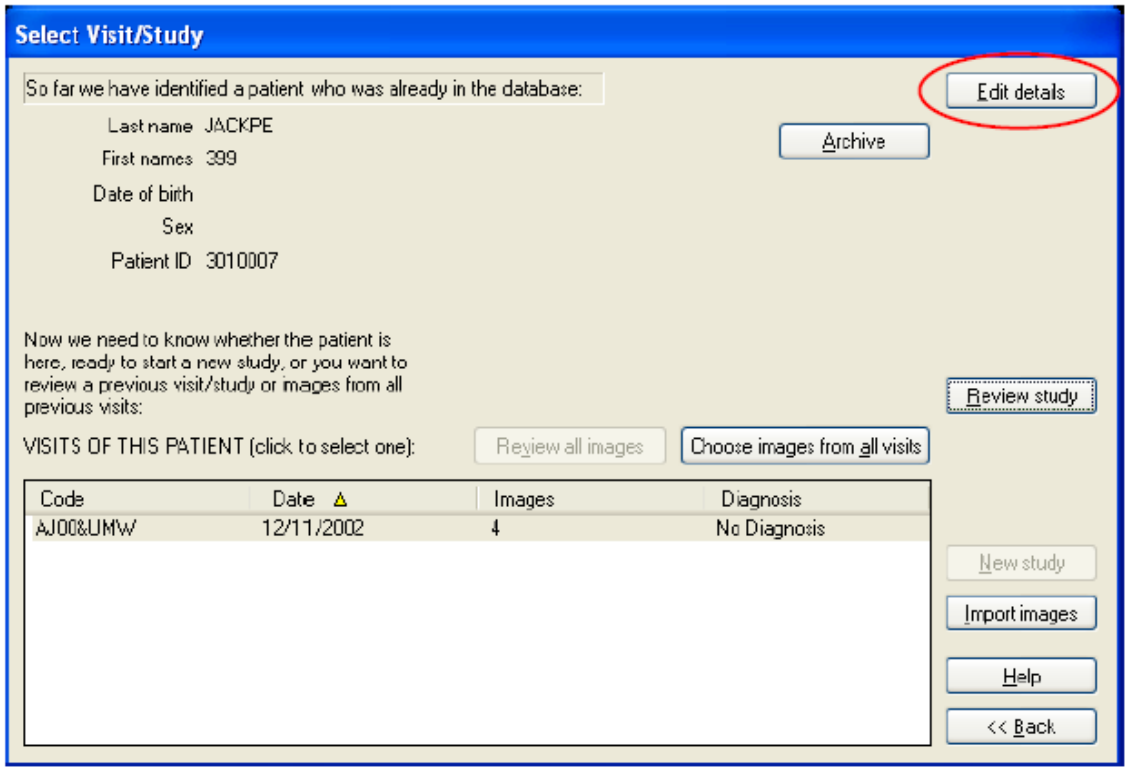
If, when reviewing unarchived visits, it is discovered that incorrect participant information has been entered, at the "Identify a Patient" screen select "Next".



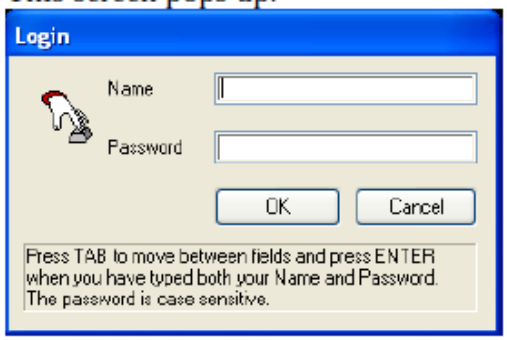
The "Patient Found in Database" screen appears. Highlight the patient you would like to edit and select "Next".



The Select Visit/Study screen appears. Select “Edit Details”.

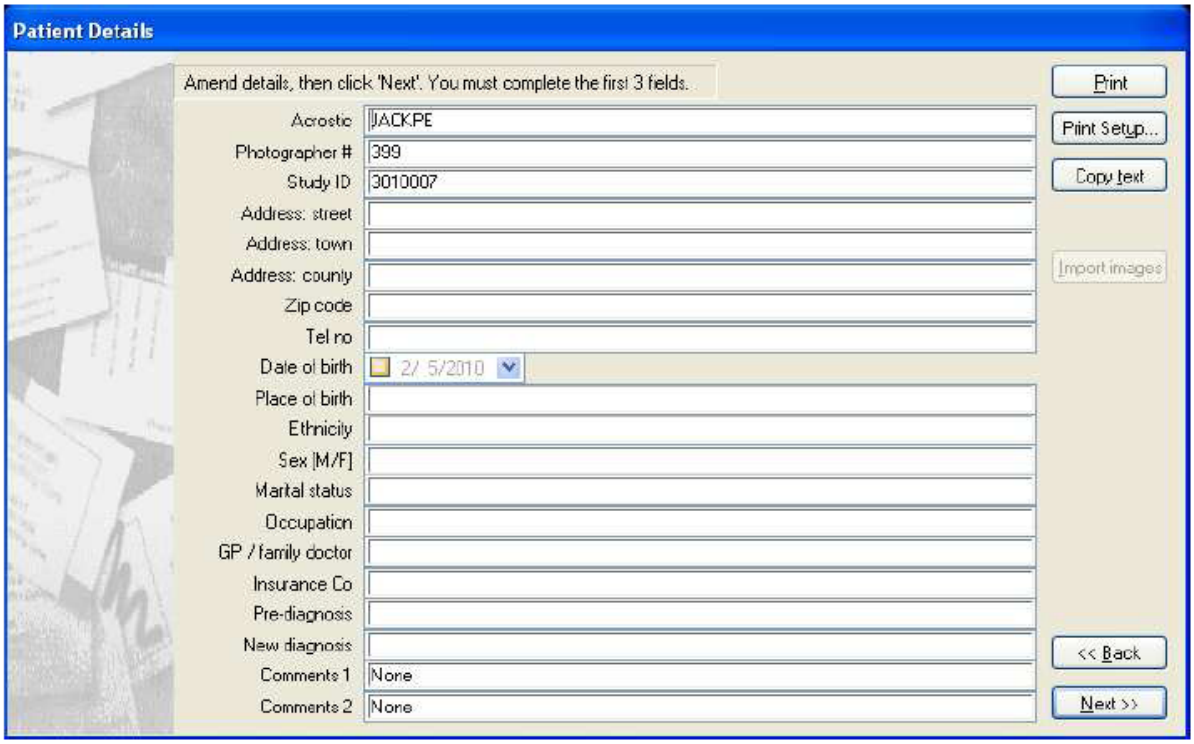


This screen pops up.



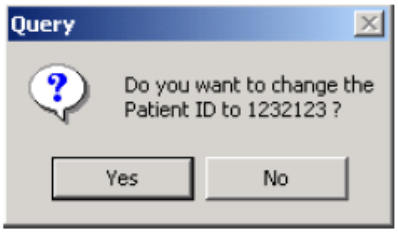
Enter Login info (caps) and select ‘ok’:
Name: X
Password: X.X

This will bring up the “Patient Details” screen.



The Acrostic, Photographer #, and Study ID information may be edited from this screen. After editing, Select “Next”. This will bring you back to the Select Visit/Study screen. Select “Back” to return to the Patient Found in Database screen. The corrected information should appear in the listed visits. Hit “Back” again to return to the Identify a Patient screen.

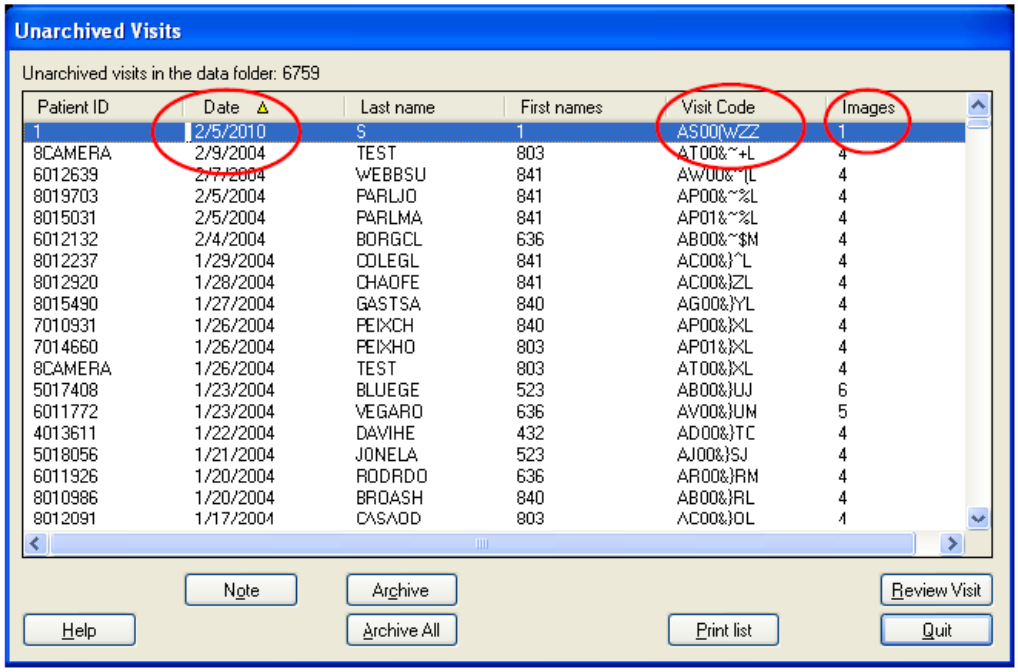
When correcting a participant’s ID number, follow the same steps as above, editing the Study number in the Patient Details screen. After correcting the number, select “Next”.
A message box will pop up, asking if you want to change the Patient ID. Confirm that you are changing the ID number to the correct number, and hit “Yes”.



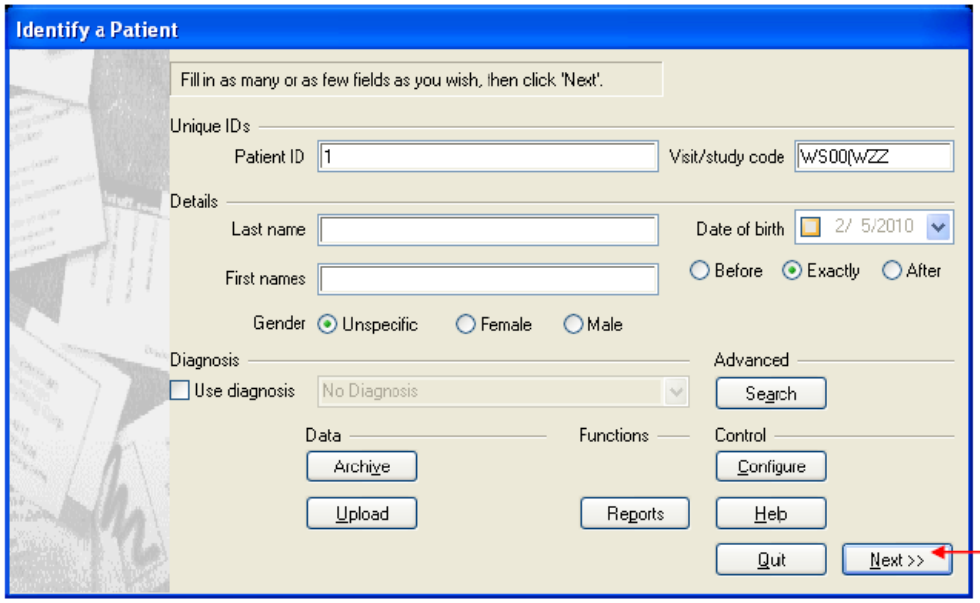
Select “Back” to return to the Patient Found in Database screen and confirm that the ID number has been corrected.

Procedure to delete a participant visit

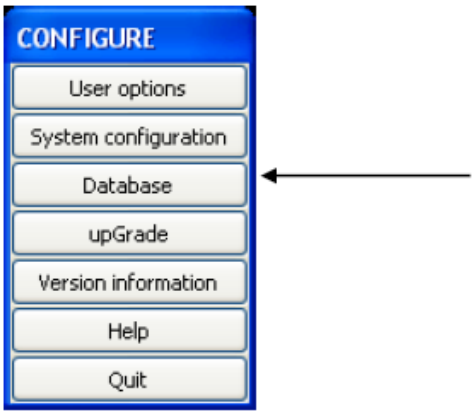
At to the "Unarchived Visits" screen. Note the (photo) Date, (number of) images and Visit Code of the participant whose images whose visit you would like to delete. For this exam, the Visit Code is an eight-character identifier assigned by the EyeQ software.



At the "Identify a Patient" screen, enter the participant's Patient ID number and the Visit Code and select "Configure".

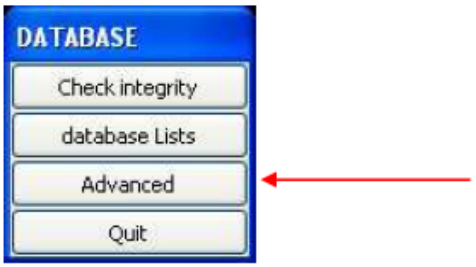


Choose "Database" from the list of options displayed.

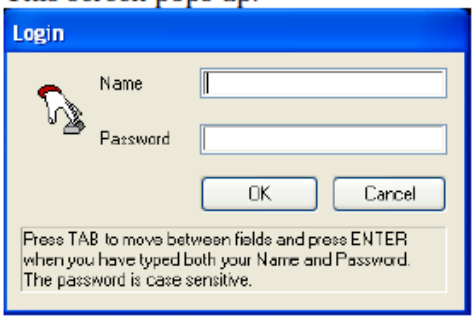


A warning box will appear. Select "OK".

Another menu will appear; select "Advanced".

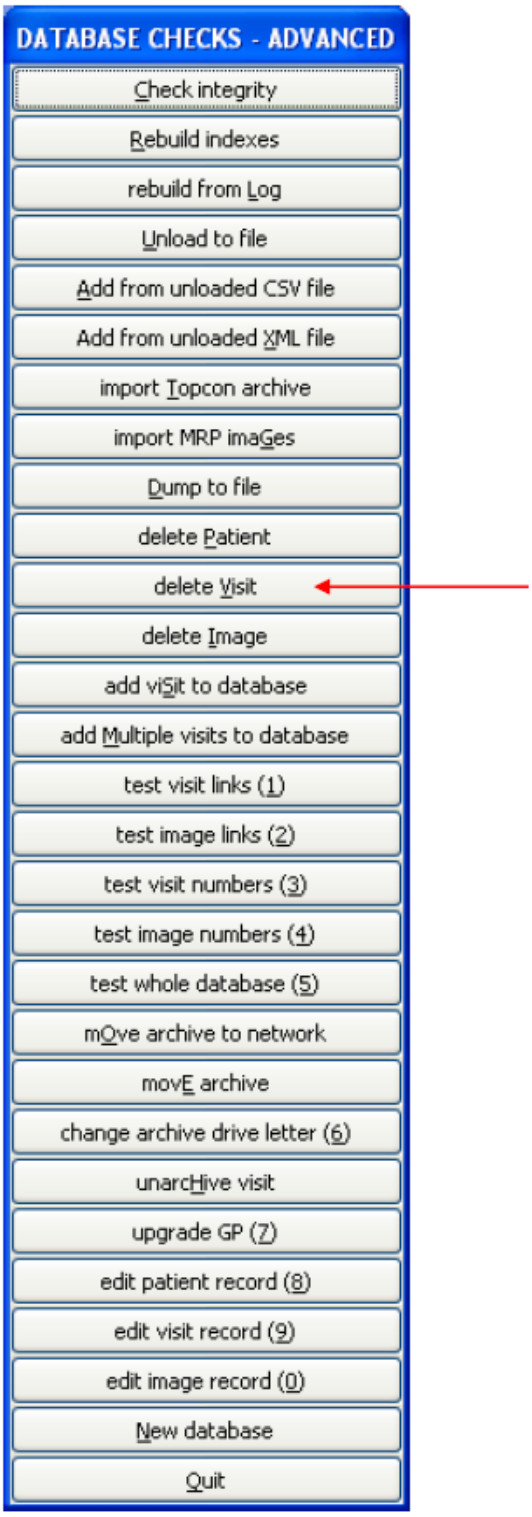


This screen pops up.

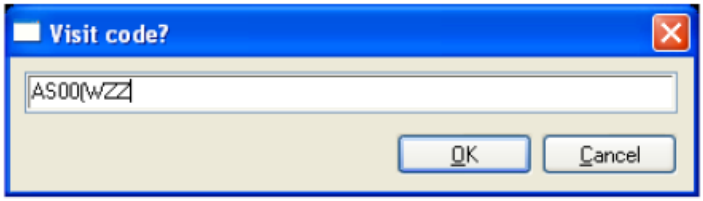


Enter Login info (caps) and select 'ok':
Name: X
Password: X.X

This screen pops up. Select ‘delete Visit’.



This screen pops up. Add the appropriate Visit code and select 'ok'.



A query (warning) box will appear asking you to confirm that you have entered the correct visit. The Study ID, acrostic, Visit Code, number of images and photo date of the visit to be deleted will be displayed in the box. Check all information to be certain that you are deleting the intended images; once you have selected "Yes", the visit will be deleted.

Hit "Quit" three times to return to the "Unarchived Visits" screen. This procedure does not delete the participant's information from the database, only the visit and the associated photographs. The participant will no longer appear in the "Unarchived Visits" screen, unless or until another photography session is completed. However, the participant's information can still be accessed through the "Identify a Patient" or the "Patient Details" screen.

Photographer Certification Request Form
MESA-Eye Study

Institution Name:	Site Number: PI: Coordinator:
Photographer's Name/Address:	Phone: Fax: E-Mail:

Quality Assurance Statement

I have read the protocols listed below, and I understand and agree to abide by the design and procedures of the trial.

Study Protocol: MESA Retinopathy Imaging Protocol

***Color Ophthalmic Photography Protocol**: MESA Non-stereo Digital Fundus Photography Protocol – Standard Field 1, Modified Field 2.*

____ ________________________________

Signature ***Date***

☐ I request certification based on prior certification for Study ______________________________

Name of Study

The following photographs are being submitted for consideration of my certification as a fundus photographer for:

The SEARCH Study

Subject ID Number								Photo Date	Eye	Photograph (circle fields present)	Fields	
								__ __ __/__ __/ __ __ __ __ Month Day Year (e.g., Nov.)	OD OS	Digital Image: Digital Image:	1 1	2 2
								__ __ __/__ __/ __ __ __ __ Month Day Year (e.g., Nov.)	OD OS	Digital Image: Digital Image:	1 1	2 2
								__ __ __/__ __/ __ __ __ __ Month Day Year (e.g., Nov.)	OD OS	Digital Image: Digital Image:	1 1	2 2
								__ __ __/__ __/ __ __ __ __ Month Day Year (e.g., Nov.)	OD OS	Digital Image: Digital Image:	1 1	2 2
								__ __ __/__ __/ __ __ __ __ Month Day Year (e.g., Nov.)	OD OS	Digital Image: Digital Image:	1 1	2 2

Fax completed form to: 608-265-5799

Export the images electronically to the secure FTP site using the upload shortcut on the camera laptop.

Questions may be directed to or Anne Goulding, phone 608-410-0628
e-mail: goulding@rc.ophth.wisc.edu

OR Michael Neider, phone 608-410-0628
email: neider@rc.ophth.wisc.edu

For Reading Center use only	Certification Approved: __ __ __/ __ __/ __ __ __ __ Month Day Year (e.g., Nov.)
Reviewer's Signature____	

3.5.7 MESA – Vision, Eye Refraction

The vision refraction component in MESA involves measuring the eyeglass prescription in people who wear glasses, testing the distance visual acuity of study participants with and without correction (glasses/contacts) and obtaining an objective refraction (incorporating also an evaluation of the corneal curvature of the eye). It is estimated to take 5-7 minutes to administer.

I. PURPOSE

The purpose of the vision component is to measure how well people see things at a distance using their glasses and/or contacts, if they wear any (usual correction = nothing, glasses and/or contacts), to measure the refractive error and corneal curvature of each of their eyes, and to determine if distance vision can be improved in the eyes of people who don't see well with their usual correction. These data will be used to look at the distribution of visual acuity and extent of visual impairment in the MESA study population and to examine associations between visual impairment, retinal changes and markers of cardiovascular disease.

II. MATERIALS AND EQUIPMENT

1. A comprehensive list of the material and equipment needed is listed in section 1.2.2. The main components are listed here.

   - Nidek Auto Lensmeter (model LM-990A)
   - Nidek Autorefractor/Keratometer (model ARK-760A)
   - Computer – linked to the Refractor

   QC items (to be circulated among the various clinics) include:
   - Test eyes/steel balls (ARK9-00-ARKJ-4)

2. Description of Equipment:

   Lensmeter
   The Lensmeter allows you to measure the prescription of single vision lenses, bifocal (trifocal) lenses, and progressive power lenses (PPL). The parameter settings to be used in MESA can be found in Appendix A.

   Autorefractor/Keratometer
   This instrument contains both a refractometer and a keratometer in one unit. The refractometer uses infrared rays to objectively measure the refractive power of the study participant's eyes and built-in charts and a cross cylinder lens for subjective measurements. It obtains the spherical, cylindrical powers, and cylindrical axis of the lens, which may correct the study participant's refractive error. The keratometer measures the corneal curvature, axis of corneal meridia, and corneal cylindrical power of the study participant's eyes. The parameter settings to be used in MESA can be found in Appendix B.

   The study participant's visual acuity is also checked with this instrument. This is initially performed aided by the study participant's usual correction, if any, whether it is glasses or contact lenses. For some study participants, you will measure the visual acuity with the

objective refraction measurements to determine whether the study participant's usual acuity can be improved.

3. Maintenance of Equipment:

There is no routine maintenance for the equipment; however, it is very important to use the dust covers when the equipment is not being used.

3.1 Daily Procedures

Lensmeter

- Inspect the Lensmeter with the penlight.
- If any debris is detected, use a puff of air from the blower brush to clean the lens.
- If necessary, use an Absorbond lens wiper to clean the lens and the screen.
- Place the dust cover over the machine when the equipment is not in use.

NOTE: DO NOT use anything but the blower brush on the Lensmeter.
DO NOT use alcohol, windex, lens cleaner, etc. or paper towels on the lens.

Autorefractor

- Inspect the Refractor's Measuring Window daily with the penlight.
- If any debris is detected, clean the window from the center outward using Absorbond lens wipers wrapped around a cotton-tipped applicator.
- If necessary, moisten the wrapped tip with an alcohol wipe.
- Dry the window using a cotton-tipped applicator wrapped with a lens wiper.
- Check again with the penlight; if streaks exist, repeat the steps above.
- Place the dust cover over the machine when the equipment is not in use.

NOTE: DO NOT clean the window with only a cotton tipped applicator – wrap it in a lens wiper. Otherwise the cotton fibers will stick to the window.

3.2 Routine Cleaning of Equipment

Lensmeter

- Lift up the nosepiece and remove it.
- Clean the protective glass by blowing off dust with the blower brush.
- If the lens is still dirty, wipe gently with Absorbond Lens wipes. DO NOT use alcohol, windex, lens cleaner, etc. or paper towels on the lens.
- If the exterior of the instrument becomes dirty, wipe with an alcohol wipe.

Autorefractor

- Wipe the forehead rest and other external parts with an alcohol wipe.
- Clean the measuring window and TV monitor with Absorbond lens wipes.
- Ensure that the chin rest has a fresh layer of tissue between study participants.

3.3 Quality Control & Quality Assurance

3.3.1 Weekly Calibration Procedures

Lensmeter

At the beginning of every week, four pairs of calibration glasses of known prescription (8 total prescriptions) should be tested to assess if the Lensmeter is working properly. See III. Methods, Section 4.1 Lensmeter Reading: Measuring Prescription of Eye Glasses. Print the Lensmeter readings to compare to the known values.

When activated, the MESA Web-based QC system will prompt you to compare data from the Lensmeter with those on the QC screens. Data to be compared includes the following:

- Lens sphere results (from 8 lenses in 4 pairs of glasses);
- Lens cylinder results (from 8 lenses in 4 pairs of glasses); and
- Lens axis results (from 8 lenses in 4 pairs of glasses).

The known values are prerecorded for the glasses. The technician must put a check in the done box to complete the QC program, but no values need to be entered.

Write the data completed on the printout and keep it in the QC Folder. The table below lists of the standardized eye glasses prescriptions and the tolerance ranges allowed.

STANDARD GLASSES Rx

	Right lens			Left lens		
	Sphere	Cyl.	Axis	Sphere	Cyl.	Axis
Pair #1	+1.50	+1.00	90	-2.50	+1.00	90
Pair #2	+1.50	+3.50	45	-5.00	+3.50	45
Pair #3	+3.00	+1.00	180	-4.00	+1.00	180
Pair #4	+3.00	+3.50	125	-6.50	+3.50	125

Tolerance range for Sphere and Cylinder is + or - .25 D

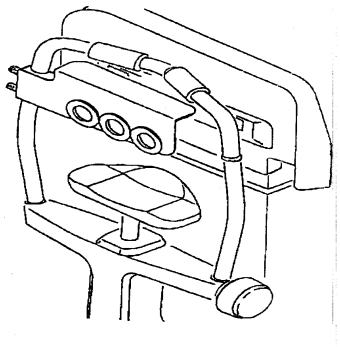
Tolerance range for Axis is + or - 3 degrees

3.3.2 Quarterly Calibration Procedures

Autorefractor

You will receive artificial test eyes prior to the start of Exam 5. Each quarter you will need to use the test eyes to determine if the autorefractor is functioning properly.

Measurement with Test Eyes



- Install the test eyes and steel balls to the head rest. To align, they must be set high—near the top of the chin rest.
- Turn the power ON and set to <OBJ.MEAS> mode by pressing the R/K button.
- Set the parameter 1: STEP to 0.01 from the default setting of 0.25. To do this, open up the trap door, press the solar system button and then use the down arrow to set to 0.01.
- Press the solar system button again.
- Aim accurately and measure the test eyes. Keep the Autofocus on to focus correctly. Then take off the Autofocus to fire manually by pressing the joystick button. Measure the right and left eye first and print these readings.
- Then measure the center eye and print the readings.
- Enter the QC data on test eyes (right, left, and center):
  - Known values for the test eyes (values are listed on the test eyes);
  - Sphere result; and
  - Cylinder result.

The results that should be entered are in the carets (< >). These values should compare with the SE values on the VD=13.75 line on the test eyes.

- Place the date completed on the printout and keep it in the QC Folder.

NOTE: A slightly incorrect alignment will affect the measured value especially when the test eyes of +10D and -10D are used. Errors of the order of -0.1D to +0.1D will be caused in measurement like that. Be sure to make the alignment as accurate as possible.

- Return to the default setting of 0.25 by setting the parameter 1: STEP to 0.25.

Tolerance of Autorefracter

Compare the SE value on the printout with these values. →

	TOLERANCE	
	SPHERE	CYLINDER
+10D	± 0.25D	± 0.25D
0D	± 0.25D	± 0.25D
-10D	± 0.25D	± 0.25D

Also verify that the VD (vertex distance) value on the printout is 13.75 mm.

NOTE: The values table is stuck on each of the test eyes. Check the tolerance in measurement comparing the measured value to the reference data in the table. Use the values corresponding to the line where VD = 13.75 mm. The cylinder value should be close to zero. [See sample in boxbelow]

SAMPLE→

VD = 13.75mm
-10.27D Left
-0.16D Center
+10.08D Right

Measurement with Steel Balls

- Install the test eyes and steel balls to the chin rest.
- Turn the power ON and set to <K> mode.
- Aim accurately and measure the steel balls.

NOTE: A slightly incorrect alignment will affect the measured value. Errors of the order of -0.02 mm to +0.02 mm will be caused in measurement like that. Be sure to make the correct alignment as accurate as possible.

Tolerance of KM Measured data

Compare the printout with these values. →

	TOLERANCE	
	AVE	R1-R2
R5.95	± 0.02 mm	± 0.04 mm
R7.94	± 0.02 mm	± 0.04 mm
R9.13	± 0.02 mm	± 0.04 mm

Enter the results in the Steel balls QC form:

- Known values for the steel balls (known values are listed above the steel balls)
- Result 1 for the steel balls (R1 on the tape);
- Result 2 for the steel balls (R2 on the tape); and
- Average result for the steel balls (AVE on the tape).
- The tape from the refractor will have 4 sets of values for the steel balls listed. Only enter the set of values (R1, R2, AVE) that are between the carets (< >).

After you have checked to determine if the Autorefractor is within tolerance, **make sure to return to the <K/M> mode by pressing the R/K button.**

If any piece of equipment is out of tolerance, first reposition the glasses (lensmeter) or test eyes/steel balls (autorefractor) and repeat the measurement. If the equipment is still out of tolerance, enlist the assistance of another Vision-certified technician at your field center to

completely retry the QC procedure from the beginning. If the equipment continues to be out of tolerance, contact a MARCO Technology representative and the Coordinating Center to report the problem.

3.4 Malfunctions and Troubleshooting

Malfunctions will need to be investigated by field center staff. If trouble-shooting tests cannot diagnose the malfunction, the coordinating center should be contacted so that MARCO Technologies can be notified.

The following are solutions to common problems that may occur with the equipment:

Lensmeter

Problem	**Solution**
The display disappears suddenly.	The auto-OFF function has been executed. Press any button to recall the ON state.
The display is unclear.	Adjust the contrast knob.
"Data Err" appears.	Clean the protective glass and the measuring lens.

Autorefractor

Problem	**Solution**
The TV monitor display disappears suddenly.	The auto-OFF function may have been executed. To recover the ON condition, press any button.
The main body cannot be moved laterally.	The locking knob may be fixing the main body. Loosen the locking knob, which is beside the joystick
The instrument does not print.	Check the printer paper. If the paper is out, set in a new printer roll. Also the printer roll may be set with the wrong side up. Set up with the correct side up.

3.5 Certification of Technicians

3.5.1 Obtaining initial certification

To obtain certification for conducting the MESA-Vision component, technicians must attend a central training session (or some other approved equivalent) wherein there is didactic training on eye anatomy, basic optics, and errors of refraction as well as hands-on training on use of the equipment to conduct the measurements according to the MESA-Vision Protocol. The technician should practice as much as possible and must complete the entire protocol on at least 5 volunteers. Certification is given after completing a written examination and a practical exam during which the technician demonstrates the ability to complete the MESA-Vision protocol without assistance.

Certification can be obtained locally. The technicians must receive similar didactic training as above as well as hands on training i.e. practice as much as possible and must complete the entire protocol on at least 5 non-MESA volunteers. Passing the written and practical exam are required.

3.5.2 Maintaining certification

To maintain certification, technicians need to:

- perform the vision component at least once a week and six times per month
- accurately read the set of 4 pairs of standard glasses once a month

It is recommended that certified technicians at each field center take turns completing the required weekly lensmeter calibration (section 3.1) to retain their certification status.

3.6 QC folder

Each field center must maintain a dated, hard copy printout of each QC activity organized by date in a folder marked QC. This folder is to document that QC activities were performed. It will be reviewed for accuracy and completeness by the field center's study coordinator or other designated field center staff member and must also be available for MESA site visitors. Note that the printout for the lensmeter QC should have <Progessive> printed on the top of the pape if the procedure was performed correctly.

3.7 QC repeats

none

3.8 Monitoring

3.8.1 Functionality of Equipment
Reports of equipment failure during a MESA-Vision Assessment or on the basis of routinely scheduled equipment calibration activities will be closely monitored by coordinating center staff. Corrective action will be taken as necessary to repair equipment and/or retrain the technician(s) as appropriate.

3.8.2 Site Visits
Site visits will be conducted as needed to inspect the organization and maintenance of vision equipment, observe technicians performing the Vision assessment, and/or review the printouts in the QC and Unsuccessful data transfer folders.

3.8.3 Accruing data
Data will be monitored regularly as they accrue with particular attention paid to outliers, data inconsistencies within a given assessment, and to QC repeat measurements.

III. METHOD

General Instructions:

Eligibility Criteria - All study participants providing informed consent are eligible for the vision assessment.

Specific Instructions:

1. **Step 1: Pre-test Procedures – Section 1 of Vision Completion Form**

- Check the form for Participant ID, Acrostic and the date.
- On the vision computer, select the vision icon from the introductory window on the automated computer system. Input the study participant's MESA identification number

and other requested information.
- Begin with section 1 of the Vision Completion Form.

2. **Step 2: Section 1 - Exclusions based on Technician observation:**

There are two categories of medical exclusions:
- those who are completely blind in both eyes and
- those with a severe eye infection in either eye.

The Technician will make these determinations by observation and enter the answers onto Section 1 of the MESA Vision Completion Form:

Is the study participant blind in both eyes?
Signs that the study participant is blind include use of a seeing-eye dog, complete reliance on a tapping cane or on another person to guide them.

If the answer is "**yes"**, then enter this information into the completion form and **STOP** the vision component. The participant will be excluded from the vision component. Inform the participant that the exam cannot be completed and thank the participant.

If only one eye is blind, the study participant will not be excluded. The not-blind eye will be tested.

Does study participant appear to have a severe eye infection in one or both eyes?
Use the following signs & symptoms as guidelines: redness, swelling, discharge, and/or pain. See also Appendix C.

If the answer is **"yes",** record which eye(s) is/are affected. If either eye has sign of an infection, **STOP** the vision component. The study participant will be excluded from the vision component at this time. An appointment can be made for the participant to return for the examination if the participant agrees.

NOTE: The presence of subjunctival hemorrhage (area of blood covering the white of an eye, sometimes taking up the whole side to the left or right of the iris) is NOT a reason to exclude a participant (see Appendix C).

Is the study participant wearing an eye patch?
If the answer **"Yes",** record which eye(s) is/are affected. You will not collect any information on the patched eye. However, the study participant is not excluded from the vision component if the other eye is not excluded by the above criteria.

Continue with Section 2 of the Vision Completion Form- if the participant has not been excluded.

3. **Step 3: Section 2 of the Vision Completion Form**

Explain the vision component to the study participant in your own words, but be sure to include the following facts:

- The eye component is to find out how clearly the person sees things at a distance using

their glasses or contact lenses (if they wear any), how well they see when they take them off, and whether their distance vision could be improved. An automated machine is used to do this and to take measurements on the shape of their eyes. The machine does this without touching their eyes. It will not hurt.

- Study participants may be asked to remove their glasses and/or contact lenses during the course of the procedures. An automated machine will be used to look at the prescription in their glasses.
- This vision assessment is not a substitute for a full eye examination performed by the person's eye doctor.
- Visual acuity results will be given to the study participants as part of the MESA exit report.

The following script is the example from the completion form which you could read to the participant:

The eye refraction component of MESA is not a complete eye examination. We want to understand how well you see at a distance using your glasses or contact lenses if you wear them, how well you see when you take them off, and whether your distance vision could be improved. An automated machine is used to do this. A visual acuity results will be given to you as part of the MESA exit report. Before we begin with the examination, I would like to ask you a few questions. But first, do you have any questions?

Participant Questions & Answers

Below are some questions that may come up, along with some suggested wording for dealing with these questions.

What exactly are you testing for?
We are interested in how well you can see far away. We are also interested in the shape of your eyes. Finally, we would like to test your glasses, if you have them, to learn more about your prescription.

Will you give me a prescription for new glasses?
No, this is not a complete regular eye exam. Therefore, we will not be able to give you a prescription for new glasses today. This exam is not a substitute for your regular eye exam. You should see an eye doctor if you are concerned about your current prescription.

Will the examination hurt?
No, the examination will not hurt. First, you may be asked to read some numbers on a card. Second, you will be asked to look into a machine and read the numbers and letters that are shown. (If asked: Unlike other vision examinations you may have experienced, the machine will not puff air onto your eyes.)

Will you check for glaucoma? (Will you check my eye pressure?)
No, we will not be checking for glaucoma today. Today's assessment is not a complete eye exam. You should see an eye doctor if you are concerned about this.

Are you an eye doctor/ophthalmologist/optometrist?
No I am not. I am a health technician specially trained to perform these tests.

Do I have to take my contact lenses out?

We would prefer if you would take your contact lenses out because one of the things we want to measure is what your vision would be without them. However, you can leave your contacts in if you prefer. It is completely up to you. If you do decide to take them out, we have saline solution that is preservative-free and safe for both hard and soft lenses or if you prefer, you may use your own.

How does vision relate to the MESA study?
The blood vessels in your eyes help influence how clearly you see. In MESA we will study whether vision is a factor related to heart disease.

Continue with questions in Section 2 of the Vision Completion Form

Ask the study participants the following question:

Have you ever had laser vision correction or refractive surgery to treat nearsightedness or myopia? If so, which eye had the surgery to improve your distance vision?
This question is asked exactly as it is worded here. **NOTE:** We are only interested in laser correction surgery or radial keratotomy (RK) surgery used to improve nearsightedness. Some other terms to describe this surgery include: PRK, RK, Lasik, Excimer, or Refractive surgery. You may need to probe a response of "laser surgery," since this may possibly be for another reason (for example, for treating diabetic retinopathy.
If the answer is "Yes," ask, "which eye(s) had the surgery," record which eye(s) was/were operated on.

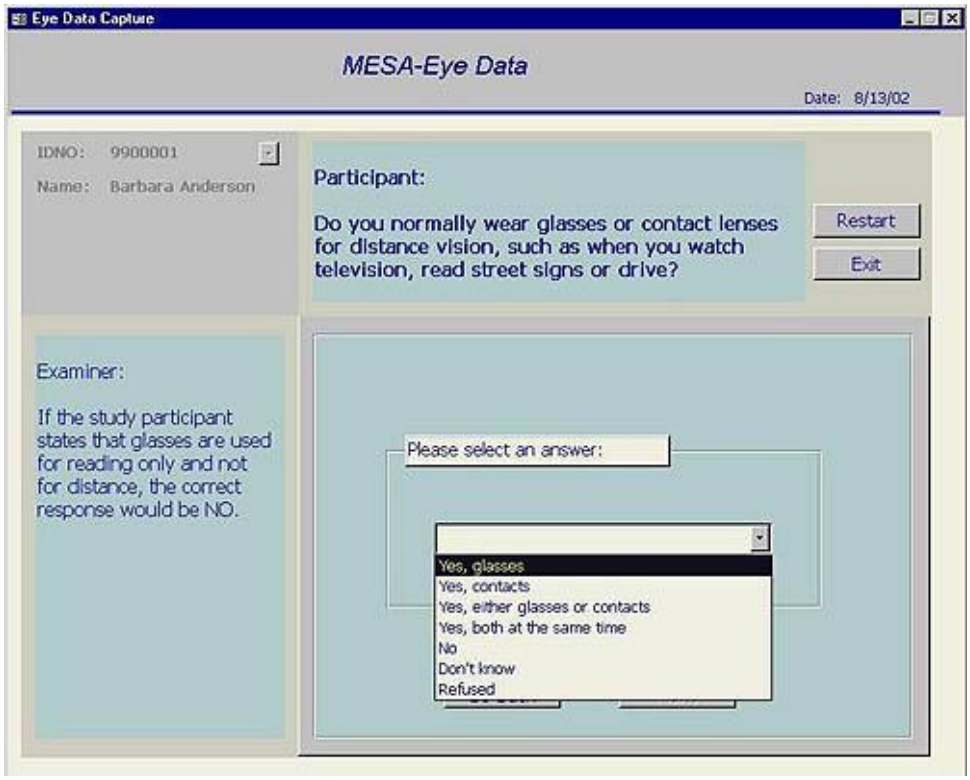
4. **Step 4: Following the Computer Screen Procedures together with the Lens meter procedure and Autorefractor.**

The flow of the visual assessment protocol is dependent on the presence and type (glasses or contacts) of distance correction normally worn by the study participant and their level of visual acuity. The computer screens are set up to help facilitate walking you through the protocol. There are four different variations in the set of computer screens depending on the different scenarios listed below:

- Study participants with **No Glasses** (i.e. People who do not wear distance glasses or contact lenses as well as those who do but did not bring their distance glasses or contacts with them. People who wear reading glasses only and do not use them for distance fall into this category);
- Study participants with **Distance Glasses** (i.e. People who brought distance glasses with them);
- Study participants with **Contact Lenses** (i.e. People who wore or brought contact lenses with them); and
- Study participants with **Contact Lenses and Glasses** (i.e. People who wore or brought both their contact lenses and distance glasses with them).

In order for the correct series of computer screens to prompt you, it is essential to accurately ask and enter the initial data. Otherwise, incorrect skip patterns may be initiated.

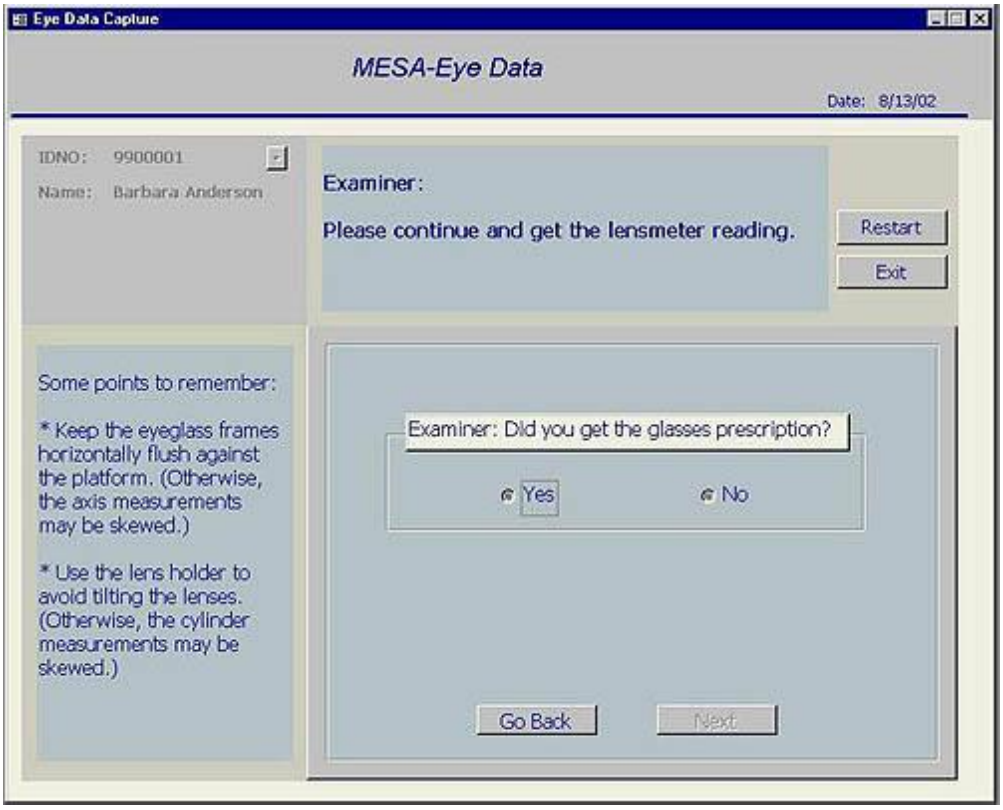
The following instructions will serve as a guide to assist you with the next steps. The first data screen with a question for the participant is shown below.



One other item worthy of note, study participants who do not know the English alphabet will not be able to complete the visual acuity measurements. If they wear distance correction, obtain the prescription of their eyeglasses and proceed directly to the Objective refraction. The computer data entry screens will remind you to do this.

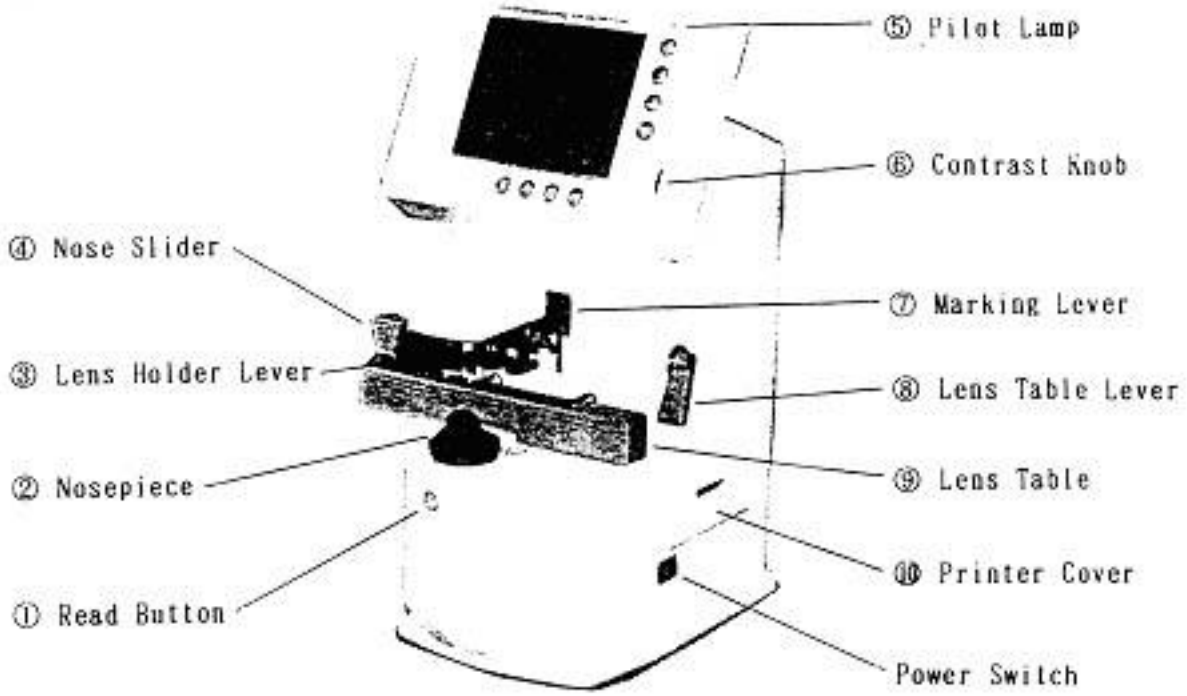
NOTE: REMEMBER TO CLEAR THE LENSMETER AND AUTOREFRACTOR!!!! The autorefractor should be set so the [CYL +] appears on the LED screen.

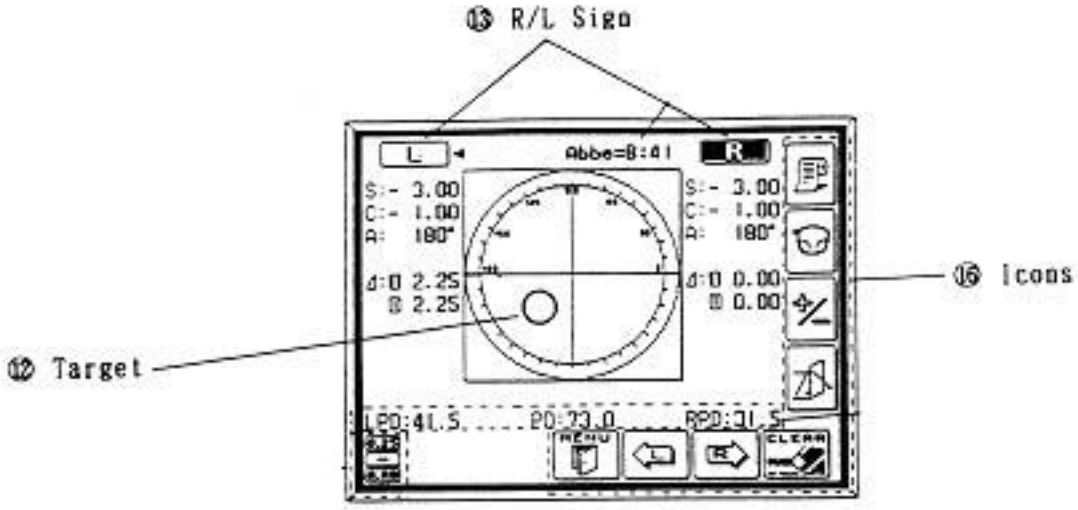
Proceed with the Computer screen procedure until you are prompted with the screen below,



4.1 **Lensmeter Reading: Measuring Prescription of Eye Glasses**

This test is conducted for all study participants who brought their distance glasses with them. The procedures for conducting the test with the Nidek Lensmeter are provided below. Refer to the Nidek Auto Lensmeter Model LM-990A Operator's Manual for more detailed specifications if necessary.





Nidek Lensmeter LM 990-A

- Ask the study participant to hand you the eyeglasses he/she wears to see distant objects.

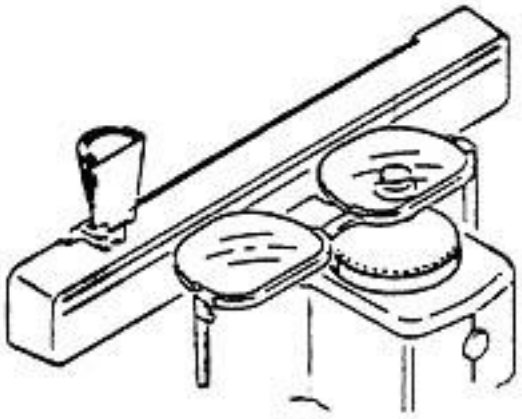
- Inspect the lenses. Clean them with lens cleaner and dry them with the Kimwipes available. Do not use paper towels, tissues, or other cloths to dry the glasses since they are not lint-free.

4.3 **Setting the Lens**

- Be sure the lensmeter is turned on before you place the glasses on the nosepiece; otherwise, you will receive an "Init Err" message because the lensmeter will not calibrate properly. If this occurs, remove the glasses, shut the machine off, wait 10 seconds, and turn it on again.

- Set the nose slider on the lens table by pulling down on it. It will lower into place in front of the lens table. Place the nose slider to the left of the nosepiece. This will allow you to test the right lens first.

- Place the eyeglass frame on the nose slider with the top of the glasses frame facing you and the bottom of the glasses frame against the platform. This will position the glasses so that the right lens is held in your right hand and the left lens is held in your left hand (similar to holding glasses upside down). Because the lensmeter will automatically detect the position of the slider, you do not need to "tell" the lensmeter which lens you are reading; it should indicate that you are testing the right lens.



- Move the lens table forward until the bottom edges of both lenses touch.
- "Fix" the lens by first raising the lens holder lever to its uppermost position and then lowering it slowly until it makes contact with the lens.

Some points to remember:

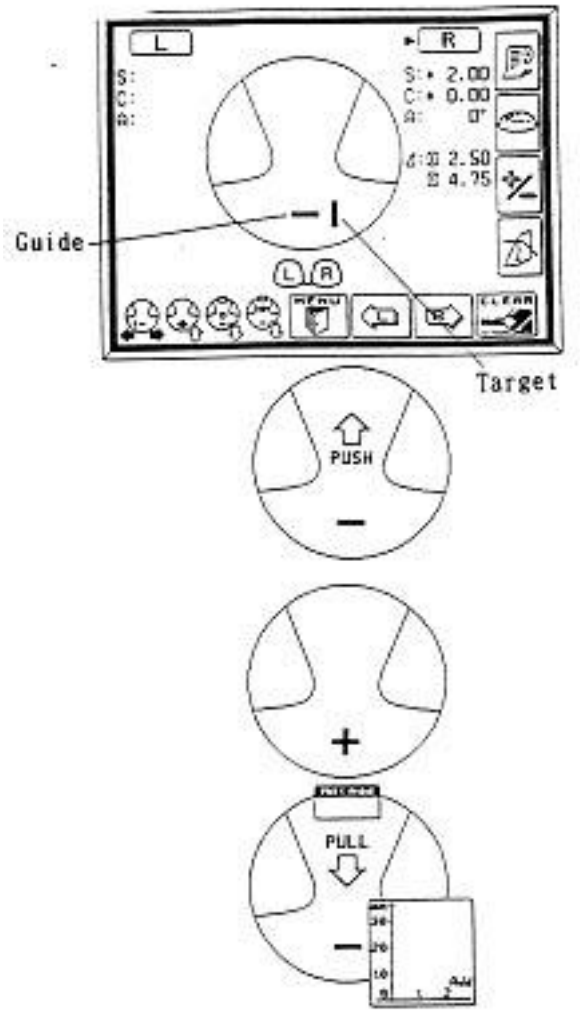
- Keep the eyeglass frames horizontally flush against the platform. (Otherwise, the axis measurements may be skewed.)
- Use the lens holder to avoid tilting the lenses. (Otherwise, the cylinder measurements may be skewed.)

4.3.1 **Measuring the Lens**

Progressive power eyeglasses have gradually increasing strength as you go from the middle to the bottom of the lens. Because you cannot readily see the different lens areas, the eyeglasses look just like single vision lenses. Bifocal and seamless bifocals have both a

distance portion and near portion on the lenses. The lensmeter has the capability to measure these in separate modes. However, all lenses may be measured in Progressive Power Lens (PPL) mode. Thus, for simplicity and to save time, **you will read the lenses in the Progressive Power Lens mode**.

Press the PPL mode button (the second button on the right hand side of the display screen). Four lens graphics will appear on the bottom left hand side of the LED screen to indicate that you are now in the PPL mode. If the lensmeter is turned off, you will have to return it to Progressive Power lens mode as it is not the default mode.



Set the lens as described above.

- Place the upper third part of the lens on the nosepiece in order to position the distance portion properly.
- Align the target (vertical line) by moving the lens sideways so that the target is brought to the middle of the guide (horizontal line).
- When the target is within an acceptable range, the “PUSH” sign with an up arrow will appear and the target line will disappear.
- Push the lens forward slowly until the target changes to a plus sign.

- When the distance value is stable, a small beep sounds and the "PULL" sign with a down arrow appears.
- Lift the lens holder off the lens. Remove the glasses and move the nose slider to the right. Place the glasses so that the left lens is in position under the lens holder. Remember to start with the distance third of the eyeglasses so that you are measuring the distance portion.
- When moving from side to side, pull the table closer to you slightly and repeat these steps for the left lens.

Some points to remember:

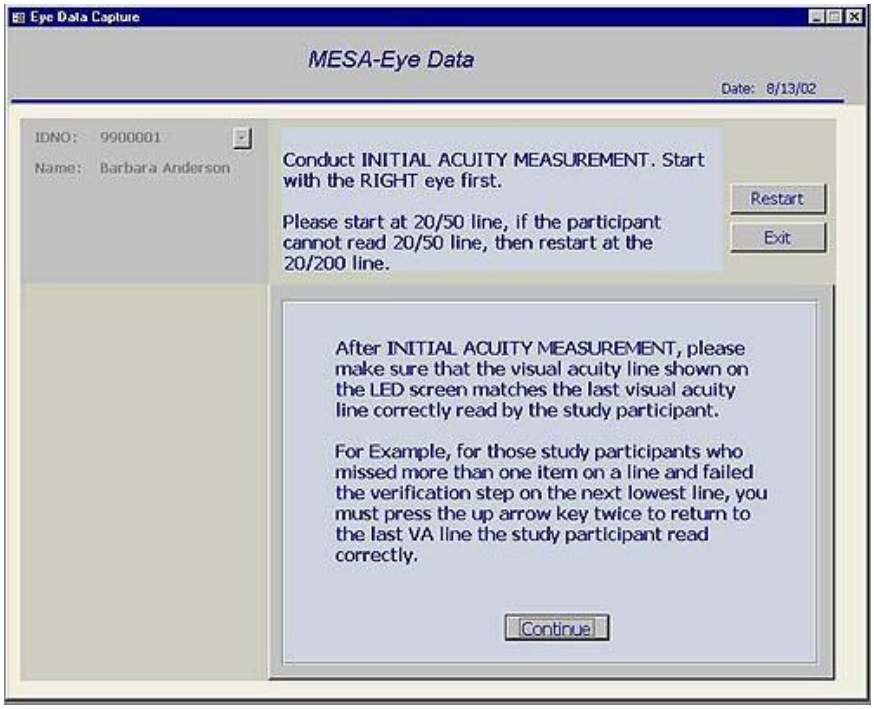
- If you forget the order of the steps, follow the lens graphics on the bottom left hand side of the screen.
- Always remember to start in the top distance portion of the lens, especially when switching to the left lens.
- If the plus sign never appears in Step 6 and the PUSH sign appears all the way to the top of the frame, remove the glasses and start a little bit lower in the lens.
- If the plus sign still does not appear after trying a second time, the prescription may not have a sphere component. In this case, measure the distance portion approximately 5 mm below the top of the frame, centering the lens as best as possible.
- Pressing CLEAR on the lensmeter resets values from both lens back to zero (not just one eye's values). Redo both lenses.

IMPORTANT NOTE FOR MEASURING ANY LENS

If the sphere is equal to or greater than /±5D/ for any lens type, use the auto-Abbe compensation when "reading" the lens as follows: Align the lens as described in this section. When pressing the "Read" button, depress it for a count of 2 seconds. This reduces error of high power lens measurements.

Once the Lensmeter procedure is completed and the eyeglasses prescription has been obtained, return to the Computer Screen procedure and enter data into the screen. Leave the lensmeter on as the data will transfer to the Autorefractor for the objective refraction measurement.

You will then be prompted to "Conduct INITIAL ACUITY MEASUREMENT. Start with the RIGHT eye (see figure below). Follow the instruction on the computer screen as you enter in data.



4.4 **Automated Refraction and Visual Acuity Measurements**

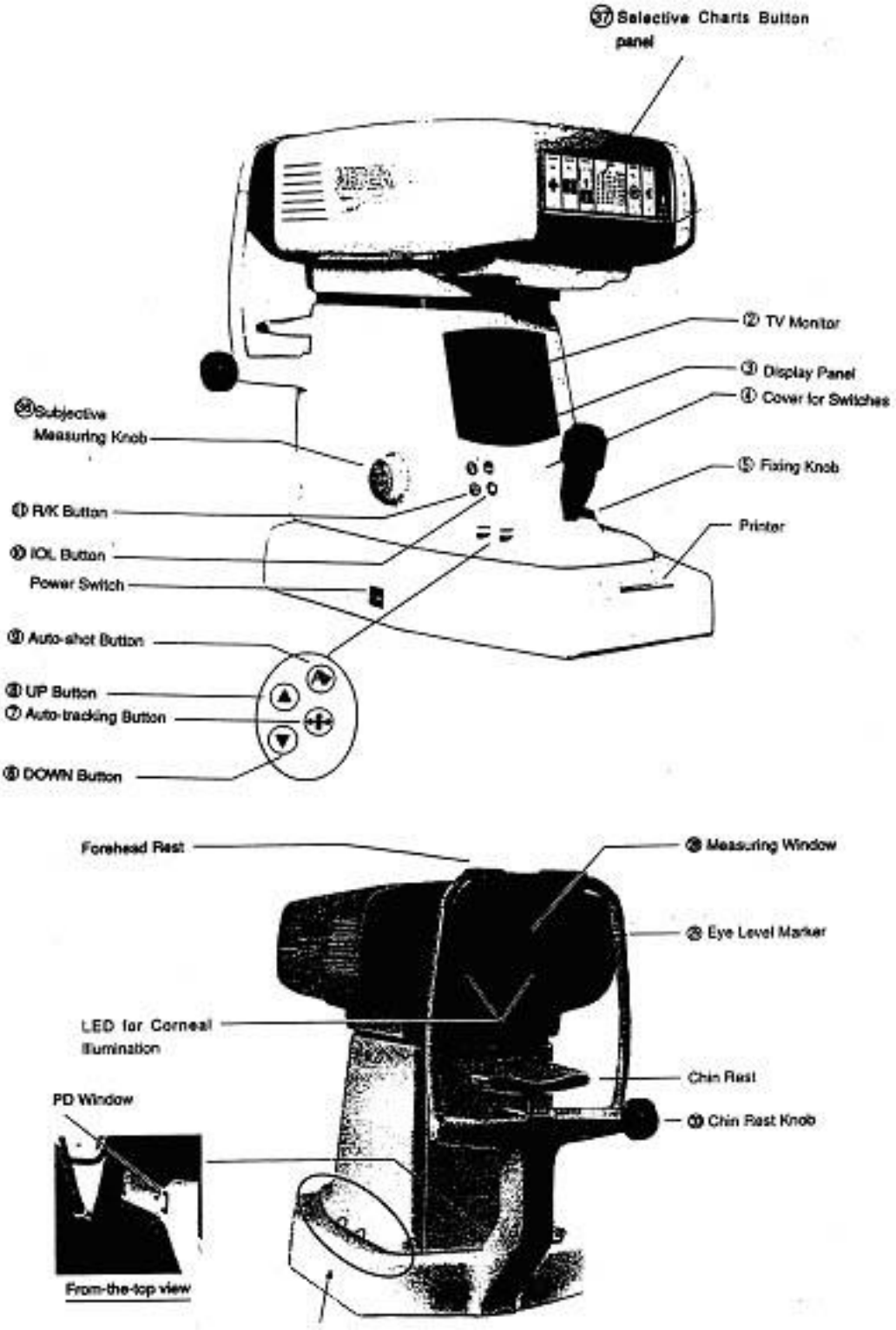
Perform the visual acuity and enter data into the computer screen as prompted as you move along the visual acuity protocol.

Positioning the Study participant

- Make sure that the moveable top portion of the autorefractor is positioned closest to you and not the study participant.
- Ask the study participant to remain seated in the chair and lean forward to place his/her chin in the chinrest and his/her forehead against the forehead rest.
- Adjust the height so that the study participant is comfortable.
- Align the center of the study participant's eye with the eye level marker (red band) by using the chin rest knob.

Some points to remember:

- Always make sure that the STUDY PARTICIPANT keeps his/her forehead on the forehead rest or you will be unable to focus the eye for any of the upcoming tests.

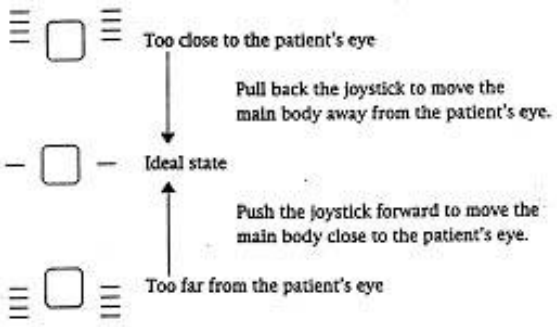


Autorefractor ARK760

Focusing the Study participant's Eye

Move the autorefractor to the appropriate eye as specified on the computer screen. [&*X CAN WE PROGRAM TO SELECT THE EYE AT RANDOM?]. A red "R" or "L" will appear at the edges of the autorefractor when you have moved the unit far enough to measure the eye. These letters are also used to remind you which eye is currently being measured.

Use the focusing indicator, called a mire ring, to focus on the center of the eye. Move the top portion of the unit forward slowly. A series of lines will appear to the left and right of the mire ring.



1. When one line appears on either side of the ring, the autorefractor is in position to focus the eye.

2. If one or more lines appear below this centerline, the unit is too far from the study participant's eye. Move the unit closer to the study participant.

3. If one or more lines appear above this centerline, the unit is too close to the study participant's eye. Move the unit away from the study participant.

4. Use the joystick to raise or lower the unit as necessary to raise or lower the mire ring in order to focus on the study participant's eye.

5. If the <LIMIT> message appears on the LED screen, move the joystick and/or the chin rest in the direction indicated by the arrows on the screen.

4.5 **Visual Acuity Measurement with Distance Correction**

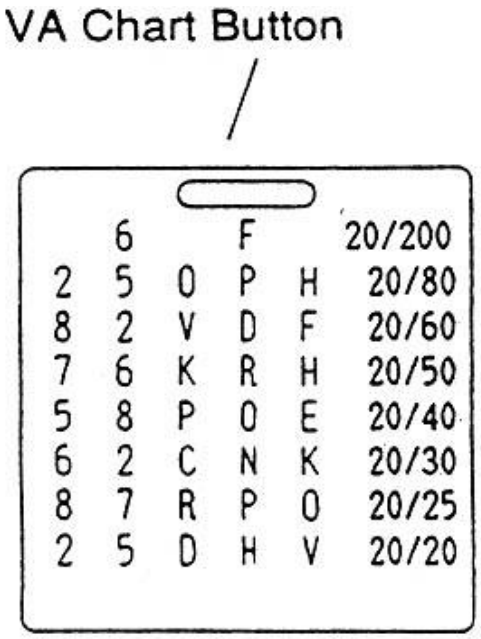
Ask the study participant to wear the glasses or contacts they use for distance vision. For study participants who wear glasses, make lens cleaner spray and Kimwipes available if they ask to clean their glasses.

Measuring Visual Acuity with Distance Correction

1. Move the autorefractor to the start eye as specified on the computer screen.

2. Begin by pressing the Visual Acuity (VA) Chart Button on the Selective Chart Buttons Panel. Note that the LED screen tells you that the whole VA chart is displayed.

3. Use the arrow buttons on the base of the autorefractor to change the whole VA chart to the 20/50 line.
4. Introduce this test as you proceed with a statement similar to the following: ***The screen will appear blurry for a few moments. Then I will ask you to read it to me as best as you can. Please note that there may be numbers and letters on the screen.***
5. Focus the eye as specified in the previous section.
6. Ask the study participant to read the line: ***Please read the numbers and/or letters on this chart slowly, starting from left to right.***
7. The study participant must read the entire line in order for you to score it. If he/she stops in the middle of the line, prompt him/her to continue with a best guess. Caution study participants to read slowly to avoid misstatements.
8. The study participant is allowed to miss one number/letter on any one line to continue. Therefore, the study participant may proceed to the next line whenever he/she reads 4 or 5 items correctly.
9. If the study participant corrects himself/herself immediately after a miss, accept the correction. If the study participant proceeds to the next item and wants to change the reading of a previously read item, do not accept the correction.
10. After the study participant reads the 20/50 line, continue as follows:
    - If the study participant reads the 20/50 line correctly, that is with one or fewer misses, press the down arrow key once. The 20/40 line is displayed. Repeat steps 6-9. If the study participant reads this line correctly continue down the visual acuity chart one line at a time using the down arrow key.
    - If the study participant cannot read the 20/50 line correctly, restart at the 20/200 line and proceed down the chart using the rules in Steps 6-9. Use the up arrow key to move to the 20/200 line. (If the study participant cannot <u>see</u> to read the 20/200 line, check "20/200+" in the data entry program for that eye and leave the chart line in the Refractor at 20/200. For any situations where the study participant was not able to read the 20/200 line that is NOT related to poor or impaired vision, check "CNO" for that eye. Examples of reasons for this could be "no time" or "lack of cooperation.")



11. Whenever the study participant misses more than one item on a line, you must "verify the miss." To do this, present the next lowest line on the chart and ask the study participant to continue.

    - If the study participant misses more than one item on this "verification" line, end the test.

    - If the study participant misses no more than one item on the line (that is he/she "passes"), continue down the chart until the next time the study participant misses more than 1 item or he/she reads the 20/20 line correctly.

12. Stop the test under the following circumstances:

    - The study participant reads the 20/20 line correctly; or
    - The study participant has missed more than 1 item on a line two lines in a row.

13. Make sure that the visual acuity line shown on the LED screen matches the last visual acuity line correctly read by the study participant. For example, for those study participants who missed more than one item on a line and failed the verification step on the next lowest line, you must press the up arrow key twice to return to the last VA line the study participant read correctly.

14. Move the autorefractor to the second eye and repeat these steps.

15. Make sure that you select the appropriate acuity line on the computer screen which reflects the acuity chart line in the Refractor.

Some points to remember:

- Make sure the study participant does not squint while reading the VA chart. You will actually see the eyes close on the LED screen when this occurs.
- Make sure that study participant understands that there are letters and

numbers on the chart.

- You will not be able to "verify a miss" when the study participant misses more than one item on the last line (20/20). In this instance, enter "20/25" as the last line the study participant read correctly.
- Make sure to move the VA screen back to the last line read correctly.

4.5 Automated Objective Refraction Measurements

Pre-Test Procedures:

1. Ask the study participant to remove his/her glasses or contacts except if they have had a cataract surgery. **Study participants with cataracts are to leave their lenses in.** For study participants who wear contact lenses, contact lens solution that is safe for all types of contact lenses should be made available. Direct study participants who wish to wash their hands before removing their contacts to a sink. Offer the disposable contact lens case for study participants who do not have a lens case of their own. If the study participant refuses to remove his/her lenses, proceed with the lenses in but note this in the drop-down comment box of the computer screen, selecting "Contact Lenses worn for OR".

2. Ask the study participant to reposition himself/herself in the autorefractor.

Measuring Objective Refraction (with No Correction):

1. Move the autorefractor to the same start eye as specified for visual acuity (see prompt on the computer display).

2. Begin by pressing the Scenery Chart Button on the Selective Chart Buttons Panel. Note that the LED screen displays the <R/K> symbol and space for several readings.

3. Introduce this test while completing steps 1-2 with a statement similar to the following: ***The screen will appear blurry for a few moments. Once it is in focus, you will see a hot air balloon on the screen. I'd like you to focus on the balloon. Please blink a few times for me while I focus on your eye. When you hear a beep, the test will begin; try not to blink for me at that time.***

4. Focus the eye.

5. Once focused, the autorefractor will automatically take the required readings. You will hear a series of three quick beeps and then a series of three longer beeps. The first three beeps indicate that the autorefractor is taking three keratometry readings. The three longer beeps indicate that the autorefractor is taking three refraction readings.

6. The word <FINISH> will appear when the test is done.

7. Move the autorefractor to the next eye, focus, and then take the required readings according to the above instructions in steps 3 - 6.

Some points to remember:

- **NOTE**: The study participant's pupil must be a certain size in order for the readings to be completed. Some medications may cause the pupil to contract. If the pupil does not fill the mire ring, you will be unable to take these readings. Make a note of this in the computer system.
- If the study participant cannot see the red hot air balloon, ask him/her to focus on the red blur. If the study participant cannot see a red blur, ask him/her to focus on where your voice is coming from and look straight through the eyepiece.
- Make sure the study participant does not blink or squint during step 5 (above) or the autorefractor will be unable to take the required readings. For study participants with baggy or limp eyelids, ask the study participant to hold the upper lid up with his/her finger, making sure that he/she continues to hold his/her forehead against the forehead rest.
- If the autorefractor is unable to take any of the three required readings in step 5, it will automatically switch to the IOL mode and try again. If the readings are not obtained at this step, you will attempt to take the readings manually.
    - Press the auto-tracking button () once to switch to the manual mode.
    - Focus on the eye and press and hold the button on top of the joystick until three keratometry and three refraction measurements are taken.
    - There are some eye conditions which cause problems in one area of the eye but not in another. Therefore, if you are having trouble taking manual readings from the center of the eye, move the joystick in a circle while pressing the button in order to "walk" around the box. You may be able to obtain the readings off-center.
    - Remember to press the auto-tracking button once more in order to return to the auto-tracking mode for the next study participant.
- If the autorefractor is unable to take <u>all</u> of the required readings, in step 5 the readings that were taken will flash on the screen. (Sometimes you will see the <KM?> indicator flash as well.) You will not see the <FINISH> indicator. If all the readings are not obtained, press the button on top of the joystick and the autorefractor will make another attempt at the readings. If you are still having difficulty, you may want to ask the study participant if they had cataract surgery. However, make only one attempt after getting the <KM?> indicator. Additionally, if you have no reading under the R (i.e., 0:3), try once more manually.
- If you are unable to get any KM data the unit will not go to the automatic 20/25 line with the sphere flashing.
- To get to that screen, press VA Chart, and then press the IOL button (next) .
- If you are able to get at least one <R> value, press the down arrow key to bring up the 20/25 line and retain the objective refraction values in place.
- If the study participant wears contacts for the OR portion of the exam, make sure to comment this in the data entry screen.
- If the study participants wears glasses and you got the prescription on the lensmeter you will hear a beep during the OR measurement indicating the data transferred.
- NEVER print to the tape BEFORE transferring the data from the equipment to the computer. **Printing erases the person's data.**

Visual Acuity with Objective Refraction Measurements

The Visual Acuity with Objective Refraction test is only done if prompted by the Vision computer program. It is performed only for study participants whose initial acuity

measurement was worse than 20/25 in either eye. Please note that you may be asked to conduct this test for one or both eyes as appropriate. The study participant should **not** wear his/her distance correction for this test.

Conducting the Measurement:

1. Immediately after conducting the Objective Refraction measurement, position the autorefractor on the eye indicted by the computer. Note that the 20/25 Visual Acuity line is displayed.

2. Focus the eye.

3. Ask the study participant to read the line for you: ***Now please read this line slowly and from left to right for me. Remember that you may see both numbers and letters on the screen.***

4. Enter the results on the data entry screen. Please note that the guidelines specified in previous section also apply here. The study participant may miss one or fewer items to "pass". It is also necessary to "verify a miss" during this test.

    - If you have no objective refraction measurements for an eye, you cannot assess the VA with OR. Answer "CNO" to the question "Can the study participant read 20/25?"

    - If the refractor was able to get only one of 3 readings you will need to manually bring up the 20/25 line by pressing the VA chart, and then the IOL button.

    - If the study participant can read the 20/25 line, you will answer: "Yes" to the question "Can the study participant read the 20/25 line?"

    - If the study participant cannot read the 20/25 line, you must change the chart line to the 20/50 visual acuity line. Also answer "No" to the question "Can the study participant read the 20/25 line?" A pop-up will remind you to go to the 20/50 line and begin the acuity check.

5. Using the up arrow keys, change the visual acuity chart to the 20/50 line.

6. Ask the study participant to read the line: ***Now please read this line slowly and from left to right for me. Remember that you may see both numbers and letters on the screen.***

    - If the study participant can read the line, you will move down the chart one line at a time, asking the study participant to read each line. Continue down the chart until the SP fails to read the line by missing more than one number or letter on two lines or you reach the 20/25 line, whichever occurs first.

- **Make sure that the final visual acuity line on the LED screen matches the last visual acuity line correctly read by the study participant.** For example, if the study participant missed more than one item on the previous two lines, you must press the up arrow key twice to return to the last VA line the study participant read correctly.

- Some study participants may fail to read the 20/25 line initially. Yet, when you go to the 20/50 line and work down they may then pass the 20/25 line. In these instances, **do not** change the answer to the question 'Can SP read 20/25' to 'Yes'. This answer should remain 'No', and you should leave the 20/25 line in the Autorefractor and in the computer system as the last line read correctly.

- Do not go below the 20/25 line, to the 20/20 line.

- If the study participant cannot read the 20/50 line, move the chart line up to the 20/200 line.

- Follow the steps above, moving down the chart until the study participant fails to read 2 lines. If the study participant cannot see to read the 20/200 line, enter "20/200+" in the data entry screen for that eye and leave the chart line in the Autorefractor at 20/200.

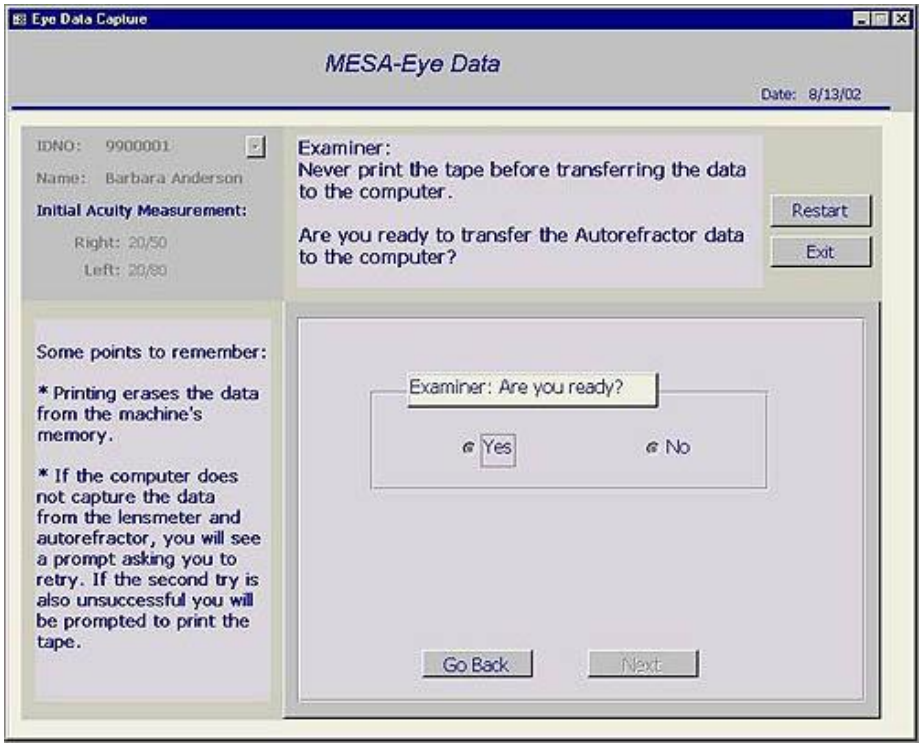
7. Repeat steps 1 – 6 for the second eye, if prompted by the computer to do so.

8. **Make sure that you select the acuity line in the computer screen that corresponds to the acuity chart line in the Autorefractor.**

4.7 Recording the Results from the Automated Equipment

Data will be recorded into a database on the field center computer using the computerized Vision data entry screens.

After you have completed all portions of the exam, the computer will ask whether or not you are ready to have the data from the lensmeter and autorefractor transferred to the computer. If you reply in the affirmative, the computer will automatically captured and transferred the information from the equipment to the MESA –Vision database (see below).



If the computer does not captured data from the lensmeter and autorefractor, you will see a prompt asking you to retry. If the second try is also unsuccessful you will be prompted to print 2 copies of the study participant's data, write their MESA study identification number and the date; send a copy to the data coordinating center for manual entry and keep the other copy in the unsuccessful data transfer folder.

NEVER print the tape before transferring the data to the computer unless prompted to do so by the computer. Printing erases the data from the machine's memory.

The actual screens and detailed instructions for use are provided in a section below.

4.8 **Finishing the Vision Component -** Return to Section 3 of the Vision Completion Form.

Note the status of the vision assessment as being "complete," "partially complete," and "not done." If either of the two latter categories is selected, check the appropriate reason for this status.

Note, also, whether the data was successfully transferred from the equipment to the computer. If this transfer was not successful, mark it as such. Remember to print 2 copies of the study participant's data, write the MESA study identification number and date on both copies; send one copy to the data coordinating center for manual entry and keep the other copy in the unsuccessful data transfer folder.

Use the Comments box to note any other observations, particularly those which may be helpful in interpreting the data.

Complete the MESA Field Center Use only box according to standard MESA protocol.

5. **Print the personalized Report of Vision Findings**.

   All examined persons with a "complete" vision assessment will have a personalized report generated by the computer based on information collected during the procedure. Study participants will receive their report either at the end of the vision component or in the MESA exit interview, depending on the procedure decided at the field center. Appendix D shows an example of this report.

6. **Post-Vision Procedures**

   - Make sure the study participant has his/her eyeglasses or contact lenses before leaving the room.
   - Change the chin rest tissue and wipe the head rest with alcohol wipes. You may elect to do this in front of the next study participant; however, it is always preferable to leave the machine clean at the end of the day.
   - Wash your hands.
   - Place the dust cover over the machines when the equipment is not in use.

7. **Report of Findings**

   All examined persons with a "complete" vision assessment will receive a report of their acuity findings in the MESA exit interview. Appendix D shows an example of this report.

APPENDIX A: LENSMETER PARAMETER SETTINGS

Note: Some settings differ from the machine's defaults. Recheck after power is disrupted.

TO REVIEW SETTINGS OR CHANGE THEN, BEGIN BY PRESSING THE MENU BUTTON.

Step	0.25
Cylinder	+
Prism	OFF
Abbe Sel	A:58
	A set
Wavelength	e
Target	o
Auto Read	off
Auto R/L	off
A. Prt R/L	off
A. Prt S	off
Prog Guide	on
Far a. read	on
Add graph	curve
Max add	off
V. drop	off
C. width	off
Pd	off
Net prism	on
Lens dia	off
Single	on
Convex add	off
Near	Add
Contact	off
Printer	on
Title	on
Density	3
Beep	lo
Auto off	15 min
RS- 232C	NIDEK
Baud Rate	9600
Parity	Odd
Data bits	8
Stop bits	1
CR code	off
Prism TX	Display

APPENDIX B

AUTOREFRACTOR PARAMETER SETTINGS FOR MESA-VISION

(Note: Some settings differ from the machine's default. Recheck after power is disrupted.)

INSTRUCTIONS:

1. Open the "trap door" on the front of the unit.
2. Push the symbol that looks like the "solar system".
3. Push the print button. (The top left button of 4 to the left of the "trap door")
4. This will print the first "page" of the parameters.
5. To go to the next page, push the symbol that looks like an eye print.
6. Press the print button again.
7. Repeat steps 5-6 until all seven parameter pages have been printed.
8. Exit by pressing the "solar system" symbol.
9. Close the "trap door"

PRINTOUTS SHOULD LOOK LIKE THE FOLLOWING:

PARAMETERS (1/7)

---

1 : Step 0.25 D
2 : Vertex D. 13.75mm
3 : KM Unit mm
4 : KM Display R1.R2
5 : Axis Step 1
6 : Meas. Mode Con.
7 : Print Manu.
8 : Al Mode YES
9 : Econo. Print NO
10 : Print Format User

PARAMETERS (2/7)

---

11 : Patient No. YES
12 : Patient No. 0709
13 : Name Print YES
14 : Date Format M/D/Y
15 : AR Print All
16 : KM Print All
17 : SE Print YES
18 : Eye Print NO
19 : TL Print NO
20 : CL Print NO

PARAMETERS (3/7)

---

21 : Auto Subj. YES

22 : RG1 program NO
23 : RG2 program NO
24 : Fog for RG1 0.50 D
25 : Fog for RG2 0.50 D
26 : Recall sel. Unaided
27 : Subj Axis 1
28 : VA Display Frac
29 :
30 :

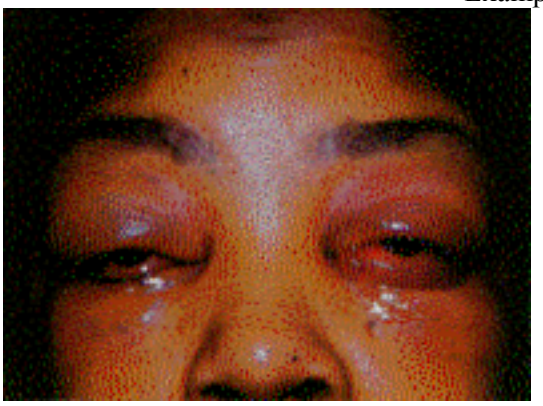
PARAMETERS (4/7)
---
31 : Obj. Chart Scene
32 : Cor. VA Check 20/25
33 : Cor. VA Test 20/20
34 : ADD VA 20/40
35 : XC Chart Dot
36 : XC test CAC NO
37 : XC auto VA NO
38 :
39 :
40 :

PARAMETERS (5/7)
---
41 : Conf. Index YES
42 : Near PD NO
43 : Working D. 14 inch
44 : Auto IOL YES
45 : Auto PD YES
46 : KM Continue 3
47 : Beep Low
48 : TV Auto-OFF YES
49 : Ref. Index n=1.3375
50 :

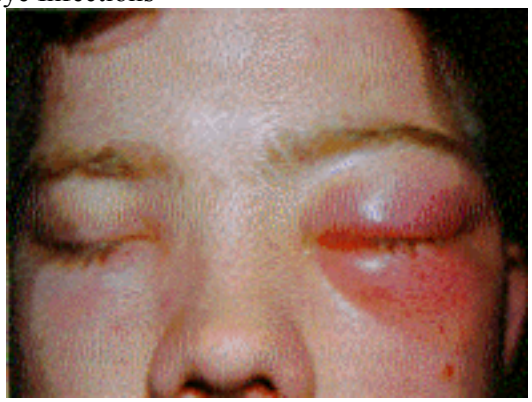
PARAMETERS (6/7)
---
51 : I/F Mode NIDEK
52 : I/F Format All
53 : Baud-Rate 9600
54 : Bit Length 8
55 : CR Code NO
56 : Error Print NO
57 : Error Code YES
58 : Error Data YES
59 :
60 :

Appendix C

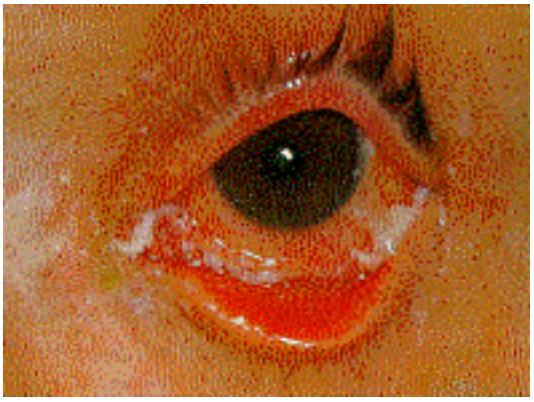
Examples of Eye Infections



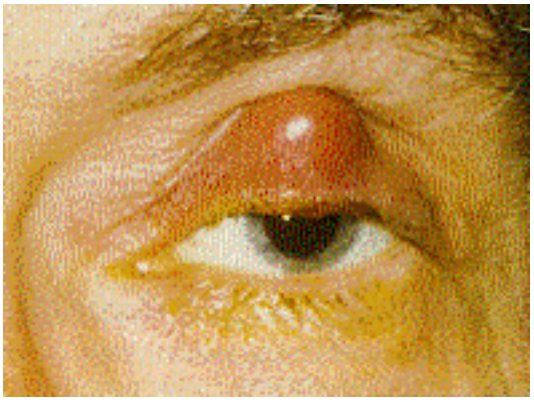
(1) Acute conjunctival chemosis.
Excluded from vision assessment



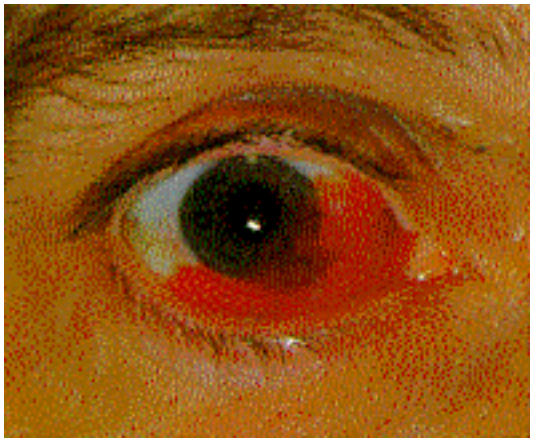
(2) Acute anterior cellulitis.
Excluded from vision assessment



(3) Acute conjunctivitis.
Excluded from vision assessment.



(4) Acute chalazion of the upper eyelid and an associated ***preauricular adenopathy.***
Included in vision assessment.



(5) Subconjunctival hemorrhage. Most are spontaneous and require no treatment or diagnostic studies.
Included in vision assessment.

Ophthalmology : Principles and concepts By Newell FW, M.D., M.Sc.(Opth)
Eighth edition, 1996 Mosby-Year Book, Inc . St. Louis, Missouri

Appendix D

Examples of MESA Vision Exit Report

Example No 1 –
FOR PEOPLE WHOSE PRESENTING VISION IS 20/25 OR BETTER IN BOTH EYES

MESA Report of Vision Findings

Vision

We have done a quick check of your vision today. Our exam is not as precise as an eye exam done by an eye doctor. These values may differ from a vision exam you may have by an ophthalmologist, optometrist, or optician.

With glasses:

In your right eye your distance vision is **20/25.**

In your left eye your distance vision is **20/20.**

This is a good level of vision. We have not done a full eye examination, so you should continue your usual schedule of periodic examinations by your eye doctor.

Example No 2 –
FOR PEOPLE WHO HAVE WORSE THAN 20/25 PRESENTING VISION IN EITHER EYE

MESA Report of Vision Findings

Vision

We have done a quick check of your vision today. Our exam is not as precise as an eye exam done by an eye doctor. These values may differ from a vision exam you may have by an ophthalmologist, optometrist, or optician.

Without glasses:

In your right eye your distance vision is **20/50.**

In your left eye your distance vision is **20/80.**

This level of vision is not as good as most people. If you were not already aware of this, you should see an eye doctor for a full eye examination and to see if he/she can improve your vision.

3.6. Cognitive Assessment

I Purpose: Inclusion of assessment of cognitive functioning in the MESA cohort offers a number of significant advantages not only to MESA but more broadly to understand cognitive aging in what is rapidly becoming a more multi-ethnic population of older adults in the US. The battery of three tests described here will (1) allow for direct evaluation of relationships between underlying cardiovascular disease and cognitive aging; (2) provide unique data on cognitive aging among major ethnic groups including those contributing most to the rapidly growing population of older adults in the US (e.g. Hispanics, African-Americans, Asians); (3) put in place a platform of "baseline" cognitive data allowing for later augmentation through longitudinal exams for evaluating patterns of cognitive aging overt ie in these ethnic groups; and (4) contribute significantly to available data on patterns of performance in Hispanics and Asians, two groups for whom current normative data are based on small samples. MESA results would be among the largest samples for whom such cognitive data would be available across cultures and languages.

The MESA Cognitive assessment is meant to provide measures of cognitive function using standardized tools that produce scores that can be compared to standardized norms and to other studies. The scores themselves are not sufficient to determine presence of dementia or other clinical disorders, but rather may be used as a means to evaluate a range of abilities within the cohort. Scores are expected to decline with age; however, once adjusted for age evaluations may be used to determine risk of other conditions or as outcomes themselves. In the MESA Study, three tests have been selected to evaluate cognitive function as described below:

1. Global Cognitive Functioning: **Cognitive Abilities Screening Instrument (V.2)** (CASI; Teng et al, 1994). This instrument was explicitly developed for cross-cultural use and has a validated Chinese version (Li et al, 1994; Tsai et al, 2007). Though we are not aware of a formal validation for a Spanish version of the CASI itself, the content of the CASI draws from standardized tests (e.g., Modified Mini-Mental State [3MS] that have been validated in Hispanics (Bird et al, 1987; Valle et al, 1991). Indeed, CASI items were explicitly chosen for their cross-cultural applicability (Teng et al, 1994).
   The CASI provides brief assessments of major domains of cognitive function including attention, concentration, orientation, short-term memory; long-term memory, language abilities, visual construction, verbal fluency, abstraction, and judgment which when summed provide a measure of global cognitive functioning (see Appendix of Teng et al, 1994 for copy of CASI). Importantly, the CASI has been used to screen for dementia as well as to track within-individual changes over time in cognitive functioning (Teng et al, 1994). We will increase the sensitivity of our battery by modifying one of the CASI items. Item # 14 asks participants to list as many 4-legged animals as possible in 30 sec up to a maximum of 10. The task assesses verbal (semantic) fluency. We will administer item #14 according to CASI instructions but we will allow the participant an additional 30 seconds to continue naming animals. This modification will eliminate potential ceiling effects and match precisely a widely used verbal fluency task thereby giving us an additional assessed cognitive domain in the battery. For the CASI total score only the number of words generated in the first 30" counts.

2. Speed of Processing [**Digit Symbol Coding Test**; Wechsler, 1996]. The DSCT, a subtest of the Wechsler Adult Intelligence Scale-III (formerly called the Digit Symbol Substitution Test), measures how quickly simple perceptual or mental operations can be performed, which along

with working memory (see next test) mediate a large proportion of age related variance in memory (Salthouse, 1991; Park et al, 1996), reasoning (Salthouse, 1991) and other cognitive abilities (Lindenberger & Baltes, 1994). For 120 seconds the participant fills in the symbols (e.g., +, >) that correspond with numbers according to a key provided at the top of the page.

3. Working Memory [**Digit Span Test** (Wechsler, 1**996)** is a sub-test of the Wechsler Adult Intelligence Scale-III). This test which is administered in two parts requires the participant to repeat spans of numbers that increase in length first forwards and then backwards. Translations into Spanish and Chinese are provided by the publisher (The Psychological Corporation).

II. Cognitive Abilities Screening Instrument

1. **INTRODUCTION:** The Cognitive Abilities Screening Instrument (CASI) consists of 25 test items and provides brief assessments of major domains of cognitive function including attention, concentration, orientation, short-term memory, long-term memory, language abilities, visual construction, list-generating ability, abstract thinking, and judgment which when summed provide a measure of global cognitive functioning. Importantly, the CASI has been used to screen for dementia as well as to track within-individual changes over time in cognitive functioning.

Administration time is approximately 15-20 minutes. The maximum total score is 100.

The CASI includes items derived from several screeners: Hasegawa Screening Test for Dementia (HSTD), The Mini-Mental State Examination (MMSE), and The Modified Mini-Mental State Test (3MS). In addition to providing CASI subscale and total scores, it is possible to compute comparable scores for the HSTD, the MMSE, and the 3MS.

The CASI was explicitly developed for cross-cultural use. Versions have been translated into Japanese, Chinese, Spanish, and French among other languages. For MESA, the Spanish, Chinese and English versions are being administered.

2. Equipment

- CASI Questionnaire
- Pen
- Stopwatch
- Soft-lead pencil with eraser
- Blank sheet of paper
- Five objects: toothbrush, spoon, key, comb, coin (quarter) for naming and recall
- Piece of blank cardboard (for covering the five objects during recall)

3. General Considerations

3.1 Examiners should thoroughly familiarize themselves with the testing procedures and the scoring criteria before using the CASI in formal assessment. The CASI record sheet contains highly condensed information from this manual. Users of the record sheet must first study this manual carefully; otherwise misunderstandings of the condensed information on the record sheet can easily occur.

Tests should be administered in a **quiet place with minimal distractions**. Distractions could affect participant performance and should be noted on the record sheet.

In the administration of the CASI, **do not offer extra help or wait too long for responses**. Although sometimes it is appropriate to re-present or rephrase a question, in general if a participant gives an incorrect answer, says "I don't know," or is unable to give an answer, the examiner should just score accordingly and proceed to the next item.

Time limits are set for some items. **Subjects should never be told of any time limit on any item.**

Practicing the administration of the CASI is highly recommended and certification by the Coordinating Center is required. Written materials (the MOP), observations, and live practice will be helpful. Questions about administration of the cognitive tests should be directed to the CC staff.

Please note the following:

3.2 If the participant makes a mistake but is able to spontaneously correct him/herself, please give him/her credit for the answer.

3.3 The aim of the CASI is to assess the participant's best cognitive performance. Assisting a participant to be comforatable and free from distractions helps obtain optimal performance.

3.4 Ask the questions using the **exact wording** on the CASI form.

3.5 **Speak loudly and enunciate clearly**.

3.6 Write down the participant's answers whenever possible. If there is any question later on, it is easier to settle if the answers are written down.

3.7 Do **not** allow the participant to see the CASI score sheet as it may affect perforamance.

3.8 Do **not** give the participant feedback about his/her performance. When a participant asks if his answers are correct, you should tell him/her, "Sorry, I cannot tell you how you did on any item"the truth. In case the answer is incorrect, you may soften the impact by saying like "this item is meant to be difficult" or "many people have trouble with this one".

3.9 There are certain items that should only be said to the participant once, and should not be repeated even if the participant did not hear or was not paying attention. However, please do NOT tell the participant "I can only ask you this question once" before these items.

3.10 Use multiple choices ONLY if specified, for example, questions (5) and (12) both state "may provide 4 choices".

3.11 Prior to starting the CASI, do NOT say "There are no write or wrong answers…" However, you should say "These tests are designed to be challenging. We do not expect you to get every question right, so just do the best you can".

3.12 When timing the participant for certain items, try not to make it too obvious. The participant should not know that he/she is being timed.

3.13 Some participants may seem upset or defensive and make statements like, "Do you think I'm senile?" or "You must think I'm stupid.". You should answer them by saying , "We do not expect you to answer each question correctly, so try not to worry about how you are doing, just do your best."

3.14 When the informed consent is completed, please advise whoever is collecting it to NOT tell the date to the participant if asked. If necessary, the technician should enter the date him/herself.

4. Special Circumstances

4.1 Scoring for a Vision-Impaired Participant: If the participant's vision is very poor, please write "RAISE YOUR HAND" and draw the pentagons **on a separate white paper, very large, and with a thick black pen.** Sometimes, this will help the person to see better. If they are still unable to see, score 0 for all of these items and for **validity code "3 = probably invalid: poor eyesight".**

4.2 Scoring for a Hearing-Impaired Participant: Before starting the CASI, if it is determined that the participant has a hearing impairment, optimize the participant's hearing as much as possible by keeping room as quiet as possible and/or by speaking louder for the entire test. If the participant is still unable to perform some of the CASI items due to poor hearing, score these items as 0 and code that the CASI is **probably invalid due to poor hearing**. Certain items, like 17 a and b (repeating sentences), should **not** be repeated even if the participant was not able to hear the first time.

4.3 CASI Administration for a Participant who is Too Severely Impaired Cognitively to Take the Test: Begin by asking as many of the CASI questions as you can, and code them as "0" if the participant cannot provide the correct answer. This will still provide a score for comparison with others in the cohort.

5. ADMINSTRATION

5.1 Start Time of the Test: Enter the testing start time in military time right before asking the first question. At the end of the exam you will be asked to record end time.

5.2 Introductory Text: Prior to starting the CASI, it is very important to inform the participant that you will be performing a memory test and the reason why. The following simple explanation should be provided as an introduction to the CASI: :

"In this next examination, we are asking you to perform tasks related to memory and other thinking abilities. Some of the questions I will ask you are easy while others are hard. Nobody gets all correct. I have to ask you ALL the questions, so just do the best you can."

This simple explanation helps to put the participants at ease and may prevent them from getting defensive if they miss certain items.

5.3 Questions 1: PLACE OF BIRTH: These items are a measure of long-term memory. It is assumed that all individuals have had repeated opportunities to learn and report their date and place of birth.

1. WHERE WERE YOU BORN?
    a. City/Town (Town/Village) _____ (2)
    b. State/Country _____ (2)

After the opening question, if the participant has provided only a part of the information, PROBE FOR THE MISSING PART(S).

Example: "Where were you born?" "Sunnyvale" "What state is it in?" "California"

If the participant was born in the US, state is sufficient. If he/she was born outside of the US, country will be accepted.

The computer screen will show state or country of birth that was collected at the MESA baseline exam. If it matches, mark it as correct.

We do not have information on city/town of birth. Therefore, this will be asked again at the end of the exam. If the same response is given easily, assume it is correct and mark it at that time

5.4 Questions 2: DATE OF BIRTH: These items are a measure of long-term memory. It is assumed that all individuals have had repeated opportunities to learn and report their date and place of birth.

2. WHEN WERE YOU BORN?
    a. Year _____
        i. Accurate = 2
        ii. Missed by 1-3 years = 1
        iii. Missed by > 3 years = 0
        iv.

    b. Month _____ (1) Date _____ (1)

Date of birth collected from the MESA baseline exam will be shown on the computer screen. Please score according to this information.

5.5 Question 3: AGE: HOW OLD ARE YOU?

3. Age _____
    i. Accurate = 2
    ii. Missed by 1-3 years = 1
    iii. Missed by > 3 years = 0

    If the question is answered "I will be xx years old this year", e.g. someone born 12/12/1940 may say "I will be 70 this year" instead of saying "I'm 69 years old". If they are correct, give full credit for either answer.

5.6 Questions 4 and 5: COMMON KNOWLEDGE. This item is also intended to be a measure of long-term memory. It is assumed that the participant has had many opportunities to learn these facts.

4. HOW MANY MINUTES ARE THERE IN AN HOUR? _____ (2)
   Or HOW MANY DAYS ARE THERE IN A YEAR?

If the participant correctly answers "minutes in an hour", skip asking "days in a year". If the participant fails "minutes in an hour", ask "days in a year". Credit 2 points (full credit) if EITHER is answered correctly.

5. IN WHAT DIRECTION DOES THE SUN SET? _____ (2)

   If the participant seems confused, provide the four choices of north, south, east, west. If the participant POINTS in a direction or names a landmark (e.g. the sun sets in the direction of the xx mountain"), ask him/her: "Is that north, south, east or west?" If still incorrect, score 0.

5.7 Questions 6a and 6b: REPEATING THREE WORDS.

I AM GOING TO SAY THREE WORDS FOR YOU TO REMEMBER. REPEAT THEM AFTER I HAVE SAID ALL THREE.

SOCKS _____ (1) BLUE _____ (1) CHARITY _____ (1)

Say each word clearly at the rate of 1.5 seconds per word. If the participant repeats after each word, request at the end of your presentation "Repeat the three words again" and score according to the response to this request. When you present the three words for the participant to repeat, sometimes they interrupt you by asking "what did you say?" or "I did not hear that, can you say that again?" before you have finished saying all three words. When this happens, do not stop in the middle of the three words even when the participant asks "what?" Finish the three words and say "repeat what you think I said". Score the number of words correctly repeated.

Please remember that if the participant is able to repeat all 3 words the first time, you DO NOT have to repeat the three words three more times. If the words are registered the first time, this should be scored as spontaneous recall. If the participant cannot repeat all three words, re-present all three words UP TO THREE MORE TIMES. Then, whether or not the participant can repeat all three words, proceed to the next item. During the first RE-presentation of the three words, it is advisable to clarify the words in order to ensure understanding. For example "Let me say the three words again. They are… SOCKS – something to wear, BLUE – a color, and CHARITY – a good personal quality. Now say the three words again.

After the participant has repeated the three words, do **not** tell him again "Now remember these words" He/she has already been told to remember them earlier. This is different from the same item on the Folstein mental status exam and this subtle difference is intentional.

Minor variations in these words are acceptable. Accept the word as correct whether it is in the singular or plural form. For example, accept "socks" for "socks".

5.8 Questions 7: DIGITS BACKWORD.

I SHALL SAY SOME NUMBERS, AND YOU REPEAT WHAT I SAY BACKWARDS. FOR EXAMPLE, IF I SAY 1-2, YOU SAY 2-1, OK? REMEMBER, YOU REPEAT WHAT I SAY BACKWARDS.

A. 1-2-3 (1) Coach for 3-2-1 if needed
B. 6-8-2 (2)
C. 3-5-2-9 (2) Skip this if A and B cannot be repeated backward.

For the first digit span only, if the participant cannot correctly repeat A. 1-2-3 backward, score as 0 but coach for the correct response of 3-2-1.

If the participant fails the first two digit spans, skip the third one and score it 0.

For each digit span, give credit only if the response is entirely correct; score 0 otherwise.

If the participant says the numbers forward only, do not clue further, score 0. If he/she says the numbers forward, then asks if he should say them backwards, you can say "yes". And score according to his next answer. If he./she say the numbers forward, then backwards simultaneously and correctly, give him/her credit.

5.9 Questions 8: FIRST RECALL OF THREE WORD.

WHAT THREE WORDS DID I ASK YOU TO REMEMBER EARLIER.

A. Shoes:	Spontaneous recall (3) After "one word was something to wear" (2) After "was it shoes, shirt or socks" (1) Still incorrect and does not know (0)
B. Blue	Spontaneous recall (3) After "one word was a color" (2) After "was it blue, black or brown" (1) Still incorrect and does not know (0)
C. Charity	Spontaneous recall (3) After "one word was a good personal quality" (2) After "was it honesty, charity or modesty" (1) Still incorrect and does not know (0)

The order in which the 3 words are remembers is NOT important.

For each word not spontaneously recalled, provide the **category** cue followed by the multiple choice cue, if necessary. Wait up to 3 seconds for spontaneous recall and category cued recall before providing the next level of cuing. When you give the participant 3 choices to choose from, allow up to 5 SECONDS for the response.

For category cuing, please be careful not to provide non-verbal cues (e.g. pointing to your shirt).

When you prompt "one word was a good personal quality", if the person does not understand what that means, you can explain "it refers to something good about a person".

If the participant starts out giving an incorrect answer in the correct category (e.g. reports "shoes" or "coat" when the correct answer is "shoes"), proceed to provide the three multiple choices and score 1 if the choice is correct. IN THIS SITUATION YOU SKIP CATEGORY CUING.

If the participant cannot get the correct answer from the multiple choices, score 0 and TELL HIM/HER THE CORRECT ANSWSER for the benefit of the second recall to be requested later.

If he/she has not reported all three words correctly without help (less than spontaneous recall), SAY ALL THREE WORDS CORRECTLY once more before proceeding to the next item.

Examples:

5.10 Questions 9: SERIAL SUBTRACTION OF 3. This item measures the ability to successfully perform a series of mental operations.

NEXT I'M GOING TO ASK YOU TO DO SOME MENTAL SUBTRACTION

A. FROM 100, TAKE AWAY 3 EQUALS HOW MANY?	Answer = 97
B. AND TAKE AWAY 3 FROM THAT EQUALS ?	Answer=94
C. TAKE AWAY 3 FROM THAT EQUALS?	Answer = 91
D. TAKE AWAY 3 FROM THAT EQUALS?	Answer=88
E. TAKE AWAY 3 FROM THAT EQUALS	Answer=85

The participant is supposed to KEEP MENTAL TRACK OF THE PREVIOUS ANSWER and to perform the next MENTAL SUBTRACTION from that. If the participant forgets the previous answer, and asks the examiner to provide the answer from the previous step, please comply but score the current step "0" even if the subtraction is correct. If the participant forgets the previous answer more than once, the same scoring rule applies each time.

After the first step, if the participant confirms "subtract 3 from 97?", you can say "yes". If he/she tries to write the answers, say "please try to do this in your head, don't write".

For the FIRST error in subtraction (this refers to the first error, not necessarily the first step): If the participant does not know the correct answer or gives an incorrect answer, SCORE 0 BUT SUPPLY THE CORRECT ANSWER. Here the first does NOT refer to the first step, rather it refers to the first time a mistake is made in subtraction. If the participant makes an error in subtraction, that step is scored 0; give him a point for the second step if he subtracts 3 correctly from that initial error.

DISCONTINUE after two consecutive 0's for any reason and score the remaining steps as 0.

SCORING: For each step, score 0 for an incorrect subtraction from the previous (corrected) answer; also score 0 if the participant requests the examiner to repeat the answer from the previous step even if the subtraction for the current step is correct.

Example.

5.11 Questions 10-12: TEMPORAL ORIENTATION.

WHAT IS TODAY'S DATE?

YEAR:	Accurate (4) Missed by 1 year (2) Missed by 2-5 years (1) Missed by 6 or more years (0)
MONTH:	Accurate or within 5 days (2) Missed by 1 month (1) Missed by 2 or more months (0)
DAY	Accurate (3) Missed by 1 or 2 days (2) Missed by 3-5 days (1) Missed by 6 days or more (0)

Today's date will be shown on the computer screen. After the opening question and the participant's response to it, PROBE FOR ITEMS NOT REPORTED .

IF HE/SHE ATTEMPTS TO LOOK AT HIS WATCH, PHONE OR A CALENDAR, tell him "Please try without looking".

Scoring: Answers are scored in a graded manner according to closeness of the response to the correct answer. Follow the criteria listed above. The year is scored completely separately and the month/date are scored together.

WHAT DAY OF THE WEEK IS TODAY?	Correct (1) Incorrect (0)

For day of the week, don't provide choices.

WHAT SEASON OF THE YEAR IS IT ?	Accurate within 1 month (1) Missed by > 1 month (0)

For season, do not accept "Christmas season" or similar responses, but it is permissible to provide the names of the four seasons for the subject to choose from.

Scoring: Give 1 point if the answer is correct or if the change of the season occurred (will occur)within 1MONTH and the participant reports the last (next) season.

See CHART to assist in correct response.

5.12 Questions 13: SPATIAL ORIENTATION.

WHAT STATE ARE WE IN? (2)

WHAT CITY/TOWN/VILLAGE ARE WE IN (2)

Score 2 points for the correct answer.

IS THIS PLACE A CLINIC, STORE OR HOME? (1)

You may replace clinic with hospital if that is the location of the exam. Use either, not both. If testing occurs at another location, i.e. senior citizen's service center, adult daycare center, church, etc, substitute it for the middle alternative (store).

IF YOU ARE PERFORMING THE CASI IN A NURSING HOME OR CAREHOME, SUBSTITUTE IT FOR THE THIRD OPTION (HOME).

5.13 Questions 14: GENERATING ANIMAL NAMES.

WHAT ANIMALS HAVE FOUR LEGS? TELLME AS MANY AS YOU CAN.

Start timing at the end of the request. If the participant says "All animals have 4 legs" say "tell me their names" but do not re-start the timing.

Discontinue after 30 seconds. Do not tell the participant about the time limit.

Write all names provided by participant into the space provided on the form. SCORE 1 POINT FOR EACH CORRECT ANIMAL NAME (excluding duplicates).

IF THE PARTICPANT GIVES NO RESPONSE IN 10 SECONDS AND THERE ARE STILL AT LEAST 10 SECONDS OF REMAINING TIME, gently remind him ONLY ONCE "What (other) animals have 4 legs?"

After the first incorrect answer, remind the participant "I want you to name four-legged animals".

Do not provide hints like "can you think of animals in the zoo or on a farm". Also, don't say "you should name 10 animals" or keep prompting "one more" until they get all points. IT'S IMPORTANT THAT WE BE CONSISTENT IN HOW THIS IS ASKED AND IN PROMPTING.

Scoring: Score one point for each correct animal name. Different names for the same animal of different ages or sexes receive no credit (e.g. kitten and cat, puppy and dog, deer and doe). If a participant says "bear", "brown bear" and "polar bear", give only 1 point. Accept marginal answers such as monkeys, chimps, baboons, etc. Donkey and mule are different and can be awarded 1 point each. Imaginary animals such as "unicorn" can be accepted as long as they have 4 legs. "Man" should receive 0 points.

IGNORE INCORRECT ANSWERS, count only the correct answers. IF THE SAME ANIMAL NAME IS REPEATED MORE THAN ONCE, IT SHOULD ONLY BE COUNTED ONCE.

5.14 Questions 15: SIMILARITIES

This item measures abstract reasoning ability.

THE NEXT SET OF QUESTIONS IS ABOUT HOW THINGS ARE ALIKE TO EACH OTHER. FOR EXAMPLE, AN 'ORANGE' AND A 'BANANA' - THE WAY THAT THEY ARE SIMILAR IS THAT THEY ARE BOTH FRUIT. SAY:

A. AN ARM AND A LEG ARE BOTH?	Body parts, limbs, extremities (2) Long, bend, muscles, bones, etc (1) Incorrect, tells difference (0)
B. LAUGHING AND CRYING ARE BOTH?	Expressions of feelings/emotions (2) Other correct answer (1) Incorrect/tells difference (0)
C. EATING AND SLEEPING ARE BOTH?	Necessary body functions (2) Other correct answer (1) Incorrect/tells difference (0)

A: For the first pair (arm and leg) only: If the participant's answer is anything less than 2 points, score them either 0 or 1 as appropriate COACH FOR A 2-POINT ANSWER, i.e. tell him/her "What I'm trying to get at is: an arm and a leg are both parts of the body, or limbs, or extremities". Even though you scored him/her 0 or 1, this coaching serves as another example before going on to the next pair. However, do NOT coach if he/she scores 0 or 1 on the remaining two pairs.

For B and C: NO COACHING. If the participant gives an incorrect similarity, or tells the difference, or says "they are different" or "I don't know" , just score 0 and proceed to the next part.

Scoring: ARM and LEG:	2 points =	Body parts Limbs Extremities Appendages "They are part of a human"
	1 point =	Long Bend Have muscles Have bones "They move" "You use them both" Skin color Pairs Have flesh Have nails
	0 points =	Don't know They're different Have fingers Other incorrect
LAUGHING and CRYING:	2 points =	Must mention "emotions" or "feelings" Sentiments

Sentimental

1 point = Expressions (alone)
Reactions
Human
Make sounds
Natural instinct
Behavior

0 points = Don't know
They're different
They're opposite
It's a habit
Sometimes you cry when you laugh
Sometimes you laugh when you cry
Things you do
Senses
Other incorrect answer

EATING and SLEEPING: 2 points = "Necessary" or "essential" bodily functions
Things that you have to do
For subsistence
For survival
Anything implying "necessary"
Basic needs
For living or for live cycle

1 point = "Bodily function" alone with out necessary
Refreshing
Relaxing
Nourishing
Things you do everyday
It's a habit
I enjoy both
They both feel good
Natural instinct
Natural or natural doings
What a person does
For your health

0 points = Don't know
They're different
They are opposite
Other incorrect answer

In general, be lenient in awarding 1 point. Participants are given credit as long as they can see beyond the differences and come with a reasonable similarity.

5.15 Questions 16: JUDGMENT.
This item assessed common knowledge. SAY:

A. WHAT ACTIONS WOULD YOU TAKE IF YOU SAW YOUR NEIGHBOR'S HOUSE CATCHING FIRE?

Score number of appropriate answers up to 2

One point for each CATEGORY of correct action:

- Call 911, inform the fire or police depts. or other appropriate authority
- Try to save or alert the residents
- Try to help put out the fire
- Safeguard own property/family
- Alert other neighbors
- Try to help

If the participant stops before earning 2 points, ask once only: WHAT ELSE MIGHT YOU DO? If the participant does not know, do not give examples.

B. WHAT ACTIONS WOULD YOU TAKE IF YOU LOST A BORROWED UMBRELLA?

Score 1 point for each category of actions

- Inform/apologize
- Replace/compensate

If the participant says "I'll look for it", ask: "What would you do if you cannot find it?" and score according to his/her next answer.

If the participant answers "I would forget about it, it's only an umbrella", score 0 points.

C. WHAT WOUD YOU DO IF YOU FOUND AN ENVELOPE THAT WAS SEALED, ADDRSSED AND HAD A NEW STAMP?

Score according to the answer provided:

2 points = Mail it
1 points = Try to locate the owner
0 points = Inappropriate action

If the participant asks "Where did I find the envelope?" you can say "on the street", then score according to his next answer.

If the participant says "I would not do anything because it's none of my business" or "I would not touch it because it's a federal offense to tamper with the US mail" or "I would look inside of the envelope", score 0 points. These answers may not mean that a person is demented, but they are not the answers usually given and may indicate impaired judgment form other causes. We should try to be consistent here.

If the participant answers "I would take it to the police station", please score 1.

"Give it to the mailman" = 2 points

"Take it to the Post Office" = 2 points

5.16 Questions 17: REPEATING SENTENCES.
This item assesses attention and the ability to follow a command. SAY:

REPEAT EXACTLY WHAT I SAY**:**

A. HE WOULD LIKE TO GO HOME, NOW REPEAT…

Say the sentence in a normal pace (about 2 seconds). Score 2 points if correct, 1 point if 1-2 words are missed/wrong, and 0 if 3 or more missed/wrong words are cited. READ THE FOLLOWING:

B. THIS YELLOW CIRCLE (1)
IS HEAVIER (1)
THAN BLUE SQUARE (1)

Say the sentence in about 3 seconds. IT SHOULD BE SAID AS A CONTINUOUS SENTENCE, not broken up into three parts. This is intended to be an anomalous sentence without the article "the" before "blue square".

Do not say "I will not repeat this statement again". Do not stop in the middle of a sentence even when the participant asks "What?" Finish the sentence and say "Repeat what you think I said".

For both A and B, take off a point for extra words.

Examples.

5.17 Questions 18: READ AND OBEY "RAISE YOUR HAND". This item tests the ability to follow a simple written command. The command is printed in all capital letters approximately 2.5 cm in height within the top third of a sheet of paper. Point to the command and say "PLEASE DO THIS". Keep the paper close to the surface of the desk.

Score:	3 points =	Raises hand without prompting
	2 points =	Raises hand after prompting
	1 point =	Reads correctly but does not raise hand
	0 points =	Can neither read nor obey

If the participant does not respond, repeat the instruction once, and if he does it then you can still give the 3 points. If the participant merely reads the command aloud, say "PLEASE DO WHAT IT SAYS". Do NOT say "Please raise your hand". If he/she does it at this point, give 2 points. If he/she still doesn't do it, but only reads it aloud, give 1 point. READING ALOUD IS NOT REQUIRED.

ALLOW UP TO 5 SECONDS for a response at each stage.

If the participant askes "which hand?", reply "either one".

As soon as he/she raises his/her hand, say "THANK YOU, YOU CAN PUT IT DOWN NOW".

5.18 Questions 19: WRITING "HE WOULD LIKE TO GO HOME". This item tests the ability to correctly spell and write five simple and common words. Tell the participant:

I WOULD LIKE TO HAVE A SAMPLE OF YOUR HANDWRITING. WRITE
"HE… WOULD… LIKE… TO …GO… HOME"

Allow either cursive or printing. Say each word of the sentence slowly and distinctly. If the participant seems to have trouble remembering the sentence, dictate word by word as the participant writes. It is ok to repeat individual words.

Allow up to 1 minute for response. If at the end of one minute the participant is still working on the task in earnest, allow him/her to finish for the sake of maintaining rapport and morale, but note how many words are finished . Do not credit parts finished after 1minute. DO NOT TELL the participant there is a time limit on this or any other item.

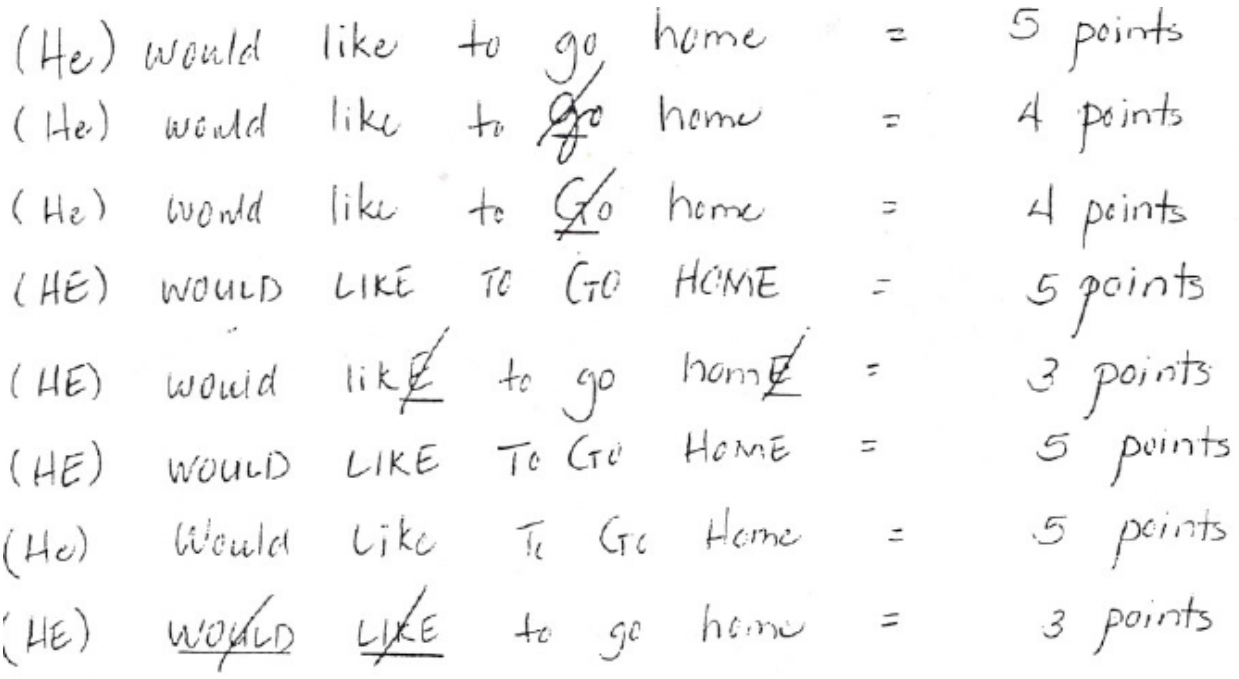
Scoring: 1 point for each word except do not score the first word (He) of the sentence. Score each word according to whether or not it can be readily identified without the context. For each word, score 0 if there is a spelling error or mixed capitalization. Do not give credit if the "i" does not have the dot. Do not penalize if the participant prints all letters in the uppercase. Do not take points off for extra words in the sentence.

Depending on a person's education or habit, capital letters are often written differently. For example, the capital letter "G" can be written the following ways.

Therefore, in order to decide whether or not a letter is capitalized or not, please consider the size of the letter relative to the size of the other letters in the word.

For the word "like", the dot does on the "i" does not necessarily have to be exactly above the "i". Give 1 point if it is a bit off-centered.

EXAMPLES:



NOTE ON THE FORM THE HAND WHICH THE PARTICIANT USES TO WRITE THIS SENTENCE.

5.19 Questions 20: COPYING PENTAGONS. Show the sample pentagons and say:

PLEASE COPY THIS

For right-handed persons, present the sample on their left side. For left-handed persons, present the sample on their right side. This way the sample will not be blocked by the drawing hand. You can observe which hand the person writes with at Question 19 (writing the sentence).

Only say "Please copy this" and do not say "make it the same shape" or "please copy these pentagons" since that provides an extra cue.

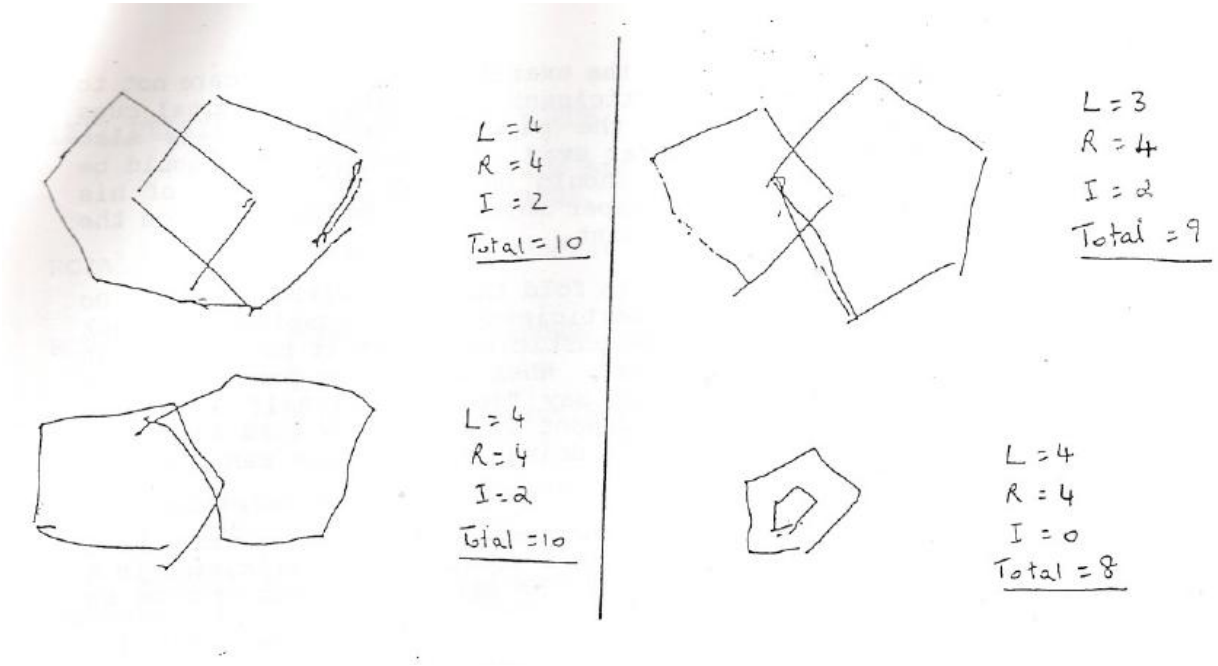
Allow up to ONE MINUTE for response, then move on to the next item. DO NOT tell the participant there is a time limit on this or any other item. If at the end of one minute the participant is still working on the task in earnest, consider allowing him/her to finish for the sake of maintaining rapport and morale. Note how much has been completed after 1 minute. For scoring, do not credit parts finished after one minute.

Sometimes the participant is not satisfied with the product and wants to try again. This is permitted but do NOT re-start the timing. At the end of one minute, score for the BETTER product. DO NOT PROMPT HIM/HER TO TRY AGAIN, BUT LET HIM/HER DO SO IF DESIRED.

Scoring: Scoring will be done for the left and right pentagons and for the intersection. The pentagons will have a maximum of 4 points and the intersection 2.

Pentagons: 3 points……if it is five-sided but the longest side is longer than twice the length of the shortest side. Do not penalize for non-straight or crooked lines, or minor gaps or over-shoots at the corners, that seem to be caused by poor motor control. Also, DO NOT PENALIZE FOR EXTRA LINES DRAWN.

EXAMPLES:



5.20 Questions 21: FOLLOWING THREE-STAGE COMMAND.
This item tests the participant's ability in understanding, remembering, and executing a three-part oral command. Please hold a piece of paper in front of your chest (not near the participant) and say:

TAKE THIS PAPER WITH YOUR (Left) (Right) HAND (1)
FOLD IT IN HALF, AND (1)
HAND IT BACK TO ME (1)

The three parts of the command are spoken clearly and without interruption in approximately 6 seconds. THIS ITEM SHOULD BE SAID AS A CONTINUOUS SENTENCE and not broken up into 3 parts. If the participant interrupts with "What did you say?" or similar, continue to finish the command, then say "Do what you think I asked you to do".

Use a blank piece of rectangular-shaped paper (about half the size of a standard 8.5" x 11" sheet) for this test.

The first stage of the command asks the participant to take the piece of paper with his NON-PREFERRED HAND (the hand not used in the preceding wring and drawing tasks).

The examiner holds the piece of paper in hand, in plain view of the participant, while giving the command. Some participants tend to reach for the paper right after hearing the first part. When this

happens, the examiner should temporarily move his hand away from the participant to be out of reach while continuing to state the next two parts of the command without interruption.

After saying the command, the examiner should take care not to move the paper toward the participant to provide non-verbal cues for the participant to take the paper. However, it is also important NOT TO HOLD IT TOO FAR AWAY. The participant should be able to reach it easily and should not have to get out of his chair to reach it. Also, the paper should not be laid down on the table but given to the participant.

Some participants attempt to fold the paper with one hand. Do not disrupt them if you think the participant can accomplish the task despite clumsiness. If the participant asks if both hands can be used for folding the paper, answer "yes". When asking the participant to fold the paper in half, do NOT say "fold it in half with both hands". However, if the participant is trying to fold the paperwith one hand and is having trouble doing it, say "you can use both hands for this".

After the participant has taken and folded the piece of paper, the examiner should NOT move his hand toward the participant in a gesture to receive the paper UNTIL the participant has started to hand the paper back.

DO NOT REPEAT ANY PART OF THE COMMAND. If the participant requests the examiner to do so and it is desirable to oblige for the same of maintaining rapport, score according to the responses executed before the repeat presentation of t he command.

IN THE MIDDLE OF THE TASK, if the participants asks "should I give this to you?", say "Do what you think I said before" and score according to his/her response.

Scoring: 1 point for each part of the command. The use of the non-preferred hand is required only for the first part.

A. No credit if the participant uses the preferred hand
B. No credit if the participant folds the paper more than once. No credit if he/she tears the paper in half.
C. No credit if the participant simply puts the paper down instead of handing it back to the examiner. No credit if he/she shoves it toward or throws it toward the examiner. He/she MUST HAND it back to get credit for the third part of the command.

SPECIAL CIRCUMSTANCES: e.g. paralysis of one hand. Usually you should ask the participant to take the piece of paper with his non-preferred hand (hand that is NOT used for writing and drawing). However, there may be occasional exceptions to this rule. For example, if the person has paralysis of his/her right arm and used his left hand to draw/write previously, he obviously can't take the paper with his/her paralyzed hand, so you can t ell him/her to take the paper with his left hand, even though this is the preferred hand.

5.23 Questions 22: DELAYED RECALL OF THREE WORDS.

WHAT THREE WORDS DID I ASK YOU TO REMEMBER EARLIER?

A. Shoes: Spontaneous recall (3)

	After "one word was something to wear" (2) After "was it shoes, shirt or socks" (1) Still incorrect and does not know (0)
B. Blue	Spontaneous recall (3) After "one word was a color" (2) After "was it blue, black or brown" (1) Still incorrect and does not know (0)
C. Charity	Spontaneous recall (3) After "one word was a good personal quality" (2) After "was it honesty, charity or modesty" (1) Still incorrect and does not know (0)

Administer this item even if the participant scored 0 on the First Recall (Question 8). Scoring is the same.

Follow the testing procedure for Question 8, First Recall. However, do NOT REPEAT the correct answer no matter what the participant scores.

The order in which the three words are remembered is not important. When you prompt "One word was a good personal quality", if the person does not understand what that means, you can explain "it referees to something good about a person".

5.24 Questions 23: NAMING BODY PARTS.
This section tests whether or not the participant can PROMPTLY name five parts of the body. Please say:

WHAT DO WE CALL THIS PART OF THE (FACE) (BODY)?

The examiner asks the above while pointing directly to the appropriate part on his/her own body: "What do you call this part of the fact (pointing to the middle of the forehead)…and what do you call this part of the body (pointing to the chin)… and this part of the body (pointing to shoulder)…and this part (pointing to the elbow)….and this part (pointing to the wrist)."

Allow 2 seconds for each body part. Score 1 point for each correctly identified:

A. Forehead (1) (accept brow)
B. Chin (1) (accept jaw)
C. Shoulder (1)
D. Elbow (1)
E. Wrist (1)

If the participant cannot name a part within 2 seconds or if the answer is incorrect, do not help or question again; just score 0 and move on to the next part.

For forehead, accept brow. Do NOT accept head, eyebrow, or temple.
For chin, accept jaw.

If the participant gives you a wrong answer, but manages to correct himself right away, give him the points. If he says "arm" instead of "shoulder", you can clarify by saying saying "what do you call the part that moves?" If he still says "arm", score 0.

5.25 Questions 24: NAMING OBJECTS .

This item assesses if the person can PROMPTLY name five common objects. Please say:

WHAT IS THIS?

Spoon (1)
Coin (1) accept coin, quarter, 25 cents, 2 bits
Toothbrush (1)
Key (1)
Comb (1)

Present one item at a time and ask the participant to name it. Lay the objects directly in front of the participant where they are easily visible. If the participant cannot name an object in 2 seconds, put it in the participant's hand and ask "What do you call this?" If he/she still cannot name it, wait 4 seconds, say (for key) "It is a key…say key". Asking the participant to repeat is to check if they register the input and can say the word.

Avoid any items with outstanding colors. Do not use keys or coins that are small (in the US use a quarter).

The exact order of presenting the objects in not important.

If the participant describes the object, ask "What do you call it?" or "What is its name?"

For coin, accept "coin" or the name of the specific denomination of the coin (quarter or 25 cents or 2 bits). Do not accept "money". If the participant says "money", ask "what is the specific name for this kind of money?", and score according to his/her response.

For toothbrush, do not accept "brush" as correct. If the participant says "brush", ask "what is the specific name for this kind of brush?" and score according to his/her response. By asking for the specific name of the object, we can differentiate between the participant not being able to name things correctly and just being global by using a general name rather than a specific one.

If the participant gives an incorrect name, tell the correct name and ask the participant to repeat it.

After naming each item, put it back down in front of the participant on a clean, uncluttered surface.

Scoring: 1 point for each correctly named object without coaching by the examiner. If the participant recognizes the item (describers it s use) but cannot name it, score 0.

If the participant gives you a wrong answer but manages to correct him/herself right away, give him/her the points. Please make sure that he can see the objects well. If his/her vision is very poor, hand him the objects, and if he/she can name them correctly, give the points.

5.26 Questions 25: IMMEDIATE RECALL OF FIVE OBJECTS .

After all five objects from the previous naming have been replaced back in front of the participant, say:

PLEASE REMEMBER THESE FIVE OBJECTS

WAIT 5 SECONDS and then cover all five objects with a blank piece of cardboard (something opaque and stiff that will hide all visual cues from the objects) and ask:

WHAT FIVE OBJECTS DID I JUST SHOW YOU?

Do not give the participant longer than 5 seconds even if he/she asks to see the objects again.

Terminate testing when t he participant has reported five objects (including incorrect names) or when the participant cannot recall any additional item in 5 seconds.

Score 1 point for each correctly name object recalled:

Spoon (1)
Coin (1) accept coin, quarter, 25 cents, 2 bits
Toothbrush (1)
Key (1)
Comb (1)

While before, "money" and "brush" were not acceptable answers – HERE THEY ARE ACCEPTABLE. Please provide a point for each if named as such.

5.27 CLOSING . Thank participant for completing the exam

THOSE ARE ALL THE QUESTIONS WE HAVE ON THIS EXAMINATION. THANK YOU FOR COMPLETING THEM FOR US.

5.28 Recording Time test Ended and Validity.

5.28.1 Finish time: Remember to fill in the time that the exam was completed in military time.

5.28.2. Validity: At the end of the test, enter whether or not the CASI was a valid assessment of the participant's cognitive abilities. The CASI should be scored as invalid only if the impairment interferes with the total score. If the participant is hard of hearing but his hearing impairment does not interfere with the score, you should code "1 = valid". The total score does not have any relation to validity, e.g. a low score due to dementia does not mean that the test is invalid, and a high score does not necessarily mean that the test is valid. **If not valid,** pick the reason that the test was invalid. Only one reason should be coded. If there are 2 or more reasons that the CASI score is not valid, pick the most important reason. You can write in the other reasons, but do not circle more than 1 number as only 1 entry can be put into the computer**. If you are unable to perform the CASI at all, because of severe dementia or agitation, code "3", because of sever deafness, code "4", because of non-verbal conditions either due to coma or aphasia, code "5", because of any other reason code "6" and specify.**

5.28.3 Validity and CASI Status Questions must always be completed unless the participant refused the CASI completely**.**

3.6.2 DIGIT SYMBOL - CODING

1. **INTRODUCTION.** This test is part of the WAIS-III battery and was formerly called the Digit Symbol Substitution Test. It assesses speed of mental processing, learning and working memory.

2. **Materials**
   Answer sheet
   Stopwatch
   Two No. 2 graphite pencils without erasers
   Digit Symbol Scoring Template

3. **Description**

   For Digit Symbol – Coding[2], the examinee copies symbols that are paired with numbers. Using a key at the top of the form, the examinee copies each symbol in a box under its corresponding number. The examinee's score is determined by the number of symbols correctly drawn within the 120 seconds,

   DSC begins with an explanation of the task and completion of sample items. Timing begins with the participant's attempt at coding after learning the task. **Discontinue after 120 seconds.**

4. **General Directions**

   4.1 A smooth drawing surface must be provided. If the table has a rough surface, the Record Form should be placed on a clipboard, a piece of cardboard, or another flat surface.

   4.2 To introduce the test, say:

   In this section, I'm going to ask you to copy some symbols.

   4.3 If the examinee asks what she/he should do if they make a mistake, encourage them to continue to work as fast as they can. However, do not discourage examinees from making spontaneous corrections unless they do so repeatedly and it impedes their performance.

   4.3 If, after completing a row, an examinee tries to complete the next row in reverse order, remind the examinee to start at the beginning of the row and not to skip any.

5. **Item Instructions**

   Turn to the Digit Symbol – Coding page in the Record Form. Fold the Paper Form so only Digit Symbol – Coding is showing, and place it in front of the examinee. Hand him or her a pencil without an eraser, point to the key above the test items, and say:

   > **Look at these boxes. Notice that each has a number in the upper part and a special mark in the lower part. Each number has its own mark.**

   Point to 1 and its mark in the key, then 2 and its mark. Then point to the seven squares located to the left of the heavy black line and say:

Now look down here where the squares have numbers in the top part but the squares at the bottom are empty. In each of the empty squares, but the mark that should go there. Like this.

Point to the first Sample Item, then point back to the key to show its corresponding mark, and say:

Here is a 2; the 2 has this mark. So I put it in this empty square, like this.

Write in the symbol. Point to the second Sample Item and say:

Here is a 1; the 1 has this mark (point to the second Sample Item, then to the mark below the 1 in the key)**, so I put it in this square.**

Write in the symbol.

Point to the third Sample Item and say:

This number is a 3; the 3 has this mark (point to the third square and to the mark below the 3 in the key)**, so I put it in the square** (write in the symbol)**.**

After marking the first three Sample Items, say:

Now you fill in the squares up to this heavy line.

If the examinee makes an error on any of the Sample Items, correct the error immediately and review the use of the key. Continue to provide help if needed. Do not proceed with the subtest until the examinee clearly understands the task.

When the examinee completes a Sample Item correctly, offer encouragement by saying **Yes** or **Right**. When all the Sample Items have been completed, say:

Now you know how to do them. When I tell you to start, you do the rest of them.

Point to the first square to the right of the heavy line and say:

Begin here and fill in as many squares as you can, one after the other without skipping any. Keep working until I tell you to stop. Work as quickly as you can without making any mistakes.

Sweep across the first row with your finger and say:

When you finish this line, go on to this one.

Point to the first square in the second row. Then point to the heavy black line and say:

Go ahead.

Begin timing.

If the examinee omits an item or starts to do only on type (e.g., only the 1's), say:

Do them in order. Don't skip any.

Point to the first item omitted and say:

Do this one next.

Provide no further assistance except to remind the examinee to continue until instructed to stop.

At the end of 120 seconds, say **Stop.**

6. Scoring

Record 1 point for each correctly drawn symbol completed within the 120-second time limit. ***Responses to the seven Sample Items are not included in the examinee's score.*** Do not give credit for items completed out of sequence.

Use the Digit Symbol Template to check the examinee's responses and record the score on the Record Form.

A response is scored as correct if it is clearly identifiable as the keyed symbol, even if it is drawn imperfectly or if it is a spontaneous correction or an incorrect symbol.

Maximum Score: 133 points

IV. DIGIT SPAN TEST

1. **General Directions.** This test, also a part of the WAIS III, is a working memory test involving repeating spans of numbers forward and then other spans backward. Please use the scripts, coaching and scoring as follows:

2. **Digits Forward**

   2.1 After saying the instructions, administer the Digit Spans in order

   2.2 Do not repeat a span once read.

   2.3 Administer both spans of the same length regardless of how the participant performs.

   2.4 Say the digits at a rate of 1 digit about every 1 second.

   2.5 Use a monotonic voice without inflections at the end.

   2.6 Do not 'chunk' spans (e.g., 34-729) when you read them

   2.7 Discontinue after failure on BOTH trials of any item (e.g. 5a and 5b).

   2.7. **SCRIPT**

   I am going to say some numbers. Listen carefully, and when I am through say them right after me. For example, if I say 7-1-9, what would you say?

   - If the participant responds correctly, 7-1-9, say "**That's right**" and proceed to Item 1.
   - If the participant fails the example, say "**No, you would say 7-1-9. I said 7-1-9 so to say it forward you would say 7-1-9. Now try these numbers. Remember, you are to say them forward. 3-4-8.**
   - Whether the participant succeeds or fails with the second example (3-4-8), proceed to Item 1. Give no help on this second example or any of the items that follow.

   2.8 **Scoring**. Each span is scored 0 or 1. Give 1 point if the participant passes the trial and no points if the participant fails the trial. ONLY DISCONTINUE THE TEST WHEN PARTICIPANT HAS FAILED BOTH TRIALS OF THE SAME SPAN LENGTH (e.g. 5a and 5b).

3. **Digits Backward**

   3.1 Administer the digit spans in order.

   3.2 Do not repeat a span once read.

   3.3 Administer both spans of the same length regardless of how the participant performs.

   3.4 Say the digits at a rate of 1 digit about every 1 second.

3.5 Do not ‘chunk’ spans (e.g., 34-729) when you read them

3.6 Use a monotonic voice without inflections at the end.

3.7 **SCRIPT**

Now I am going to say some numbers, but this time when I stop I want you to say them backwards. For example, if I say 7-1-9, what would you say?

- If the participant responds correctly, 9-1-7, say “**That’s right**” and proceed to Item 1.
- If the participant fails the example, say “**No, you would say 9-1-7 I said 7-1-9 so to say it backwards you would say 9-1-7. Now try these numbers. Remember, you are to say them backwards. 3-4-8.**
- Whether the participant succeeds or fails with the second example (3-4-8), proceed to Item 1. Give no help on this second example or any of the items that follow.
- Discontinue after failure on BOTH trials of any item.

3.8 **Scoring**. Same as Digit Forward. Each span is scored 0 or 1. Give 1 point if the participant passes the trial and no points if the participant fails the trial. ONLY DISCONTINUE THE TEST WHEN PARTICIPANT HAS FAILED BOTH TRIALS OF THE SAME SPAN LENGTH (e.g. 5a and 5b).

MESA Exam 5
Manual of Operations

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MESA Exam 5 Table of Contents

Mesa Exam 5 Specimen Collection and Processing
MESA Manual of Operations: EXAM 5

Collection, Processing, and Shipping of MESA Classic Blood Samples

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 (maximum of 90 mL from participants at Exam 5) that are 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 samples are not correctly drawn and processed, the laboratory results may not be precise or valid. Consistency in this step across the multiple clinical centers is vital to the study.

In addition to the sample collection on the MESA Classic cohort, there are two ancillary studies (MESA Epigenomics and MESA COPD) and a cohort subset selected for MRI-GAD, that will involve the collection of additional blood samples specific to their research aims. Further details and protocols specific for MESA Epigenomics are explained in the chapter, Lab Protocol for MESA Epigenomics Study at Exam 5" in this Manual of Operations.

II. EQUIPMENT & SUPPLIES

For the MESA Classic collection CBAL will provide the following supplies in bulk:

Styrofoam Shipping Containers
5 mL SCAT-I tubes (1 per participant) Must be stored 4°C until used.
15 mL centrifuge / pooling tubes
Cryovials / tubes – 0.5 mL, 2.0 mL, & 10.0 mL with colored caps for coding.
HbA_{1c} Sample Preparation Kit (BioRad), Cat. No. 270-2167 includes supplies sufficient for 100 test samples:

1. Sample Preparation Vials, 100 per kit, each vial contains 1 mL of an aqueous solution of EDTA and potassium cyanide (0.25 mmol/L). Store at 5-30^{o}C.
2. Capillaries, one glass dispenser vial containing 100 sodium-heparinized capillary tubes (5µL). Reorder capillary tubes, 10 x 10 tubes/vial, Cat. No. 196-12052. BioRad

3. Capillary tube holder, one holder for manipulating 5 µL capillary tubes. Reorder box/20 holders, Cat. No. 196-1054.

For the MESA Classic urine collection and the MESA Epigenomics ancillary study see study specific chapters in the MESA Manual of Operations.

1. MESA Classic Urine Collection - MESA Manual of Operations chapter, "Collection, Processing, and Shipping of MESA Urine Samples".

2. MESA Epigenomics Study - MESA Manual of Operations chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5".

Blood Collection Supplies - 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 (BD# 367281)
Vacutainer barrels
Tourniquets
Alcohol prep pads
Gauze (2x2)
Surgical tape - paper tape (easier on participants)
Band-Aids
Blood collection tubes:

MESA Classic (keep extras on hand):

2 – 10 mL Serum tubes (BD# 366430)
2 – 10 mL EDTA tubes (Monoject# 8881311743)
1 – 4.5 mL Citrate tubes (BD# 366415)
1 – 5 mL SCAT-I (Haem-Tech# 222031) (*provided by CBAL*)
1 – 2 mL EDTA tube (BD# 367841)

MESA MRI-GAD subset (local creatinine testing - site specific)

1 – 3.5 mL Serum tube (BD# 367983) MRI-GAD sample collection will vary per site but 3.5 mL is the max volume expected.

MESA COPD blood collection tubes:

1 – 10 mL Heparin tube (BD# 366480)
1 – 2 mL EDTA (BD# 367841). (For COPD overlap with MESA Epigenomics, only one tube for CBC/Diff will be collected.)

MESA Epigenomics blood collection tubes (refer to MESA Manual of Operations chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5"):

4 – 8 mL CPT (BD# 362753)
1 – 2 mL EDTA (BD# 367841) (*provided by Lab Corp*)

Draw tube rocker
Draw tube racks
Ice bucket and crushed ice - filled 10 min before draw
Stopwatches or timers
Scissors
Pens and Sharpie pens
Participant barcode labels
MESA Phlebotomy and Processing Forms
Blood Spill Kit
Biohazardous waste container
Needle/sharps container

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 who handle blood. Documentation of vaccination, or technician's refusal 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 participant ID number barcode labels to use for labeling draw tubes, pooling tubes, cryovials, and freezer boxes. There will be a total of 71 labels - for MESA Classic collection including labels needed for

urine collection and processing, and draw tubes for MESA MRI-GAD and MESA COPD. MESA Classic QC labels for the blind duplicate aliquots will have a QC ID number barcoded on them and are separate from the labels listed below. Details regarding the labels needed for the MESA Epigenomics are explained in the chapter, Lab Protocol for MESA Epigenomics Study at Exam 5".

Mesa Phlebotomy & Processing Forms (2)
MESA Classic Draw tube labels (7)
MESA Classic Cryovial labels (52)
MESA Classic EDTA Pooling tube (1)
MESA Classic Serum Pooling tube (1)
MESA Classic Urine Collection container (1)
MESA Classic Shipping Forms (1)
MESA Classic Freezer boxes (1)
MESA GAD Draw tube (1)
MESA COPD Draw tubes (2)
MESA COPD Shipping Form (1)
Extra labels in case needed for replacement draw tubes, etc. (3).

MESA Epigenomics Study (35)

Each set of participant barcode labels has the same 7-digit sample identification number. (The first digit identifies the clinic. Wake Forest is 3, Columbia 4, John's Hopkins 5, University of Minnesota 6, Northwestern 7, UCLA 8). The exam year, "5", is represented as the 8th digit in the 10 digit number on the cryovial labels. The last two digits on the cryovial label represents the number assigned to that cryovial. This 2-digit number (01 to 52) uniquely identifies each cryovial within a participant ID which is of key importance in tracking the repository. See Appendix for proper orientation of the barcode label on the cryovial and an example set of labels.

There will also be special QC ID labels for the blind duplicate samples. See the section 5.6, "Processing Blind Duplicates" for further information about this procedure.

Blood samples must 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, and cross-check the labels with each participant's ID number prior to the phlebotomy and again at time of processing.

3. Forms

The MESA Phlebotomy Form and Processing Form provide the vital link between the Participant ID number and the samples. (For details on the required forms for MESA Epigenomics, refer to chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5"). 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 5 Phlebotomy Form will be printed at the time the participant gives consent and will identify the draw tubes to be collected. The phlebotomist will then need the form to complete draw tube labeling and set-up for that participant's blood collection. The form is completed legibly in ink by the phlebotomist at the time of phlebotomy, and accompanies the filled draw tubes to the sample processing area.

The MESA Processing Forms are used to record the processing times and to account for all the MESA Classic aliquots (blood & urine).

Completed MESA Phlebotomy and Processing Forms, plus a Shipping Form identifying all the participant samples in a shipment, are sent with the sample shipments to CBAL. It is critical that all forms are labeled with the correct participant ID label and all appropriate sections completed legibly.

MESA COPD has a special Shipping Form that is completed and shipped with the filled Heparin draw tubes to the CBAL the same day the blood is collected.

4. Blood Collection

4.1 Preparation

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

Initial preparation for specimen collection occurs prior to the arrival of participants.

Make sure venipuncture supplies are stocked (see section "II. EQUIPMENT & SUPPLIES") for Mesa Classic as well as all Ancillary Studies
Make sure tubes and cryovials are labeled for MESA Classic. Have tubes, cryovials (where applicable), and labels for ancillary studies (and/or substudy) ready to use based on participant's eligibility and consent.

Make sure the phlebotomy room is tidy and stocked with all items needed, and that the draw tube mixer is working.

Make sure the sample processing station is equipped with all items needed (see "Processing Specimens", Section 5.2 "Daily Preparation").

Approximately 10 minutes before the scheduled specimen collection, fill ice bucket ¾ full with crushed ice.

Draw tubes and aliquot racks: Correct labeling and accurate tracking of collected specimens is vital, and correct draw tube order is important. Setting up pre-labeled draw tubes in a blood collection tube rack prior to the participant's arrival is recommended. Additional draw tubes for the ancillary studies (and substudy) will need to be included in the rack in the correct order as soon as eligible participants have given consent. Setting up aliquot racks with the corresponding pre-labeled cryovials prior to processing the samples is strongly recommended. It may be helpful to have a separate aliquot rack for the serum cryovials (red capped cryovials) as they are generally centrifuged at a different time from the other tubes.

Phlebotomy Room: The blood draw is done in an isolated room, or participants are separated by room dividers. The room is equipped with all of the necessary blood drawing supplies (see section "II. EQUIPMENT & SUPPLIES").

Pre-label draw tubes (and cryovials) needed for the MESA Classic collection (Draw Type I) prior to the participant's arrival. Have draw tubes and labels (and cryovials) for the ancillary studies nearby. Once consent on any of the ancillary studies is given by an eligible participant, the draw tubes pertaining to those ancillary studies are quickly labeled and added in to the blood drawing scheme in the correct draw tube order (based on Type II through VI - See table of draw types in section 4.2, Draw Tube Order).

Participants: 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 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.

Seven to thirteen tubes of blood - depending on draw type - of various sizes are collected, each containing about 0.5-2 teaspoons (3-10 mL) of blood. Participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn will not exceed 6 tablespoons, although it may look like more. The phlebotomist may also assure participants that about five times as much blood (450 mL or 30 tablespoons) is collected when they donate a unit of blood.

4.2 Draw Tubes

A maximum of 90 mL (includes ancillary studies) of blood will be collected from each participant into 7 to 13 draw tubes. There are no additional tubes collected specifically for quality control purposes. There are six possible variations on blood collection/draw tube order depending on eligibility and participant consent for the ancillary studies. The blood draw type (Type 1 through VI listed in the table

below) is determined by the consent process. The order in which the tubes are collected is extremely <u>important</u> and must be done as follows:

	Mesa EXAM 5		Comments
Type I			
Tube order	Mesa Classic	Vol (ml)	Sites: All
1	Serum	10	Classic
2	EDTA	10	Classic
3	Citrate	4.5	Classic
4	Serum	10	Classic
5	EDTA	10	Classic
6	Scat	5	Classic
7	EDTA	2	Classic (for Whole Blood)
8			
9			
10			
11			
12			
Total Max Volume		51.5	
Type II			
Tube order	Mesa Classic/MRI-GAD	Vol (ml)	Sites: All
1	**Serum Creatinine**	**2.5 to 5**	GAD: 3.5 mL MAX
2	Serum	10	Classic
3	EDTA	10	Classic
4	Citrate	4.5	Classic
5	Serum	10	Classic
6	EDTA	10	Classic
7	SCAT	5	Classic
8	EDTA	2	Classic (for Whole Blood)
9			
10			
11			
12			
Total Max Volume		54	

Type III			
Tube order	Mesa Classic/ Epigenomics	Vol (ml)	Sites: WFU, Columbia, JHU, Uminn
1	Serum	10	Classic
2	EDTA	10	Classic
3	Citrate	4.5	Classic
4	CPT	8	Epigenomics
5	CPT	8	Epigenomics
6	Serum	10	Classic
7	EDTA	10	Classic
8	CPT	8	Epigenomics
9	CPT	8	Epigenomics
10	EDTA	2	Epigenomics: CBC Diff (Lab Corp)
11	SCAT	5	Classic
12	EDTA	2	Classic (for Whole Blood)
Total Max Volume		85.5	

Type IV			
Tube order	Mesa Classic/ Epigenomics / MRI-GAD	Vol (ml)	Sites: Columbia, JHU, Uminn, WFU
1	**Serum Creatinine**	**2.5 to 5**	GAD: 3.5 mL MAX
2	Serum	10	Classic
3	EDTA	10	Classic
4	Citrate	4.5	Classic
5	CPT	8	Epigenomics
6	CPT	8	Epigenomics
7	Serum	10	Classic
8	EDTA	10	Classic
9	CPT	8	Epigenomics
10	CPT	8	Epigenomics
11	EDTA	2	Epigenomics: CBC Diff (Lab Corp)
12	SCAT	5	Classic
13	EDTA	2	Classic (for Whole Blood)
Total Max Volume		88	

Type V			
Tube order	Mesa Classic /COPD/MRI-GAD	Vol (ml)	Sites: JHU, Columbia, UCLA, NW
1	**Serum Creatinine**	**2.5 to 5**	GAD: 3.5 mL MAX
2	Serum	10	Classic
3	EDTA	10	Classic
4	Citrate	4.5	Classic
5	Heparin	10	COPD sent immediately to Vermont
6	EDTA	2	COPD for CBC/Diff
7	Serum	10	Classic
8	EDTA	10	Classic
9	SCAT	5	Classic
10	EDTA	2	Classic (for Whole Blood)
11			
Total Max Volume		66	

Type VI			
Tube order	Mesa Classic/ COPD /Epigenomics/MRI-GAD	Vol (ml)	Sites: JHU, Columbia
1	**Serum Creatinine**	**2.5 to 5**	GAD: 3.5 mL MAX
2	Serum	10	Classic
3	EDTA	10	Classic
4	Citrate	4.5	Classic
5	Heparin	10	COPD sent immediately to Vermont
6	EDTA	2	COPD for CBC/Diff
7	Serum	10	Classic
8	EDTA	10	Classic
9	CPT	8	Epigenomics
10	CPT	8	Epigenomics
11	CPT	8	Epigenomics
12	SCAT	5	Classic
13	EDTA	2	Classic (for Whole Blood) May not collect if > 90 mLs
Total Max Volume		90	

MESA Classic Draw Tubes:

- Serum 10 mL red-topped tubes (BD# 366430). After collection, these tubes remain upright at room temperature for a minimum of 40 minutes (maximum 90 minutes) to allow the blood to clot. Serum from the 10 mL centrifuged draw tubes is pooled then aliquoted into seventeen red-capped cryovials (#s 28–44). Participants selected for blind duplicate purposes will have 0.5 mL serum aliquoted into a labeled Blind Duplicate 0.5 mL cryovial after Cryovial # 28 and 29 are completed. Once the Blind Duplicate vial is made then continue with aliquoting the remaining serum vials starting with Cryovial #30.

- EDTA 10 mL purple-topped tubes (Monoject# 8881311743). After collection, these tubes are mixed for ~30 seconds on the tube rocker then placed on wet ice. Before centrifuging, a drop (5 µL) of whole blood is removed from one of these tubes to add to a fixing solution for Hemoglobin A1C analysis. After centrifuging, the plasma is pooled then aliquoted into seventeen purple-capped cryovials (#s 02-18). The packed red blood cells from both tubes are transferred

into one 10 ml tube (# 19). Participants selected for blind duplicate purposes will have 1.0 ml of EDTA plasma aliquoted into a labeled Blind Duplicate 2.0 mL cryovial after Cryovials 01 and 02 are completed. Once the Blind Duplicate vial is made then continue with aliquoting the remaining EDTA vials starting with Cryovial #03.

- Citrate 4.5 mL blue-topped tube (BD# 366415) containing 0.5mL of 3.2% sodium citrate. After collection, these tubes are mixed for ~30 seconds on the tube rocker then placed on wet ice till centrifuging. The plasma is aliquoted into four blue-capped cryovials (#24-27). No citrate blind duplicate samples will be assigned.

- "Special Coagulation" tube (SCAT-I Haem Tech# 222031), is a 5mL white-topped tube. These tubes, provided by CBAL, contain a combination of white powdered 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 µM D-Phe-Pro-Arg-chloroketone. Important to note this tube is 'non-sterile' therefore it must be collected using a butterfly apparatus with 12 inches of tubing; alternatively, a syringe may be used for the venipuncture, and the blood expressed through the cap (with great care to limit turbulence) into the SCAT tube. The SCAT-I tube must be drawn after at least one other tube has been drawn. <u>It is critical that the SCAT-I tube is mixed well (>30 seconds of gentle inversion) before being placed on ice to await further processing/centrifugation</u>. The plasma is aliquoted into four yellow-capped cryovials (# 20-23). No SCAT blind duplicate samples will be assigned.

<u>MESA Substudy Draw Tubes</u>:

- MESA MRI-GAD – Serum 3.5 mL tube (BD# 367983) is collected for local serum creatinine. Collection and processing of this tube will vary per site but 3.5 mL is the maximum volume expected. (NW will perform point of care Creatinine testing.)

<u>MESA Ancillary Study Draw Tubes</u>:

- MESA COPD – Heparin 10 mL tubes *(*BD# 366480) and EDTA 2 mL tubes (BD# 367841) are collected. After filling the Heparin tube is gently inverted 5-6 times then placed on an aliquot mixer for 3 minutes. This tube is kept at room temperature until preparation to ship. This tube will be shipped by the end of the day. The 2 mL EDTA tube will be used for local CBC/Diff testing by Lab Corp in most cases. There may be some different local arrangement made for the NW and UCLA sites.

- MESA Epigenomics – Generally four 8 mL Cell Prep Tubes (CPT BD# 362753) and one 2 mL EDTA (BD# 367841) are collected. The EDTA tube is collected for CBC/Diff and will be provided by Lab Corp. Processing requirements for the CPTs are fully explained in the MESA Manual of Operations chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5".

4.3 Procedure

There are four questions listed on the Phlebotomy Form 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.

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 non-sterile SCAT-I tubes by eliminating the possibility of blood back-washing from tube to participant. Step-by-step procedures are as follows:

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 (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.

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

5. Re-apply tourniquet and start timer. Document start time. (It is best to release the tourniquet as soon as possible after flow has been established. The tightened tourniquet should be on no longer than two minutes; if it is necessary to have it on longer than two minutes, loosen the tourniquet and then re-apply. 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.)

6. Grasp the participant's arm firmly, using your thumb to draw the skin taut to anchor the vein. The thumb should be one or two 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 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 shut-off valve and cessation of blood flow. Do not vary pressure or reintroduce pressure after completion of the draw

12. Fill each Vacutainer tube as completely as possible (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. Plasma tubes less than ½ full are not acceptable. Partially-filled serum tubes are okay but will result in a reduced number of aliquots. If a tube is not completely filled, clearly document on Phlebotomy Form. Partially filled Heparin tubes (COPD) are acceptable.

13. When the blood flow ceases, remove the tube from the vacutainer holder. The shut-off valve re-covers the point and stops blood flow until the next tube is inserted (if necessary).

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 formation of a hematoma. Discard needle into puncture-proof sharps container.

16. Record on Phlebotomy Form the duration the tourniquet was applied and the end of venipuncture time.

17. Place EDTA, Citrate, and SCAT-I tubes on the tube mixer for a minimum of 30 seconds then keep tubes on wet ice till centrifuging.

18. Serum tubes are kept at room temperature for a minimum of 40 minutes but no longer than 90 minutes.

19. CPTs - see MESA Manual of Operations chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5" for details regarding collection and processing of CPTs).

20. Heparin tubes are gently inverted 5-6 times then placed on an aliquot mixer for 3 minutes. Keep at <u>room temperature</u> till shipping.

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

22. Clean up the venipuncture area (if necessary). Dispose of needle and tubing in the appropriate biohazard needle sharps containers. Complete the Phlebotomy Form.

23. The filled MESA MRI-GAD serum tube for local creatinine is handled per site-specific instructions.

24. The 2 mL EDTA tube for CBC/Diff is also site-specific and handled according to Lab Corp or local protocol.

25. Take the filled blood collection tubes to the processing area, keeping the EDTA, citrate, and SCAT-I tubes on ice, and the serum and COPD Heparin at room temperature. Refer to the chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5" for details on collection and processing of CPTs.

4.4 <u>Blood Mixing and Handling</u>

<u>Serum</u>: do not mix; place in rack at room temperature for at least 40 minutes; no longer than 90 minutes. Serum creatinine on MESA MRI-GAD participants is performed locally; follow site specific protocol for this tube.

<u>EDTA</u>: place on mixer for ~30 seconds, then place in rack on wet ice. For the 2 mL EDTA collected for CBC/Diff required in MESA Epigenomics and

MESA COPD is site-specific. The CBC/Diff will be performed by Lab Corp; consult their protocol for handling this tube.

Citrate: place on mixer for ~30 seconds, then place in rack on wet ice.

SCAT: place on mixer for at least 30 seconds, then place in rack on wet ice.

CPT: Refer to MESA Manual of Operations chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5" for details.

Heparin: Gently invert 5-6 times then place on aliquot mixer for a minimum of 3 minutes. Keep at room temperature till shipping. Ship the tube the same day it is collected.

4.5 Venipuncture Difficulties

- Assisting participants who are extremely apprehensive about having blood drawn. Explain to the participant that the blood draw is designed to be as painless as possible. It may help 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', reassuringly say, "Oh, you have good veins; there should be no problem." Do not, under any circumstances force the participant to have blood drawn.

- Procedures for a difficult draw. If a blood sample is not forthcoming, the following manipulations may be helpful:

  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.

  If no blood appears, move needle slightly in hope of entering the vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.

  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.

  Do not attempt a venipuncture more than twice.

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

If venipuncture is unsuccessful, note on the Phlebotomy Form.

- Assisting participants who look or feel faint.

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

  Provide a basin if the participant feels nauseated.

  Place a cold wet cloth on the back of the neck.

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

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

- If a MESA Classic collection tube does not fill, try another tube of the same type. Plasma tubes < ½ full are not acceptable and should be discarded. Serum tubes less than ½ full are acceptable but will yield a reduced number of aliquots. If the tube is not completely filled, note this on the Processing Form, as this can affect future assays.

- If all tubes are not collected (blood flow ceases, difficult venipuncture, etc.), make a note of the difficulties on the Phlebotomy/Processing Form. Always fill collection tubes in the order specified. 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 attempt.

- Heparin tubes that are not completely filled – even if only a small draw - should still be shipped the same day.

- For acceptable fill volumes for the CPTs see the chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5".

5. Processing Specimens:

5.1 Overview

Initiate processing 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 recommended), and adhere to any additional safety regulations recommended or required by your institution.

5.2 Daily Preparation

The following items should be on hand before beginning to process specimens:

Lab coats, ample supply of nitrile or latex gloves, and splash shield
Lab mats and wipes
10% bleach solution or approved biohazard disinfectant
Emergency eye wash station
Biohazards waste container
Ice bucket with crushed ice, filled before start of processing
Draw tube racks
Cryovial/tube racks for 0.5 mL, 2.0 mL, 10.0 mL, and pooling tubes
Cryovials (0.5 mL, 2.0 mL, and 10.0 mL). Pre-labeled and have extras on hand.
Cryovial Caps (red, blue, yellow, purple)
Fixed-volume pipettes with tips (MLA). (Volumes to be pipetted: 0.5 mL, 1.0 mL, and 9.0 mL)
Participant ID barcode labels
MESA Processing Forms
Cryovial freezer boxes (Revco boxes 2" with 10 x 10 grids and 3" with 7 x 7 grids)
Pens and Sharpie pens
Clock
Stop watch or timer
-80°C Freezer
4°C Refrigerator
Refrigerated Centrifuge with a Horizontal (swing-out head) rotor – minimum 2000 g-force
Test tube holders (adapters) for centrifuge
Harvard trip balance/Pan balance
Water bottles for balance
HbA_{1c} Sample Preparation Kit (Bio-Rad Cat# 270-2167)
Reagents and materials for cell prep procedure (see MESA Manual of Operations chapter, "Lab Protocol for MESA Epigenomics Study in Exam 5")
Reagents and materials for MESA urine collection on Mesa Classic (see MESA Manual of Operations chapter, "Collection, Processing, and Shipping Urine Samples")

5.3 HbA_{1c} Procedure

Mix the first 10 mL EDTA blood collection tube thoroughly by inversion (invert at least 20 times). Remove purple stopper from the blood tube and place adjacent to tube. Using the plastic capillary holder provided, depress plunger to reveal hook and place capillary tube in the hook, using gloved hand if necessary. Fill the capillary end to end (5uL) with the blood sample by touching capillary to residual blood on the stopper of the EDTA tube. If there is a hanging drop of blood or any

excess blood on the outside of the capillary, remove it by touching to the EDTA tube stopper. Drop the capillary into a pre-labeled sample preparation vial provided in the kit. Cap the vial and shake it gently to rinse the blood out of the capillary and to allow hemolysis of the sample to take place. The capillaries are heparinized to prevent clotting inside the capillary. Label the vial. Place properly labeled vial in the freezer at -80^{o} C.

Samples prepared as directed are stable for 2 weeks at room temperature, 4 weeks at 2-8^{o} C, and at least 8 weeks at –80^{o} C. Do not freeze at -20^{o} C, as samples deteriorate after one week of storage at -20^{o} C.

Example of the Bio-Rad Kit contents for HbA1C collection:

- Procedure steps:

1. Take one capillary out of the capillary dispenser and attach it in the capillary holder. Fill the capillary with blood by touching it to the stopper of the EDTA tube. **IMPORTANT: The capillary must be filled end to end.**

2. If any residual blood remains on the capillary, such as a drop hanging from end, touch it to the stopper to remove it. Transfer the filled capillary into the sample preparation vial.

3. Cap the vial and shake it to rinse the blood completely from the capillary. **IMPORTANT: Make sure that no blood remains in the capillary.**

4. Confirm that the vial is correctly labeled.

5.4 Centrifugation

Instructions for centrifuging EDTA, citrate, SCAT-I, and serum tubes

- EDTA 10 mL tubes. BEFORE centrifuging, follow the steps outlined above for the HbA_{1c} Procedure (Sections 6.3 and 6.3.1) to transfer 5µl of whole blood using the capillary tube (see section 6.3) from one of the filled vacutainer tubes to the appropriate vial for HbA_{1c} testing.

- EDTA, citrate, and SCAT-I tubes should be kept upright on wet ice till centrifuging. Centrifuging these tubes should be initiated as soon as possible (0 – 30 minutes) following venipuncture. Record start time of centrifuging on the Processing Forms.
CBC/Diff tube is handled per site specific instructions.

- Serum tubes. Keep at room temperature for at least 40 minutes (but no longer than 90 minutes) to allow them to clot. Record start time of centrifuging on the Processing Forms. MESA MRI-GAD serum creatinine tube handled locally per site.

- Centrifuge EDTA, citrate, SCAT-I, and serum tubes at 4°C for at least 2000 g for 15 minutes or 3000 g for 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.

- CPTs require special processing steps. Follow protocol explained in the MESA Manual of Operations chapter, Lab Protocol for MESA Epigenomics Study at Exam 5".

- Heparin 10 mL tubes. Do NOT centrifuge. Immediately after collection these tubes are gently inverted 5-6 times then placed on an aliquot mixer for 3 minutes. The tubes are now ready to ship. Keep them at room temperature till shipping. Heparin tubes are shipped the same day they are collected.

5.5 Aliquoting

Guidelines for aliquoting MESA Classic samples:

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

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

- When aliquoting plasma and serum, 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.

- Use a new pipette tip for each draw tube.

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

- All aliquots must be immediately (< 10 minutes) frozen in an upright position at -80°C or colder. (Promptly place aliquots on dry ice for quick freezing if an ultra cold freezer is not immediately available.

- If any tubes are accidentally mixed during pipetting, so that plasma is contaminated with red cells, the tubes may be re-centrifuged.

- If there is insufficient sample of a tube type to make the full set of aliquots, fill the lowest numbered cryovials for that tube type first. Any partially-filled (less than 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.

- Discard vacutainer tubes after pooling and aliquoting are completed.

Instructions for aliquoting EDTA, citrate, SCAT-I, and serum tubes (MESA Classic samples):

- Description of aliquots – MESA Classic Aliquots

Draw Tube	Sample Type	Number of Cryovials	Color Code	Volume per Cryovial
2 x 10 mL EDTA	Whole blood (HbA1c)	1 (# 01)	Purple	5 µL (whole blood)
	Plasma	1 (# 02)	Purple	1.0 mL
	Plasma	16 (#s 03 – 18)	Purple	0.5 mL
	Packed RBCs	1 (# 19)	White	9.0 mL pRBCs
1 x 5 mL SCAT-I	Plasma	4 (#s 20 - 23)	Yellow	0.5 mL
1 x 5 mL Citrate	Plasma	4 (#s 24 – 27)	Blue	0.5 mL
2 x 10 mL Serum	Serum	17 (#s 28 – 44)	Red	0.5 mL
1 x 2 mL EDTA	Whole blood	2 (#s 45 – 46)	Purple	1.0 mL

- EDTA whole blood (HbA1c) → cryovial # 01. A drop (5 µl) of whole blood is removed from one of the 10 mL EDTA tubes before centrifuging, (following HbA_{1c} Procedure explained in Sections 6.3 and 6.3.1) for Hemoglobin A1C analysis. Freeze cryovial in an upright position at -80°C or colder within 10 minutes of aliquoting. **Important – do not store at -20°C**.

- EDTA plasma → cryovials #s 02 – 18 Keep draw tubes, pooling tube, and cryovials on wet ice during aliquoting. After centrifuging, pool plasma from the two 10 mL EDTA tubes in a 15 mL tube (or other suitable pooling tube). Aliquot, by the volumes specified in the table above, into purple-capped cryovials 02 – 18. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting.

- EDTA packed red blood cells (pRBCs) → cryovial/tube # 19. Keep all tubes on wet ice during aliquoting. After removing the plasma, transfer all of the EDTA pRBCs from both vacutainer tubes to tube # 19. Do NOT exceed 9 mL total volume in this tube. Freeze tube in upright position at -80°C or colder within 10 minutes of aliquoting. Discard the empty vacuatiner tubes in appropriate biohazard waste container.

- SCAT-I plasma → cryovials #s 20 – 23. Keep draw tubes and cryovials on wet ice during aliquoting. Aliquot 0.5 mL of the plasma

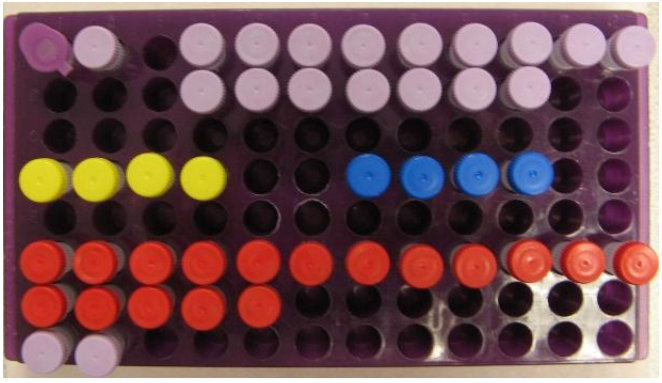
from the SCAT-I draw tube into each of the yellow-capped cryovials 20 – 23. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. Remaining red cells (and the tube itself) can be discarded in the appropriate biohazard waste container.

- Citrate plasma → cryovials #s 24 – 27. Keep draw tubes and cryovials on wet ice during aliquoting. Aliquot 0.5 mL of the plasma from the Citrate draw tube into each of the blue-capped cryovials 24 – 27. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. Remaining red cells (and the tube itself) can be discarded in the appropriate biohazard waste container.

- Serum → cryovials #s 28 – 44. Keep draw tubes, pooling tube, and cryovials on wet ice during aliquoting. Pool serum from the two 10 mL draw tubes in a 15 mL tube (or other suitable pooling tube). Aliquot 0.5 mL serum into each of the red-capped cryovials 28 – 44. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. Remaining red cells (and the tube itself) can be discarded in the appropriate biohazard waste container.

- EDTA whole blood → cryovials #s 45 – 46. Keep draw tubes on wet ice till aliquoting. Do not centrifuge this tube. Carefully remove the stopper and aliquot 1.0 mL whole blood into each cryovial# 45 and 46. Freeze cryovials in an upright position at -80°C or colder within 10 minutes of aliquoting. Discard the draw tube in the appropriate biohazard waste container.

Example of Cryovial Rack:



For instructions on processing and aliquoting MESA Epigenomics study samples, see chapter, "Lab Protocol for MESA Epigenomics Study at Exam 5".

5.6 Blind Duplicate

- A blind duplicate sample for quality control will be reserved from ~10% of participants (5% each for EDTA and serum).

- Participants will be selected for QC purposes based on the last two digits of their ID numbers. Participants whose last digit of their MESA ID numbers is "6" and the sixth digit is 0 - 4, will be selected for EDTA QC activity. Participants whose last digit of their MESA ID number is "6" and the sixth digit is 5 – 9, will be selected for serum QC activity.

- Participants will have pre-assigned QC ID numbers in case he/she is selected for QC purposes.

- Participants selected for a serum blind duplicate will have 0.5 mL serum aliquoted into a labeled Blind Duplicate 0.5 mL cryovial after Cryovial # 28 and 29 are completed. Once the Blind Duplicate vial is made then continue with aliquoting the remaining serum vials starting with Cryovial #30. Serum blind duplicate cryovials will have red caps.

- Participants selected for an EDTA blind duplicate will have 1.0 ml of EDTA plasma aliquoted into a labeled Blind Duplicate 2.0 mL cryovial after Cryovials 01 and 02 are completed. Once the Blind Duplicate vial is made then continue with aliquoting the remaining EDTA vials starting with Cryovial #03. EDTA blind duplicate cryovials will have purple caps.

- Place blind duplicate samples (from multiple participants) in a freezer box (10 x 10 grid). Ship this box, (according to instructions listed in Section 7 Shipping Blood Samples), to the University of Vermont at least one week after shipping the corresponding participant's repository cryovials (#s 01-52). (Delayed shipping prevents the laboratory from matching the blind duplicate samples with the participant.) When shipping the blind duplicate samples, include the

completed Blind Duplicate Shipping Form in lieu of the Processing Forms.

5.7 Special Circumstances

- 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. Maintain the EDTA, citrate, and SCAT-I tubes on wet ice until centrifugation. (Refer to chapter, Lab Protocol for MESA Epigenomics Study at Exam 5" for instructions regarding special processing circumstances for CPTs.)

- If cryovials can not 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 vials in an upright position) or stored at -20°C until transfer to -80°C or colder is possible. Note: whole blood with HbA1c fixing solution (cryo #01) do NOT store at -20°C. If cryovials are not frozen at -80°C within 10 minutes, record storage conditions, storage temperature and length of time at that temperature, on the Processing Forms.

5.8 Completion

- The MESA Processing Forms are kept in a temporary file. Enclose copies of the Phlebotomy and Processing Forms with each shipment of samples to the CBAL. Upon receipt at CBAL, forms and samples are examined for monitoring and quality control purposes.
- Complete MESA Processing Forms legibly in ink.
- Frozen cryovials from two participants are packed into one 2" freezer box. Participant ID labels are attached to the front cover of the freezer box. Frozen 10 mL pRBCs tube # 19, (and urine tubes #s 47 – 52*)* are packed in taller 3" freezer boxes for shipment to Vermont. (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.

COLLECTION, PROCESSING AND SHIPPING OF URINE SAMPLES

IV. PURPOSE

A random, spot urine will be collected on all Mesa Classic participants upon AM 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 weekly.

V. EQUIPMENT AND SUPPLIES

CBAL will provide the following supplies and reagents in bulk:

4 mL (Sarstedt #62.611) and 10 mL tubes (Krackler Scientific #622-T310-10A)
1 M Acetic acid, glacial

Reagent Description: Acetic acid will be used for urine processing. As acetic acid is corrosive, it is recommended that the stock solution being stored in a metal secondary container. The ratio of additive is 25 µL 1 M acetic acid to 1.0 mL urine.

Note: An alternative urine preservative may be recommended by the Central Lab. We are currently pursuing different options.

Additional supplies needed at the Field Centers:

Participant ID barcode labels (provided by the Coordinating Center)
Specimen collection containers
Sage Commode Specimen and Measuring System #2500 (optional)
4 x 4 inch gauze pads
10% solution of antiseptic soap
Pipettes – *230 µL,* 3.0 mL and 9.0 ml volumes
Wet ice
MESA Phlebotomy and Processing Form

VI. METHODS

1. Preparation:

   Collect urine as early in the visit as possible and before venipuncture. Rarely, a participant will refuse to provide a urine sample. Please keep a list of the MESA enrollment ID numbers of any participants who refuse.

   Urine samples must be precisely labeled throughout the collection and processing stages to ensure they are correctly coded. Pre-label collection containers and

cryovials prior to the participant's visit, and cross-check the labels with each participant's ID number prior to specimen collection

2. Preparation of Participants for Urine Collection:

   Collect urine before venipuncture.

   Collect urine from all participants whenever possible. Encourage participants to stay hydrated even while fasting for the visit. However, do not collect samples after acute fluid load (>24 ounces) or after participant exertion. Collection will be random and, therefore considered a 'spot' urine collection. Participants who have difficulty producing a urine specimen may be offered a glass of water, and subsequent urine specimens may be collected later in the visit to bring the total volume to the required amount.

   Female participants may use the Sage Commode Specimen and Measuring System #2500 for urine collection (follow instructions provided), or they may urinate directly into a specimen collection container, if they prefer. Male participants should urinate directly into a specimen collection container.

   Do not collect urine from females who are menstruating. Collect a sample at a later visit, if possible.

3. Forms:

   The MESA Phlebotomy Form and Processing Forms provide a vital link between the Participant ID number and the samples and facilitate the efficient collection of urine samples in addition to providing information critical to the interpretation of assay results.

   The completed MESA Processing Forms identify the urine aliquots filled and are included in the sample shipments to CBAL. Forms must be labeled with the correct participant barcode ID labels and legibly completed in ink.

4. Urine Collection:

   Containers for routine random specimens should be chemically clean, should hold about 80+ mL, (processing will need ~48-51 mLs) and must have a tight-fitting lid to prevent leakage during transportation.

   Orient the participant to the supplies (antiseptic-soaked gauze pad, collection container) and explain the procedure.

   The participant's privacy should be assured.

5. Instructions for participants:

Wash hands before and after voiding. Open or remove clothing to make voiding and collection easier. Remove the cap from the collection container and have ready.

Void directly into the container until half full.

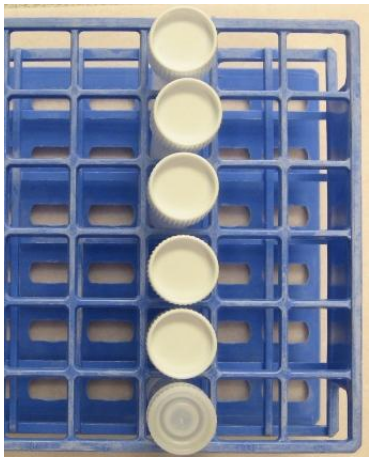
Carefully seal the cap of the container so that it is tight and leak proof.

Record if urine collected and approximate volume on the Phlebotomy Form.

6. Aliquoting:

- For urine storage, prepare one 3.0 mL aliquot using the 4.0 mL Sarstedt clear-capped tube (cryovial #47) and 5 x 9.0 mL aliquots using 10.0 mL white-capped tubes (#s 48 - 52). Process following the instructions below:
- Do not overfill the tubes. There must be room for the urine to expand when frozen.
- Pipette a minimum of 3.0 mL urine into the 4 mL Sarstedt clear-capped tube #47; pipette 9 mL urine into three white-capped tubes #48, #49 and #50. Place both on ice. (Tube# 50 will be for MESA Air repository storage.)
- Pipette 9 mL urine into two white-capped tubes #51 and #52, then add 230 µL (0.230 ml) 1 M acetic acid to each tube. Mix by inversion and place on ice.
- Double-check that urine aliquots have correct participant ID labels
- Check off on the MESA Processing Form the number of urine aliquots made (#s 47 - 52).
- Discard any extra urine.
- Freeze cryovials in an upright position at -80°C or colder, preferably with in 10 minutes of aliquoting.

Example of the urine tube rack:



7. Blind Duplicate Urine Sample:

Process a blind duplicate urine sample from all participants who are selected for an EDTA QC. The QC ID number will be the same for both the EDTA and urine blind duplicate cryovials, however the two-digit cryo identifier will be different.

After aliquoting the participant's urine into repository tubes #s 47- 52, aliquot 3 mL of the remaining urine into a 4 mL clear-capped tube (similar size to tube# 47). Carefully label this tube with the QC ID# and freeze in the upright position at -80°C immediately. Urine blind duplicates should be stored separately from the participant's original sample set, in a freezer box with a 7 x 7 grid. Ship blind duplicate urine samples with blind duplicate blood samples one week after the participant's repository samples are shipped (see MESA Manual of Operations chapter, "Collection, Processing, and Shipping of MESA Classic Blood Samples").

Complete the Blind Duplicate Shipping Form – this is of key importance! - and include a copy in the shipping container with the frozen samples.

8. Packaging and Shipping Samples:

Package and ship frozen urine specimens according to the instructions in the MESA Manual of Operations chapter, "Collection, Processing, and Shipping of MESA Classic Blood Samples", Section 6 : SHIPPING SAMPLES – Mesa Classic.

VII. SHIPPING SAMPLES

1. General Instructions for MESA Classic:

   Blood samples are shipped only on Mondays and Tuesdays to the CBAL by an overnight carrier (Federal Express is preferred). Samples are shipped on a pre-arranged schedule, which allows the laboratory to stagger the arrival of samples for easier processing. Urine aliquots are included with the frozen MESA Classic blood sample shipments to CBAL.

2. Packaging:

   Sample shipping checklist –
   Frozen MESA Classic samples (blood and urine) in labeled freezer boxes
   Styrofoam shipping containers (Polyfoam Packers) with outer cardboard sleeve.
   Rubber bands for freezer boxes
   Ziploc 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 pounds per shipping container)
   Shipping labels (FedEx address labels)
   Category B labels (UN3373 "BIOLOGICAL SUBSTANCE, CATEGORY B")
   Dry Ice labels (Dry Ice UN 1845, Class 9 - Miscellaneous Dangerous Goods Label)
   Completed MESA Phlebotomy and Processing Forms
   Completed MESA Shipping Form (also to be faxed or e-mailed with shipment tracking #)

3. Procedure:

   This shipping protocol follows procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904.

   For frozen shipments to the University of Vermont:

   1. Line styrofoam mailer(s) with absorbent material (i.e. absorbent pads).

   2. Place approximately 1/2 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 freezer boxes containing the samples.

4. Collect the freezer boxes containing samples to be shipped, and check the participant ID numbers against the Processing Forms and Shipping Form for that shipment.

5. Place a rubber band around each cardboard freezer box containing samples before enclosing each box in a ziplock plastic bag. Carefully place these bagged boxes containing samples in the shipping container. The rubber band helps prevent freezer boxes opening and spilling contents; the ziplock bag serves as an additional form of containment, and the absorbent material is essential in the event of a spill and thaw.

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 top of the styro is secured in place with tape. (Allow dry ice vapors to escape by not completely sealing Styro lid shut.) Enclose styro in the outer cardboard sleeve.

9. Place the MESA Phlebotomy and Processing Forms for all samples included in the Styrofoam shipping container and a copy of the Shipping Form for that shipment in a ziplock bag, then place on top of the Styrofoam lid before securely taping the outer cardboard sleeve closed.

10. 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).

11. A copy of the completed Shipping Form, listing all participant samples enclosed in the shipping container and <u>including the Fedex airbill number, is faxed to the University of Vermont at (802) 656-8965 the same day the shipment is shipped.</u>

Mailing Address:
University of Vermont
Department of Pathology
Colchester Research Facility, Room 154C
208 South Park Drive

Colchester, VT 05446
Attn: Elaine Cornell
(802) 656-8963
(802) 656-8965 Fax

SHIPPING SAMPLES – MESA COPD

4. General Instructions for MESA COPD:
   Samples will be shipped on a pre-arranged schedule. Blood samples are only shipped on Monday through Thursday by an overnight carrier (Federal Express is preferred). They may not be shipped on Fridays.

5. Packaging Samples: Sample Shipping Checklist (Room Temperature samples to CBAL)

STP 600:	bubble-wrap tubes holders, which hold up to 5 draw tubes
STP 150:	3" absorbent strips with a 50 mL capacity
STP 710:	inner leak proof poly bag (STP 711) and outer envelope (STP 710)
STP 308:	insulated polystyrene cooler w/outer diagnostic shipper.
STP 317:	15-30°C PCM (Phase Change Material) bricks NOTE: These should be stored at room temperature (15-30°C). If room temperature is colder than 15°C, these can be pre-warmed for ~1 hour in a 37° water bath prior to packaging for shipping.

Fill out the Shipping Log, including the FedEx tracking numbers and fax to the University of Vermont at (802) 656-8965 or scan and email to Margaret.Doyle@uvm.edu.

For shipment to the University of Vermont:

1. Insert tubes into bubble wrap tube holder (STP 600).
2. Be sure absorbent paper (STP 150) is in the inner leak-proof poly bag (STP 711).
3. Add tubes in bubble wrap tube holder to inner leak-proof poly bag containing absorbent paper. Seal the bag completely.
4. Place the completely-sealed plastic bag into outer envelope (STP 710) and seal.
5. Add two 2 phase change material packs (STP 317) flat in the bottom of the insulated polystyrene cooler (STP 308).
6. Lay the sealed bag containing the tubes on top of the phase change material packs.

7. If more than 2 participants are drawn on one day, another bubble wrap tube holder may be used and sealed, as above, in the STP 710 leak-proof bags. Place the second sealed bag in the same package as the first set.
8. If more than 4 participants are drawn, a second shipping box should be used.
9. Add 2 more phase change material packs (STP 317) on top of the tubes. If necessary to fill the package, place packing material on top (absorbent material, shredded newspaper, packing foam, etc.). Place top on insulated cooler. Tape lid to polystyrene cooler.
10. Add the completed lab forms for all samples enclosed. If placing the paperwork inside the cooler these must be sealed in a plastic bag.
11. Place cooler into a cardboard outer box. Seal the carton. Ensure that the package is labeled "Biological Substance, Category B" and has the "UN3373" label visible. If not, place a new Diagnostic Specimen and UN3373 lable onto the box.
12. Affix the shipping labels. If pickup is not immediate, DO NOT REFRIGERATE. Keep tubes at room temperature at all times.

Email Margaret.Doyle@uvm.edu notification of package shipped each day with tracking #.

After the blood is drawn and packaged, contact Federal Express and arrange for the package to be picked up. Blood drawn on Monday through Thursday should be shipped via FedEx overnight priority to the University of Vermont for next day delivery.
Keep all blood samples at room temperature until they are picked up by Federal Express.

Mailing Address:

University of Vermont
Department of Pathology
Colchester Research Facility, Room 236
208 South Park Drive,
Colchester, VT 05446
Attn: Peggy Doyle
(802) 656-8939
(802) 656-8965 Fax

VIIII. QUALITY ASSURANCE (QA)

1. 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 an effort to prevent any sample associated problems, a system 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 and certification process for each Field Center technician;
   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.

2. Technician Training and 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 at the MESA meeting in February 2010 WFU Field Center. 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 numerous draw tubes (e.g., centrifugation, temperature requirements, and aliquoting).

   Field center technician requirements: Field Center technicians who will be performing blood collection 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.

   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 and 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 three steps required for certification are:

1. Read and understand the appropriate chapters in the MESA Manual of Operations.
2. Observe the process performed by a certified technician.
3. Successful completion of the written exam (prepared by CBAL).
4. Successful completion of the practical exam (using the Certification Form/Supervisor Checklist in this Manual) which requires observation by certified personnel of complete phlebotomy/processing procedure on a volunteer.

Maintaining Certification: A technician should perform phlebotomy and/ or processing on a minimum of one participant every two weeks in order to maintain certification.

3. 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 need to be addressed. Equipment temperature logs are filed on site for future reference and reported to the CBAL twice per year. These equipment records can identify concerns with sample quality in the processing and local storage steps.

4. Field Center Supervisor Checklist

The Field Center Supervisor checklist serves as a monitoring measure. The 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. Checklists need to be completed once per month per technician. Completed Supervisor Checklists will be sent to the CBAL quarterly for monitoring purposes.

5. Sample Acknowledgement Forms

Sample Acknowledgement Forms are generated by the CBAL and faxed to the field center after each field center's sample shipment is received and processed by the CBAL. These forms serve as a tool to track possible problems and variations from protocol. Shipment condition and any protocol deviations or concerns found by the CBAL after review of the Shipping Forms, Phlebotomy and Processing Forms and physical inspection of the specimens, are summarized on the Sample Acknowledgement Form.

6. Field Center Forms

MESA Exam 5 Phlebotomy and Processing Form
MESA Exam 5 Shipping Form
MESA Exam 5 Blind Duplicate Shipping Form
MESA Exam 5 Field Center Supervisor Checklist
MESA Exam 5 Field Center Technician Certification Examinations
MESA Exam 5 Equipment Temperature Logs

Appendix: MESA Exam 5 Collection, Processing, and Shipping of Blood Samples

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

APPENDIX Item 1

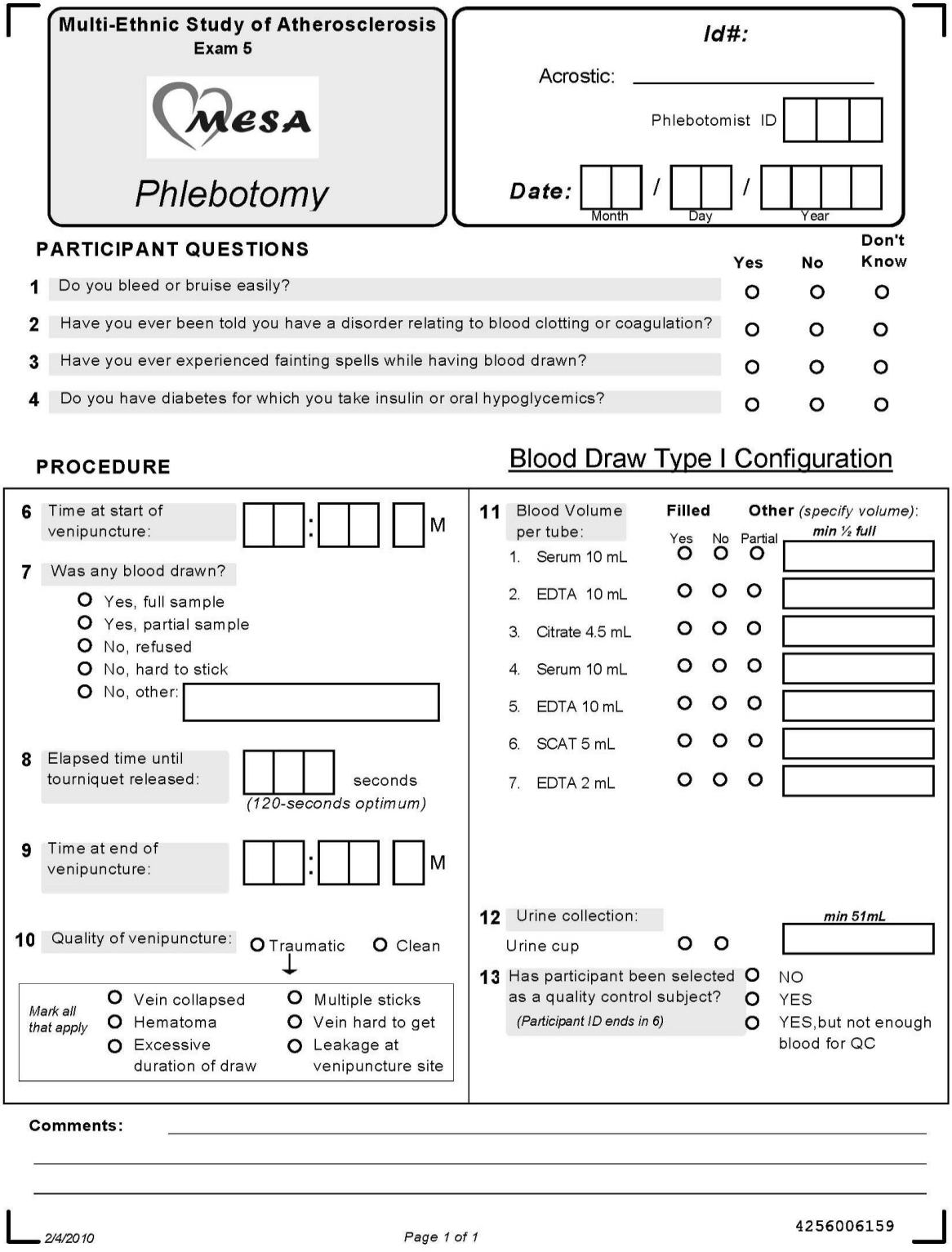
Alert Values

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

APPENDIX Item2



Multi-Ethnic Study of Atherosclerosis
Exam 5

MESA

Phlebotomy

Id#:

Acrostic: _____

Phlebotomist ID [ ][ ][ ]

Date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
Month Day Year

PARTICIPANT QUESTIONS

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

PROCEDURE

Blood Draw Type I Configuration

6 Time at start of venipuncture: [ ][ ] : [ ][ ] [ ] M

7 Was any blood drawn?
- O Yes, full sample
- O Yes, partial sample
- O No, refused
- O No, hard to stick
- O No, other: [ ]

8 Elapsed time until tourniquet released: [ ][ ][ ] seconds
(120-seconds optimum)

9 Time at end of venipuncture: [ ][ ] : [ ][ ] [ ] M

10 Quality of venipuncture: O Traumatic O Clean

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

11 Blood Volume per tube:

	Filled Yes	Filled No	Filled Partial	Other *(specify volume): min ½ full*
1. Serum 10 mL	O	O	O	
2. EDTA 10 mL	O	O	O	
3. Citrate 4.5 mL	O	O	O	
4. Serum 10 mL	O	O	O	
5. EDTA 10 mL	O	O	O	
6. SCAT 5 mL	O	O	O	
7. EDTA 2 mL	O	O	O	

12 Urine collection: *min 51mL*

Urine cup O O [ ]

13 Has participant been selected as a quality control subject? *(Participant ID ends in 6)*
- O NO
- O YES
- O YES,but not enough blood for QC

Comments: _____

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Pending Phlebotomy Forms types II through VI

PROCEDURE

Blood Draw Type II Configuration

6 Time at start of venipuncture: ☐☐:☐☐ ☐ M

7 Was any blood drawn?

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

8 Elapsed time until tourniquet released: ☐☐☐ seconds
(120-seconds optimum)

9 Time at end of venipuncture: ☐☐:☐☐ ☐ M

10 Quality of venipuncture: ○ Traumatic ○ Clean

Mark all that apply

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

11 Blood Volume per tube:

	Filled Yes	Filled No	Filled Partial	Other *(specify volume): min ½ full*
1. Serum Cre. 3.5 mL	○	○	○	☐
2 Serum 10 mL	○	○	○	☐
3. EDTA 10 mL	○	○	○	☐
4. Citrate 4.5 mL	○	○	○	☐
5. Serum 10 mL	○	○	○	☐
6. EDTA 10 mL	○	○	○	☐
7. SCAT 5 mL	○	○	○	☐
8. EDTA 2 mL	○	○	○	☐

12 Urine collection: *min 51 mL*

Urine cup ○ ○ ☐

13 Has participant been selected as a quality control subject?
(Participant ID ends in 6)

- ○ NO
- ○ YES
- ○ YES,but not enough blood for QC

Comments: _____

_____

_____

Blood Draw Type III Configuration

PROCEDURE

6 Time at start of venipuncture: ☐☐:☐☐ ☐ M

7 Was any blood drawn?

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

8 Elapsed time until tourniquet released: ☐☐☐ seconds
(120-seconds optimum)

9 Time at end of venipuncture: ☐☐:☐☐ ☐ M

10 Quality of venipuncture: ○ Traumatic ○ Clean

Mark all that apply

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

11 Blood Volume per tube:

	Filled Yes	Filled No	Partial	Other (specify volume): *min ½ full*
1. Serum 10 mL	○	○	○	☐
2. EDTA 10 mL	○	○	○	☐
3. Citrate 4.5 mL	○	○	○	☐
4. CPT 8 mL	○	○	○	☐
5. CPT 8 mL	○	○	○	☐
6. Serum 10 mL	○	○	○	☐
7. EDTA 10 mL	○	○	○	☐
8. CPT 8 mL	○	○	○	☐
9. CPT 8 mL	○	○	○	☐
10. EDTA 2 mL	○	○	○	☐
11.SCAT 5 mL	○	○	○	☐
12. EDTA 2	○	○	○	☐

12 Urine collection: *min 51 mL*

Urine cup ○ ○ ☐

13 Has participant been selected as a quality control subject? *(Participant ID ends in 6)*

- ○ NO
- ○ YES
- ○ YES,but not enough blood for QC

Comments: _____

_____

_____

Blood Draw Type IV Configuration

PROCEDURE

6 Time at start of venipuncture: ☐☐:☐☐ ☐ M

7 Was any blood drawn?

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

8 Elapsed time until tourniquet released: ☐☐☐ seconds
(120-seconds optimum)

9 Time at end of venipuncture: ☐☐:☐☐ ☐ M

10 Quality of venipuncture: ○ Traumatic ○ Clean

Mark all that apply

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

11 Blood Volume per tube:

	Filled Yes	Filled No	Filled Partial	Other (specify volume): *min ½ full*
1. Serum Cre 3.5 mL	○	○	○	☐
2. Serum 10 mL	○	○	○	☐
3. EDTA 10 mL	○	○	○	☐
4. Citrate 4.5 mL	○	○	○	☐
5. CPT 8 mL	○	○	○	☐
6. CPT 8 mL	○	○	○	☐
7. Serum 10 mL	○	○	○	☐
8. EDTA 10 mL	○	○	○	☐
9. CPT 8 mL	○	○	○	☐
10. CPT 8 mL	○	○	○	☐
11. EDTA 2 mL	○	○	○	☐
12.SCAT 5 mL	○	○	○	☐
13. EDTA 2	○	○	○	☐

12 Urine collection: *min 51mL*

Urine cup ○ ○ ☐

13 Has participant been selected as a quality control subject? *(Participant ID ends in 6)*

- ○ NO
- ○ YES
- ○ YES,but not enough blood for QC

Comments: _____

PROCEDURE

Blood Draw Type V Configuration

6 Time at start of venipuncture: ☐☐ : ☐☐ ☐ M

7 Was any blood drawn?

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

8 Elapsed time until tourniquet released: ☐☐☐ seconds
(120-seconds optimum)

9 Time at end of venipuncture: ☐☐ : ☐☐ ☐ M

10 Quality of venipuncture: ○ Traumatic ○ Clean

Mark all that apply

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

11 Blood Volume per tube:

	Filled Yes	Filled No	Other (specify volume): Partial	*min ½ full*
1. Serum Cre. 3.5 mL	○	○	○	☐
2 Serum 10 mL	○	○	○	☐
3. EDTA 10 mL	○	○	○	☐
4. Citrate 4.5 mL	○	○	○	☐
5. Heparin 10	○	○	○	☐
6. EDTA 2 mL	○	○	○	☐
7. Serum 10 mL	○	○	○	☐
8. EDTA 10 mL	○	○	○	☐
9. SCAT 5 mL	○	○	○	☐
10. EDTA 2 mL	○	○	○	☐

12 Urine collection: *min 51 mL*

Urine cup ○ ○ ☐

13 Has participant been selected as a quality control subject?
(Participant ID ends in 6)

- ○ NO
- ○ YES
- ○ YES,but not enough blood for QC

Comments: _____

_____

_____

Blood Draw Type VI Configuration

PROCEDURE

6 Time at start of venipuncture: ☐☐:☐☐ ☐ M

7 Was any blood drawn?

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

8 Elapsed time until tourniquet released: ☐☐☐ seconds
(120-seconds optimum)

9 Time at end of venipuncture: ☐☐:☐☐ ☐ M

10 Quality of venipuncture: ○ Traumatic ○ Clean

Mark all that apply

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

11 Blood Volume per tube:

	Filled Yes	Filled No	Filled Partial	Other (specify volume): *min ½ full*
1. Serum Cre 3.5 mL	○	○	○	☐
2. Serum 10 mL	○	○	○	☐
3. EDTA 10 mL	○	○	○	☐
4. Citrate 4.5 mL	○	○	○	☐
5. Heparin 10 mL	○	○	○	☐
6. EDTA 2 mL	○	○	○	☐
7. Serum 10 mL	○	○	○	☐
8. EDTA 10 mL	○	○	○	☐
9. CPT 8 mL	○	○	○	☐
10. CPT 8 mL	○	○	○	☐
11. CPT 8 mL	○	○	○	☐
12.SCAT 5 mL	○	○	○	☐
13. EDTA 2	○	○	○	☐

12 Urine collection: Urine cup ○ ○ *min 51 mL* ☐

13 Has participant been selected as a quality control subject? *(Participant ID ends in 6)*

- ○ NO
- ○ YES
- ○ YES,but not enough blood for QC

Comments: _____

APPENDIX Item 3

MESA Exam 5 Processing Form - MESA Classic samples

Processor ID:_____ **Participant ID:** _____

Processing Start Time: _____:_____ EDTA, SCAT-I, Citrate

Processing Start Time: _____:_____ Serum

Processing Start Time: ____: _____ Urine

Cryo #	Type	Color	Sample Vol	Check if Done	Comment*	Cryo #	Type	Color	Sample Vol	Check if Done	Comment*
1	EDTA	P	5 uL		(HbA1c WB)	28	Serum	R	0.5		
2	EDTA	P	1.0			29	Serum	R	0.5		
3	EDTA	P	0.5			30	Serum	R	0.5		
4	EDTA	P	0.5			31	Serum	R	0.5		
5	EDTA	P	0.5			32	Serum	R	0.5		
6	EDTA	P	0.5			33	Serum	R	0.5		
7	EDTA	P	0.5			34	Serum	R	0.5		
8	EDTA	P	0.5			35	Serum	R	0.5		
9	EDTA	P	0.5			36	Serum	R	0.5		
10	EDTA	P	0.5			37	Serum	R	0.5		
11	EDTA	P	0.5			38	Serum	R	0.5		
12	EDTA	P	0.5			39	Serum	R	0.5		
13	EDTA	P	0.5			40	Serum	R	0.5		
14	EDTA	P	0.5			41	Serum	R	0.5		
15	EDTA	P	0.5			42	Serum	R	0.5		
16	EDTA	P	0.5			43	Serum	R	0.5		
17	EDTA	P	0.5			44	Serum	R	0.5		
18	EDTA	P	0.5			45	EDTA_WB	P	1.00		whole blood
19	Red Cells	W	9.0			46	EDTA_WB	P	1.00		whole blood
20	SCAT-I	Y	0.5			47	Urine	C	3.0		
21	SCAT-I	Y	0.5			48	Urine	W	9.0		
22	SCAT-I	Y	0.5			49	Urine	W	9.0		
23	SCAT-I	Y	0.5			50	Urine	W	9.0		(Mesa Air)
24	Citrate	B	0.5			51	Urine /acid	W	9.0		
25	Citrate	B	0.5			52	Urine/acid	W	9.0		
26	Citrate	B	0.5								
27	Citrate	B	0.5								

* P = partial volume

P=purple, W=white, Y=yellow, B=blue, R=red, C=clear

Comments:

LCBR Rec'd Date: _____ Frozen: Y N

APPENDIX Item 4

MESA EXAM 5 - SHIPPING FORM for MESA Classic Samples

Date of shipment: _____
Center: _____ Prepared by: _____

FedEx Air Bill#: _____

(Include in shipment AND fax (802-656-8965) or e-mail: Elaine.Cornell@uvm.edu)

MESA ID#	VISIT DATE	BOX #

APPENDIX Item 5

MESA EXAM 5: - BLIND DUPLICATE SHIPPING FORM

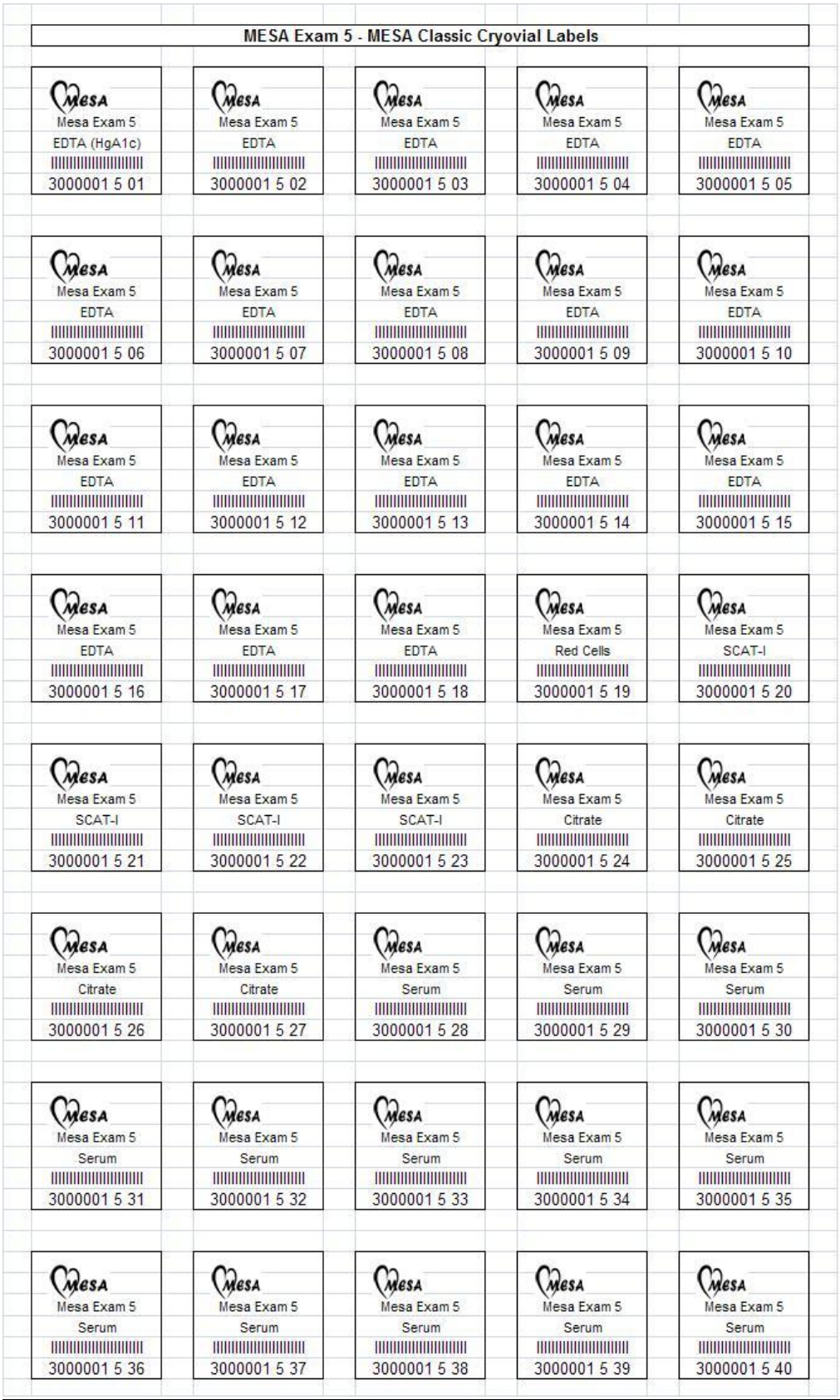
Date of shipment: _____
Center: _____ Prepared by: _____

FedEx Air Bill#: _____

(Include in shipment AND fax (802-656-8965) or e-mail: Elaine.Cornell@uvm.edu)

Blind Dupe /QC ID#	# of Cryovials	Color code (red, white, urine)

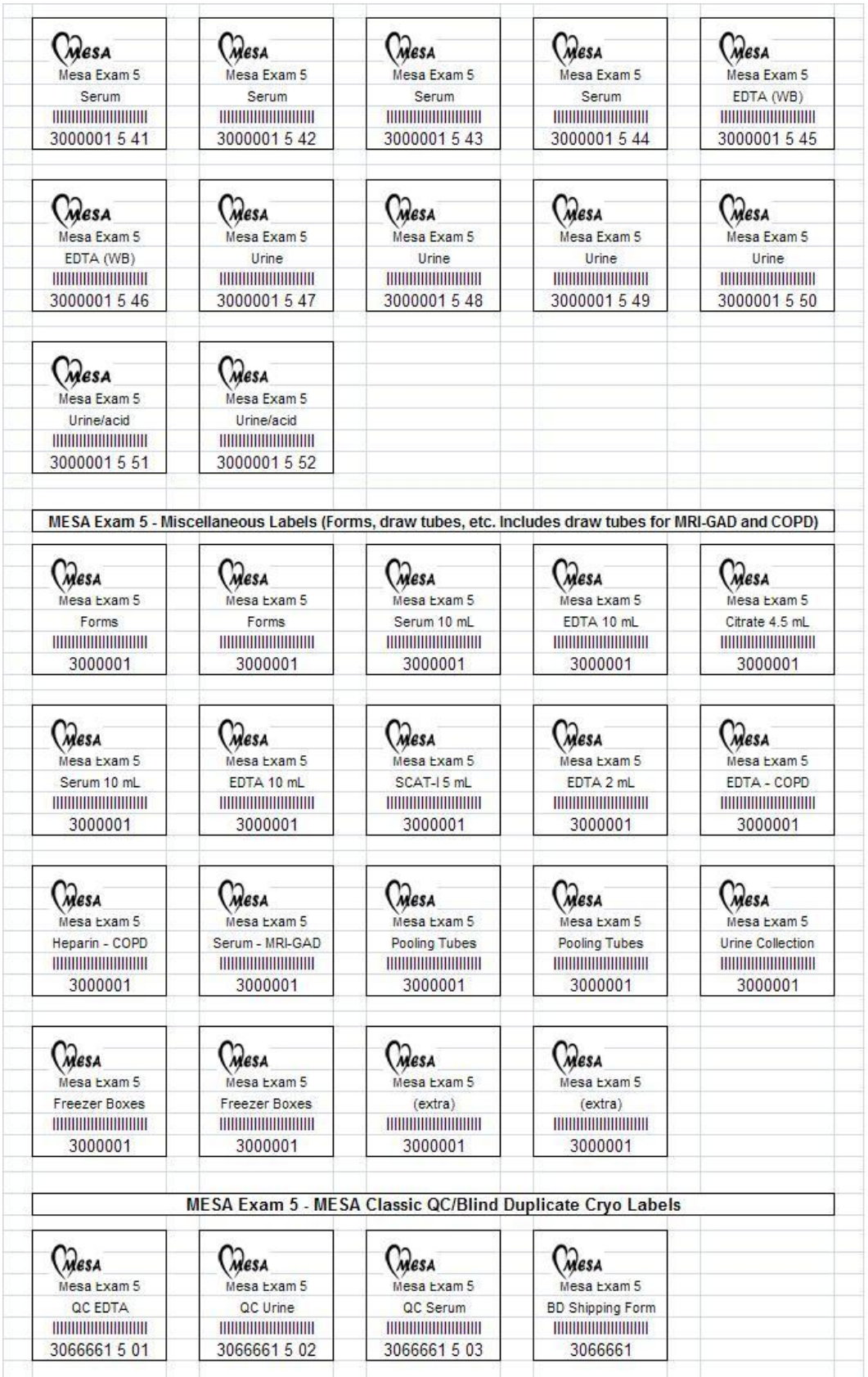
APPENDIX Item 6



MESA Exam 5 - MESA Classic Cryovial Labels

Mesa Exam 5 EDTA (HgA1c) 3000001 5 01	Mesa Exam 5 EDTA 3000001 5 02	Mesa Exam 5 EDTA 3000001 5 03	Mesa Exam 5 EDTA 3000001 5 04	Mesa Exam 5 EDTA 3000001 5 05
Mesa Exam 5 EDTA 3000001 5 06	Mesa Exam 5 EDTA 3000001 5 07	Mesa Exam 5 EDTA 3000001 5 08	Mesa Exam 5 EDTA 3000001 5 09	Mesa Exam 5 EDTA 3000001 5 10
Mesa Exam 5 EDTA 3000001 5 11	Mesa Exam 5 EDTA 3000001 5 12	Mesa Exam 5 EDTA 3000001 5 13	Mesa Exam 5 EDTA 3000001 5 14	Mesa Exam 5 EDTA 3000001 5 15
Mesa Exam 5 EDTA 3000001 5 16	Mesa Exam 5 EDTA 3000001 5 17	Mesa Exam 5 EDTA 3000001 5 18	Mesa Exam 5 Red Cells 3000001 5 19	Mesa Exam 5 SCAT-I 3000001 5 20
Mesa Exam 5 SCAT-I 3000001 5 21	Mesa Exam 5 SCAT-I 3000001 5 22	Mesa Exam 5 SCAT-I 3000001 5 23	Mesa Exam 5 Citrate 3000001 5 24	Mesa Exam 5 Citrate 3000001 5 25
Mesa Exam 5 Citrate 3000001 5 26	Mesa Exam 5 Citrate 3000001 5 27	Mesa Exam 5 Serum 3000001 5 28	Mesa Exam 5 Serum 3000001 5 29	Mesa Exam 5 Serum 3000001 5 30
Mesa Exam 5 Serum 3000001 5 31	Mesa Exam 5 Serum 3000001 5 32	Mesa Exam 5 Serum 3000001 5 33	Mesa Exam 5 Serum 3000001 5 34	Mesa Exam 5 Serum 3000001 5 35
Mesa Exam 5 Serum 3000001 5 36	Mesa Exam 5 Serum 3000001 5 37	Mesa Exam 5 Serum 3000001 5 38	Mesa Exam 5 Serum 3000001 5 39	Mesa Exam 5 Serum 3000001 5 40

APPENDIX Item 6

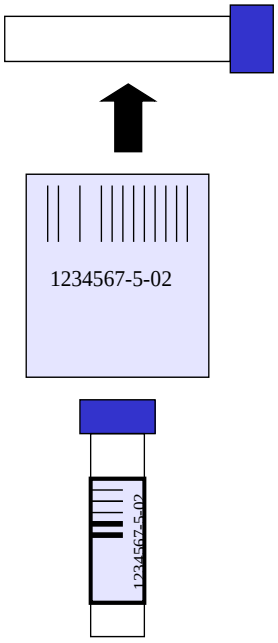


APPENDIX Item 7

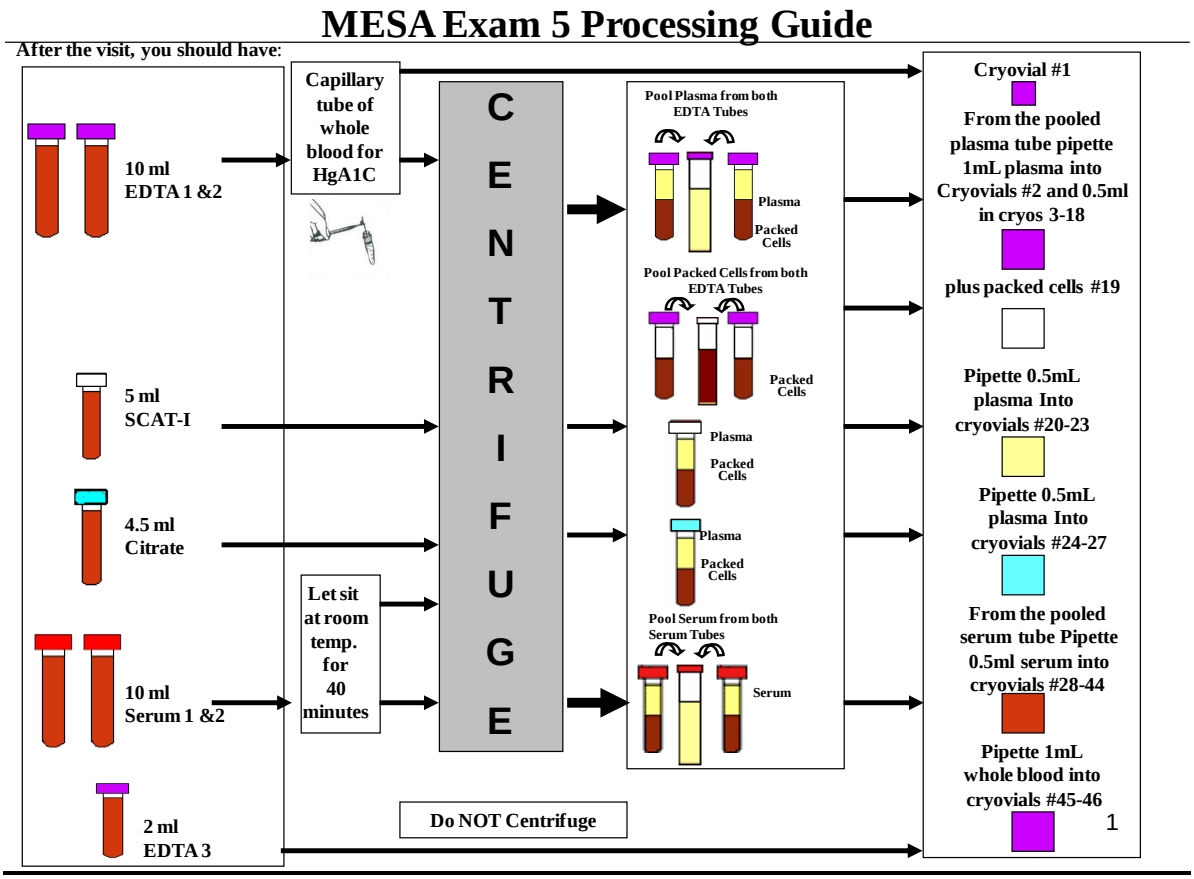
ORIENTATION of BARCODE LABEL

MESA STUDY

Label Orientation on Cryovial



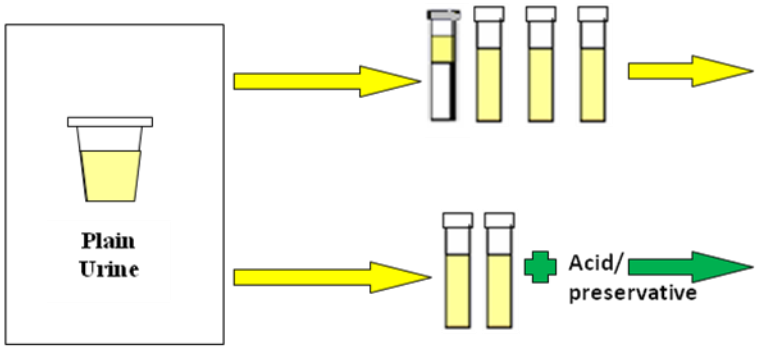
APPENDIX Item 8



APPENDIX Item 8

MESA Exam 5 Urine Processing Guide

After the visit, you should have:



Cryovial #, Size	Vol. (mL)	Cap Color
#47 (4ml)	3.0	Clear
# 48-50 (10 mL)	9.0 plain urine	White
#51 &52 (10mL)	9.0 urine + acid/preservative	White

2

APPENDIX Item 9

MESA Exam 5 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. ☐ Blood draw tubes in correct order and correctly labeled.
3. ☐ Checked Phlebotomy Form has correct participant ID.
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; time noted on Phlebotomy form.
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 protocols.
15. ☐ Filled tubes placed in the correct racks - on ice or at room temperature – ASAP per protocol.
16. ☐ EDTA, Citrate or SCAT-I tubes < ½ full discarded.

P/P Form:

17. ☐ Correct sample ID labels on both pages of Phlebotomy/Processing form.
18. ☐ Venipuncture starts and end times legibly recorded on the Phlebotomy form.
19. ☐ Elapsed tourniquet time noted on form.
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: _____

For QC activity, Make sure to complete the Web-based QC Procedures/Activities form

APPENDIX Item 9

MESA Exam 5 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
2. ☐ Non-permeable lab coats, gloves, and face shields used.

Stage 1:

3. ☐ Time checked to ensure tubes are processed within the correct time limits post venipuncture per protocol.
4. ☐ HbA1c procedure performed correctly and placed in correctly labeled cryovial.
5. ☐ Equipment is checked to ensure all tubes requiring centrifuging are centrifuged at the correct temperature and speed.
6. ☐ EDTA plasma from 10 mL tubes pooled before aliquoting into correctly labeled and color-coded cryovials.
7. ☐ New pipet tip used for each sample type and aliquots kept on ice during aliquoting.
8. ☐ Filled cryovials checked off on the Processing Form and frozen upright @ -80 ^{o}C within 10 minutes

Stage 2:

12. ☐ Time monitored to ensure serum tubes remain at room temperature for > 40 minutes and < 90 minutes.
13. ☐ Serum from 10 mL tubes pooled before aliquoting into correctly labeled and color-coded cryovials.
14. ☐ EDTA whole blood from the correct draw tube is aliquoted into cryovials #45 and 46.

Processing Completion:

15. ☐ Urine is kept refrigerated till aliquoting into correctly labeled tubes (#47 – 52), and the appropriate preservative added to tubes# 51 and #52.
16. ☐ Processing area and equipment is cleaned with appropriate disinfectant.
17. ☐ Processing Form completely filled out, including recording all blood and urine aliquots obtained and if any are less than the required volume. Any comments noted in comment section.

Comments: _____

_____

_____

Supervisor Signature _____

APPENDIX Item 10

MESA Exam 5 - Equipment Temperature Log

Equipment: _____ Year: _____

Equipment ID#: _____

Month: _____

Date	Temperature °C	Tech	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
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26			
27			
28			
29			
30			
31			

Month: _____

Date	Temperature °C	Tech	Comments
1			
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APPENDIX Item 11

MESA Exam 5 – Freezer Box Diagrams for Shipping MESA Classic Cryovial Samples

Box map for 2" boxes (10x10 divider) with all 0.5 mL and 2.0 mL cryovials for two participant's sample sets.

Pt1 Cryo 01	Cryo 11	Cryo 22	Cryo 32	Cryo 42	**Pt2** Cryo 01	Cryo 11	Cryo 22	Cryo 32	Cryo 42
Cryo 02	Cryo 12	Cryo 23	Cryo 33	Cryo 43	Cryo 02	Cryo 12	Cryo 23	Cryo 33	Cryo 43
Cryo 03	Cryo 13	Cryo 24	Cryo 34	Cryo 44	Cryo 03	Cryo 13	Cryo 24	Cryo 34	Cryo 44
Cryo 04	Cryo 14	Cryo 25	Cryo 35	Cryo 45	Cryo 04	Cryo 14	Cryo 25	Cryo 35	Cryo 45
Cryo 05	Cryo 15	Cryo 26	Cryo 36	Cryo 46	Cryo 05	Cryo 15	Cryo 26	Cryo 36	Cryo 46
Cryo 06	Cryo 16	Cryo 27	Cryo 37		Cryo 06	Cryo 16	Cryo 27	Cryo 37	
Cryo 07	Cryo 17	Cryo 28	Cryo 38		Cryo 07	Cryo 17	Cryo 28	Cryo 38	
Cryo 08	Cryo 18	Cryo 29	Cryo 39		Cryo 08	Cryo 18	Cryo 29	Cryo 39	
Cryo 09	Cryo 20	Cryo 30	Cryo 40		Cryo 09	Cryo 20	Cryo 30	Cryo 40	
Cryo 10	Cryo 21	Cryo 31	Cryo 41		Cryo 10	Cryo 21	Cryo 31	Cryo 41	

APPENDIX Item 11

MESA Exam 5 – Freezer Box Diagrams for Shipping MESA Classic Urine and Packed Cells Samples

MESA Exam 5 - MESA Classic Box Format for 3" Boxes (7 x 7 dividers).						
Ship 6 participants worth of packed RBCs, urine, and urine with acid aliquots.						
Pt 1 Tube# 19 (pRBC)	Pt 2 Tube# 19 (pRBC)	Pt 3 Tube# 19 (pRBC)	Pt 4 Tube# 19 (pRBC)	Pt 5 Tube# 19 (pRBC)	Pt 6 Tube# 19 (pRBC)	
Tube# 47 (3 mL urine)	Tube# 47 (3 mL urine)	Tube# 47 (3 mL urine)	Tube# 47 (3 mL urine)	Tube# 47 (3 mL urine)	Tube# 47 (3 mL urine)	
Tube# 48 (9 mL urine)	Tube# 48 (9 mL urine)	Tube# 48 (9 mL urine)	Tube# 48 (9 mL urine)	Tube# 48 (9 mL urine)	Tube# 48 (9 mL urine)	
Tube# 49 (9 mL urine)	Tube# 49 (9 mL urine)	Tube# 49 (9 mL urine)	Tube# 49 (9 mL urine)	Tube# 49 (9 mL urine)	Tube# 49 (9 mL urine)	
Tube# 50 (9 mL urine)	Tube# 50 (9 mL urine)	Tube# 50 (9 mL urine)	Tube# 50 (9 mL urine)	Tube# 50 (9 mL urine)	Tube# 50 (9 mL urine)	
Tube# 51 (9 mL urine/acid)	Tube# 51 (9 mL urine/acid)	Tube# 51 (9 mL urine/acid)	Tube# 51 (9 mL urine/acid)	Tube# 51 (9 mL urine/acid)	Tube# 51 (9 mL urine/acid)	
Tube# 52 (9 mL urine/acid)	Tube# 52 (9 mL urine/acid)	Tube# 52 (9 mL urine/acid)	Tube# 52 (9 mL urine/acid)	Tube# 52 (9 mL urine/acid)	Tube# 52 (9 mL urine/acid)	

MESA Epigenomics Table of Contents

Lab Protocol for MESA Epigenomics (Epi) Study at Exam 5

1. PURPOSE

pending

2. EQUIPMENT & SUPPLIES

1. WFU CHG will provide the following equipment:
   - 1 AutoMACS (Miltenyi Biotech)
   - 1 Countess Automated Cell Counter (Invitrogen)
   - 3 Mr. Frosty freezing containers (Sigma) – store at room temperature
   - 1 Cryovial Ice Bin
   - Refrigerated Centrifuge with a Horizontal (swing-out head) rotor

2. WFU CHG will provide the following supplies in bulk:
   - 8 mL cell preparation tubes (CPT) – store protected from light at room temperature (18-25°C)
   - 15 mL sterile conical centrifuge tubes
   - 50 mL sterile conical centrifuge tubes
   - 2.0 mL sterile cryovials
   - Countess Chamber Slides
   - Replacement Columns for AutoMACS (change every 2 weeks)
   - Replacement Air Filters for AutoMACS (change every 12 months)
   - Sample Storage Boxes (5x5x2 – 100 samples)
   - Styrofoam Mailers with outer Cardboard sleeves for shipping
   - Shipping labels

3. WFU CHG will provide the following reagents:
   - 10x PBS and Molecular Grade water for dilution
   - 0.4% Trypan Blue Stain
   - 70% Ethanol for AutoMACS
   - 7.5% BSA to make Running Buffer for the AutoMACS
   - 0.5mM EDTA to make Running and Rinsing Buffers for the AutoMACS
   - CD14 Microbeads for the AutoMACS
   - CD4 Microbeads for the AutoMACS
   - FBS to make Freezing Media A and B
   - DMSO to make Freezing Media B
   - Isopropanol (2-propanol) for the Mr. Frostys
   - RLT Lysis Buffer for sample storage

   - The blood processing area should have the following supplies and equipment:-70°C or -80°C Freezer
   - 4°C Refrigerator

- -20°C *non-defrosting* freezer
- Microcentrifuge for 2 mL cryovials (at 4°C if possible)
- Dedicated 2-3 feet Bench Space
- Lab coat and gloves
- Ice bucket with crushed ice, filled before start of processing
- Blood tube racks
- Participant ID Labels (provided by coordinating center)
- Conical tube racks for 15 mL and 50 mL tubes – 2 each
- 4 Cryovial racks for 2mL tubes
- MESA Epi Brief Protocol and data collection forms
- Pens
- 10% bleach and Ethanol for cleaning benchtop and pipettes
- Bleach for treating AutoMACS waste before disposal
- Clock
- Biohazards waste container
- 5 mL sterile transfer pipettes
- 1000 uL, 200 uL and 10 uL Micropipettes and DNAse/RNAse free pipette tips

3. METHODS

1. Participant ID Labels

1.1 The Coordinating Center will supply each field center with sheets of sample ID barcode and sample type labels to use for labeling draw tubes, working tubes, and cyrovials. All the tubes must be labeled prior to the sample process.

Tube Labeling Guide			
Label	**# tubes**	**tube type**	**Use**
CPT	4	CPT tubes	blood collection
CBC	1	EDTA tube	blood collection for CBC w/ Diff sent to Lab Corp
PBMC	1	50 ml conical	Pooling of cell layers from CPT tubes
Platelets	1	50 ml conical	Collection of Platelets from PBMC wash
Plasma	1	15 ml conical	Pooling of plasma layers from CPT tubes
CD14Pos Frac	1	15 ml conical	Collection of CD14 Positive Fraction (Monocytes)
CD14Neg Frac	1	15 ml conical	Collection of CD14 Negative Fraction
CD4Pos Frac	1	15 ml conical	Collection of CD4 Positive Fraction (T Cells)
CD4Neg Frac	1	15 ml conical	Collection of CD4 Negative Fraction
PLASMA	6	cryovials	Plasma Storage
PLATELETS	1	cryovial	Platelet Pellet Storage
PBMC-CRYO	1	cryovial	Cryopreserved PBMC Subset
PBMC-LYSIS	1	cryovial	PBMC Subset in Lysis Buffer
MONOCYTES	1	cryovial	Monocyte (CD14+ cells) Storage
TCELLS	1	cryovial	TCELL (CD4+) Storage
CD14/CD4 NEG	2	cryovials	Negative CD14-/CD4- Cell Storage - Cryopreserved
PBMC count	1	cryovial	PBMC Cell Count
Monocyte count	1	cryovial	Monocyte (CD4+ Fraction) Cell Count
CD14Neg count	1	cryovial	CD14 Negative Fraction Cell Count
Tcell count	1	cryovial	T Cell (CD4+ Fraction) Cell Count
CD14/CD4Neg count	1	cryovial	Negative (CD4 Negative Fraction) Cell Count
FORM	2	forms	form to sample coordination
	3	extra labels	just in case!

1.2 Each set of participant barcode labels has the same 7-digit sample identification number. It is critical that these labels be placed on all draw tubes, working tubes, and cryovials during processing to ensure that samples are not mixed up. *It may be helpful to write the last 3 digits on the top of all working tubes to make them more easily identified. It is also recommended that each participant's set of tubes be placed in a separate rack to reduce error in tube handling.*

2. Forms

2.1 The MESA Epi Data Collection Form provides a vital link between the sample ID number and the participant ID number. The form facilitates the monitoring of Sample collection, processing and other quality assurance parameters and provides information critical to the interpretation of results.

2.2 The MESA Epi Data Collection Form will be photocopied for records and original copy will be shipped with the samples to WFU Genomics. The form must be labeled with the correct pre-printed barcode sample ID label. All forms must be completed neatly in ink.

3. Processing CPT tubes

SEPARATION OF PLASMA AND PBMCS USING CPT TUBES

Blood will be collected into 4 heparin CPT tubes and 1 EDTA tube (for CBC) by the MESA phlebotomist. The EDTA tube collected at each site will be picked up by Lab Corp for processing.

When samples from several participants are run at once, it is *very important* to ensure that the samples *do not get mixed up*. *In addition, it is absolutely imperative that pipette tips and transfer pipettes be changed between samples from different participants*. Failure to do so could completely invalidate DNA and RNA results.

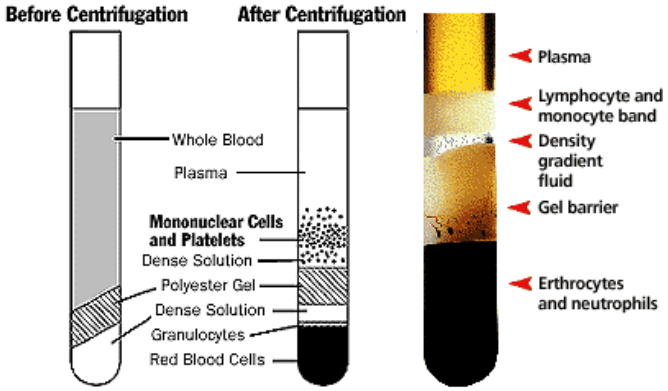
Be sure to record/copy the time at blood collection from the Blood Collection Form onto the MESA Epi Data Collection Form. This information is critical for timing the processing correctly.

Step 1: Centrifugation of CPT tubes.

If you need help calculating the centrifuge speed and time appropriate for your centrifuge, contact Megan Rudock (336-716-9842 or mrudock@wfubmc.edu).

CPT tubes MUST be centrifuged immediately. Invert each tube slowly 8 to 10 times to ensure the sample is adequately mixed (DO NOT SHAKE). The CPT tubes are then centrifuged at room temperature (20°C) in a horizontal rotor (swing-out head) for 30 minutes at 1500-1800 x g. Be sure to record the time of centrifugation on the MESA Epi Data Collection Form. *Excessive centrifuge speed (over 2000 x g) may cause tube breakage and exposure to blood and possible injury. The centrifuge must NOT be allowed to overheat. This may require a refrigerated centrifuge set at room temperature (20°C). Note that these tubes are taller (13cm) than other sample collection tubes and a different rotor may be necessary.*

Store centrifuged tubes upright at room temperature to allow batching of CPT tubes from several participants. These tubes must be stored in a safe area of the lab free from warm or cold drafts (ie. near a vent or centrifuge), away from windows, vibrations or heavy foot traffic. *Tubes MUST be process within 3 hours of the FIRST blood draw time.*



When centrifugation is complete, several layers will be evident. The top layer is plasma. Under this is a whitish cell layer (buffy coat with PBMCs) used for cell collection. Then there is a gradient gel layer that acts as a barrier to prevent contamination by the red cells which are at the very bottom of the tube.

Step 2: Instrument Start up and tube labeling

The AutoMACS magnetic separator MUST be primed every day before use. This must be started before processing the CPT tubes.
Place the labeled "waste" 50 mL conical tubes under the collection ports and the 15 mL "uptake waste" tube on the uptake port. Turn the machine on using the switch on the back right side. Initialization will take approximately 2-3 minutes. Once initialization is complete, select the "Clean" program. At this time the machine will begin flushing out all of the ethanol used in overnight storage and preparing for a sample.

Label ALL MESA Epi tubes for sample processing and storage according to the Tube Labeling Guide (See Methods section 1.1).

Step 3: Collecting Plasma

Following centrifugation, use a new transfer pipette for each sample and transfer 3 mL (or approximately half) of the plasma layer from each tube into a 15 mL conical vial "PLASMA", pooling each participant's samples. NOTE: if the tube was not filled to capacity, only remove half of the layer above the gel, which will be less than 3mL). *Be very careful not to disturb the cell layer.* Centrifuge the 15 mL conical vial at room temperature (20°C) for 15 minutes at 1500 x g. Using a fresh pipet tip for each participant, aliquot approximately 2 mL of plasma into 6 "PLAMSA" cyrovials; leaving behind a small amount of plasma and any pellet.

Step 4: Storage of plasma

Freeze the PLASMA cryovials IMMEDIATELY by placing in -70°C or colder freezer. Record the time the tubes were placed in the freezer on the MESA Epi Data Collection Form.

PBMC PROCESSING AND STORAGE

*When working with PBMCs, it is CRITICAL that all reagents and samples be kept refrigerated and **ALL WORK BE DONE ON ICE**. This dramatically reduces the amount of sample degradation and toxicity of the reagents.*

Step 5: Pelleting the PBMCs.

Next, decant or slowly pour the cell layer from each CPT tube into a 50 mL conical centrifuge tube labeled with participant ID# and "PBMCs". All 4 tubes for each individual will be pooled together into one (1) 50 mL conical tube. Fill the conical tube up to the 45 mL mark with *cold* Phosphate Buffered Saline (PBS). Mix gently by inverting tube 5 times and centrifuge for 15 minutes at 500 x g at 4°C. Record the centrifugation time on the Mesa Epi Data Collection From. Keep samples on ice between steps.

Step 6: Washing the PBMCs.

After centrifugation is complete, a small pellet should be evident in the bottom of the tube. Carefully remove the PBS solution by pipette into a new 50 mL conical tube labeled with the participant ID# and "PLATELETS". *Be extremely careful not to disturb the pellet.* Leave approx 1mL PBS behind to protect the pellet. *Centrifuge "PLATELETS" conical tube at 2,000 x g for 15 min at 4°C for Step 7.*

Add 5 mL fresh *cold* PBS to the "PBMC" tube and mix cells thoroughly by pipetting up and down with a 1000uL pipet. Verify that the cells have been completely resuspended in the PBS and cell clumps are not visible. Remove 2 aliquots of 300uL each into 2 cryovials labeled with the participant ID# and "PBMC-CRYO" and "PBMC-LYSIS" for step 8. Cap the 50 mL "PBMCs" conical tube and place in the refrigerator.

Step 7: Recovering Platelets from PBMC layer

After platelet centrifugation and being careful not to disturb the pellet, remove and discard supernatant. Leave a small amount behind in order to transfer the pellet to one cryovial, labeled "PLATELETS" using a transfer pipet. Centrifuge the platelet cryovial for 90 sec at 500 x g and draw off any remaining PBS. Place the pellet tube in a -70°C or colder freezer

IMMEDIATELY. Record this time on the MESA Epi Data Collection Form.

Step 8: Freezing small aliquot of PBMCs.

Centrifuge the "PBMC -CRYO" and "PBMC-LYSIS" cryovials for 15 minutes at 500 x g. Carefully remove all PBS without disturbing the cell pellets.

Cryopreservation: To the cryovials labeled "PBMC-CRYO" add 500 uL of *cold* Freezing Media A to each cryovial. Resuspend the pellet by flushing the pipette tip repeatedly. Add 500 uL *cold* Freezing Media B and mix by pipet until solution is homogenous. Place the cryovials in inside a Nalgene "Mr. Frosty" for slow freezing and place a -70°C or colder freezer.

Lysis Buffer: To the cryovials labeled "PBMC -LYSIS" add 350uL of RLT Lysis Buffer to each tube and mix thoroughly by pipet. Place this tube in a -70°C or colder freezer IMMEDIATELY.

Record the time tubes are placed in the freezer on the Data Collection Form. *This work must be done quickly so the medium stays cold. If the cells warm up enough to take up DMSO in the Freezing media, it will kill them.*

The "Mr. Frosty" must be at room temperature when the cryovials are placed inside him. The rack holding the tubes is place inside "Mr. Frosty" and then the entire container is placed in a -70°C (or colder) freezer overnight. Once "Mr. Frosty" is placed in the freezer, additional tubes cannot be added. When "Mr. Frosty" is removed from the freezer in the morning, remove the cryovials and place them in their appropriate freezer box at -70°C or colder. Allow "Mr. Frosty" to warm up to room temperature before using him again. Be sure to follow the "Mr. Frosty" maintenance protocol (see 6.1).

MONOCYTE ISOLATION AND STORAGE

Step 9: Determining PBMC Cell Count.

This Step may be started during the centrifugation/incubation in the previous step.

Determine the number of cells per mL by using the Invitrogen Countess automated cell counter. Push the **POWER** button to start the instrument. The start-up screen will be displayed. *Be sure the machine is set to **CELL MODE** and the MESA Epi Protocol is loaded.* Insert the provided USB

drive into the USB port. Label a Chamber slide with each sample number. *It is important to handle the chamber slides without touching the optical surfaces. Be sure to hold the chamber slides by the edges.*

Remove the "PBMCs" 50 mL conical tube from the refrigerator and put on ice. Gently swirl the sample to be sure the cells are in a homogenous suspension. Add 90uL of PBS to a cyrovial labeled "PBMC Count" and return the remainder to the ice bucket. Add 10uL of PBMC sample and 100uL of the supplied 0.4% Trypan Blue Stain to the cryovial. This is a 1:10 dilution of PBMCs. Mix gently by pipetting up and down. And 10uL of the sample mixture to the Chamber Port A. Insert the Countess Chamber Slide, sample side A first, into the slide inlet on the instrument, making sure that the slide is inserted completely. You will hear a soft click if the slide is pushed in correctly. Press the **Count Cells** or **Next Sample** button and then the **ZOOM** button. To make minor adjustments to the image, use the **Focus Knob** to gently adjust the image. ONLY VERY MINOR ADJUSTMENTS should be made to ensure the accuracy of subsequent cell counts. Optimize the image for analysis such that *Live cells have bright centers and dark edges and Dead cells have uniform blue color with no bright center.* When you are satisfied with the image, press **Count Cells.** *Cells must be counted within 3 minutes of mixing with Trypan Blue stain. This stain is toxic to cells and the cell count will no longer be accurate after 3 minutes.*

Record the cell count and viability on the MESA Epi Processing Sheet and press **SAVE** to save the image and counting data on the USB drive. Enter the file name using the keypad buttons displayed on the Save Menu. The file name will be the same as the Patient number followed "PBMC" and the date. "DIL10" will also be added to the file name if a 1:10 dilution has been done (**filename= sample#_PBMC_MMDDYY_DIL10**). After saving the data, press the **CLOSE** button to return to the main screen. Save the Chamber Slide for determining the Monocyte Count for this sample. Carefully return the slide to its original packaging for the next cell count. Once the data has been saved, the remainder of the trypan blue mixed sample may be discarded.

Complete the calculation on the Data Collection Sheet. The number of cells is multiplied by the number of mLs of sample (5.5); that will be the total PBMCs. This number will determine the amount of microbead solution to use. For example, 10×10^6 requires 80 uL running buffer to resuspend the pellet and 20 uL of Microbead solution.

Step 10: Preparation of PBMCs for Monocyte Collection.

It is important that all solutions are kept cold in the refrigerator. It is very important that all solutions and cells are kept cold. Keep the sample and

all reagents on ice when out of the refrigerator. Centrifuge 50mL conical tube of "PBMCs" at 500 x g for 10 minutes at 4°C. *Again, carefully remove as much PBS as possible without disturbing the pellet (It's OK to leave a few uL).* Resuspend in _____ uL *cold* AutoMACS Running Buffer (uL Running Buffer needed = Total PBMCs($x10^6$) x8) and put on ice.

Step 11: Adding Monocyte (CD14) MicroBeads.

Keep the cold Monocyte (CD14) MicroBeads on ice during use. Add _____uL MicroBeads (uL CD14 MicroBeads needed = Total PBMCs($x10^6$) x2) to the sample. Mix well by pipetting up and down about 10 times and incubate in the refrigerator (at 4°C) for 15 minutes. *Record the time on the MESA Epi Data Collection Form. Please stick exactly to the Magnetic Bead Incubation times and the temperature (in the fridge, not on ice). Incubating on ice leads to less efficient antibody staining, which means loss of recovery. Incubating too short times leads to same problem. Incubating too long leads to a decrease in purity because other cells (non-target cells) have the risk of getting non-specifically labeled.*

Wash cells by adding an additional 2 mL cold AutoMACS running buffer and centrifuge at 500 x g for 10 minutes at 4°C.

Remove all buffer without disturbing the pellet (leave about 0.5 mL behind) and add 1000 uL of fresh cold AutoMACS buffer and mix by pipetting up and down. Return to ice and process on the AutoMACS immediately.

Step 12: Collection of Monocytes (CD14 cells) using the AutoMACS.

Collect the 15mL tubes labeled with the Sample number and "CD14Pos Frac" or "CD14Neg Frac". Place the CD14 Positive tube into the collection port "Pos1" to collect the CD14 positive fraction. Place the CD14 Negative tub into the collection port "Neg1" to collect the depleted negative fraction. Apply tube containing sample to the "Uptake Port" of the AutoMACS.

Press the "**Separation**" button on the touch screen. Next scroll down and select "**PosSel**" for Positive Selection and press "OK". Record the time on the Data Collection Form. The AutoMACS will count down the time on the screen. Once the separation is complete, approximately 2 – 3 minutes, re-cap the labeled collection tubes and put both on ice for further processing. Be sure to record the Negative Fraction Elution volume on the MESA Epi Data Collection Form (line J).

Place the 50 mL "waste" tubes under all 3 collections ports and the 15 mL "uptake waste" tube on the uptake port. Press the "**QRinse**" button on the touch screen and the AutoMACS will perform a quick rinse of the internal tubing in preparation for the next sample. *This step must be completed between each sample.*

Step 13: Determining Monocyte Count.

Monocytes will be counted in a way similar to how PBMCs were counted. Pipette 10 uL of CD14 Positive Fraction into the cryovial labeled "Monocyte Count". Add 10 uL of the provided 0.4% Trypan Blue stain and mix gently by pipetting up and down. Add 10 uL to the chamber slide for this sample in Chamber Port B. Discard the remaining 10 uL. Insert the Chamber Slide, sample side B, into the slide inlet on the instrument, making sure to hear a soft click. Press the **Count Cells** or **Next Sample** button. Press **ZOOM** to view the cells. When you are satisfied with the image, press **Count Cells.** *Cells must be counted within 3 minutes of mixing with Trypan Blue stain. This stain is toxic to cells and the cell count will no longer be accurate after 3 minutes.*

Record the cell count and viability on the Data Collection Sheet and press **SAVE** to save the image and counting data on the USB drive. Enter the file name using the keypad buttons displayed on the Save Menu. The file name will be the same as the Sample number followed "Monocyte Count" and the date (**filename= sample#_MONOCYTE_MMDDYY**). After saving the data, press the **CLOSE** button to return to the main screen. Discard the Chamber Slide.

Step 14: Storage of Monocytes.

Centrifuge the 15mL"CD14Pos Frac" conical vials at 500 x g for 10 minutes at 4°C (may be done with the Negative fraction tubes after negative cell count). Remove all the buffer (except a few uL), being extremely careful not to disturb the cell pellet. Add 350 uL of RLT Lysis Buffer (600 uL for greater than $5x10^6$ cells). Mix thoroughly with a 1000uL pipet and transfer into 1 "MONOCYTES" cryovial. Cap the cryovial and freeze in the -70°C freezer IMMEDIATELY. Record time placed in freezer on the MESA Epi Data Collection Form.

T-CELL ISOLATION AND STORAGE

Step 15: Count the CD14 Negative Cells.

Negative Cells will be counted the same way PBMCs were counted. Pipette 90 uL of running buffer into the cryovial labeled "CD14Neg Count". Add 10 uL of CD14 Negative Fraction (from the 15 mL conical

tube) and 100 uL of the provided 0.4% Trypan Blue stain and mix gently by pipetting up and down. Add 10 uL to a new chamber slide labeled for this sample in Chamber Port A. Discard the remaining 190uL sample. Insert the Chamber Slide, sample side A, into the slide inlet on the instrument, making sure to hear a soft click. Press the **Count Cells** or **Next Sample** button. Press **ZOOM** to view the cells. When you are satisfied with the image, press **Count Cells.** *Cells must be counted within 3 minutes of mixing with Trypan Blue stain. This stain is toxic to cells and the cell count will no longer be accurate after 3 minutes.*

Record the cell count and viability on the Data Collection Sheet and press **SAVE** to save the image and counting data on the USB drive. Enter the file name using the keypad buttons displayed on the Save Menu. The file name will be the same as the Sample number followed "CD14Neg Count" and the date (**filename= sample#_CD14Neg_MMDDYY_DIL10**). *Only include "DIL10" in the file name if the sample was diluted 1:10.* After saving the data, press the **CLOSE** button to return to the main screen. Save the Chamber Slide for determining the T-Cell Count for this sample.

Complete the calculation on the Data Collection Sheet. The number of cells is multiplied by the number of mLs of sample (typically about 3 mL); that will be the total CD14 Negative Cells. This number will determine the amount of microbead solution to use.

Step 16: Preparation of CD14 Negative Cells for T-cell (CD4) Collection.

Centrifuge CD14Neg Cells in the "CD14Neg Frac" conical vials at 500 x g for 10 minutes at 4°C. *Again, carefully remove as much PBS as possible without disturbing the pellet.* Leave a few uL behind. Resuspend the cells in _____uL *cold* AutoMACS Running Buffer (uL Running buffer needed = Total Neg Cells($x10^6$) x8) and put on ice.

Step 17: Adding T-Cell (CD4) MicroBeads.

Keep the cold T-cell (CD4) MicroBeads on ice during use. Add _____uL MicroBeads (uL CD4 MicroBeads needed = Total Neg Cells($x10^6$) x2) to the sample. Mix well by inverting 10 times and incubate in the refrigerator (at 4°C) for 15 minutes. Record time on MESA EPi data collection form.

Wash cells by adding an additional 2 mL cold AutoMACS running buffer and centrifuge at 500 x g for 10 minutes at 4°C. Remove all but approximately 0.5mL buffer without disturbing the pellet and add 1000 uL of fresh cold AutoMACS buffer and mix by pipetting up and down. Return to ice and process on the AutoMACS immediately.

Step 18: Collection of T-Cells using the AutoMACS.

Collect the 15mL tubes labeled with the appropriate Sample number and "CD4Pos Frac" or "CD4Neg Frac". Place the T-Cell CD4 Positive tube into the collection port "Pos1" to collect the T-cell positive fraction. Place the T-Cell CD4 Negative tube into the collection port "Neg1" to collect the depleted negative fraction. Apply tube containing sample to the "Uptake Port" of the AutoMACS.

Press the "**Separation**" button on the touch screen. Next scroll down and select "**PosSel**" for Positive Selection and press "OK". Record the time on the Data Collection Form. The AutoMACS will count down the time on the screen. Once the separation is complete, approximated 2 – 3 minutes, the re-cap the labeled collection tubes and put both on ice for further processing.

Place the 50 mL "waste" tubes under all 3 collections ports and the 15 mL "uptake waste" tube on the uptake port. Press the "**QRinse"** button on the touch screen and the AutoMACS will perform a quick rinse of the internal tubing in preparation for the next sample. *This step must be completed between each sample.*

Step 19: Determining T-Cell Count.

T-Cells will be counted the same way PBMCs were counted. Pipette 10 uL of T-Cell CD4 Positive Fraction into the cryovial labeled "TCell Count". Add 10 uL of the provided 0.4% Trypan Blue stain and mix gently by pipetting up and down. Add 10 uL to the chamber slide for this sample in Chamber Port B. Discard the remaining 10 uL. Insert the Chamber Slide, sample side B, into the slide inlet on the instrument, making sure to hear a soft click. Press the **Count Cells** or **Next Sample** button. Press **Zoom** to view the cells. When you are satisfied with the image, press **Count Cells.** *Cells must be counted within 3 minutes of mixing with Trypan Blue stain. This stain is toxic to cells and the cell count will no longer be accurate after 3 minutes.*

Record the cell count and viability on the Data Collection Sheet and press **SAVE** to save the image and counting data on the USB drive. Enter the file name using the keypad buttons displayed on the Save Menu. The file name will be the same as the Sample number followed "T-Cell Count" and the date **(filename= sample#_TCELL_MMDDYY**). After saving the data, press the **CLOSE** button to return to the main screen. Discard the Chamber Slide.

Step 20: Determining Negative Cell Count

Negative Cells will be counted the same way PBMCs were counted. Pipette 90uL Running Buffer into the cryovial labeled "CD14/CD4Neg Count". Add 10 uL of Negative Fraction (from the conical tube) and 100 uL of the provided 0.4% Trypan Blue stain and mix gently by pipetting up and down. Add 10 uL to the chamber slide in the unused Chamber Port. Discard the remaining sample. Insert the Chamber Slide into the slide inlet on the instrument, making sure to hear a soft click. Press the **Count Cells** or **Next Sample** button. View the cells by pressing the **Zoom** button. When you are satisfied with the image, press **Count Cells.** *Cells must be counted within 3 minutes of mixing with Trypan Blue stain. This stain is toxic to cells and the cell count will no longer be accurate after 3 minutes.*

Record the cell count and viability on the Data Collection Sheet and press **SAVE** to save the image and counting data on the USB drive. Enter the file name using the keypad buttons displayed on the Save Menu. The file name will be the same as the Sample number followed "Negative Count" and the date (**filename= sample#_CD14/CD4Neg_MMDDYY_DIL10**). *Only include "DIL10" in the file name if the sample was diluted 1:10.* After saving the data, press the **CLOSE** button to return to the main screen. Discard the Chamber Slide after both Chambers have been used

Step 21: Storage of T-Cells

Centrifuge the 15mL "CD4Pos Frac" conical vials at 500 x g for 10 minutes at 4°C (may be done with the Negative fraction tubes after Negative cell count). Remove all the buffer (except a few uL), being extremely careful not to disturb the cell pellet. Add 350 uL of RLT Lysis Buffer (600 uL for greater than $5x10^6$ cells). Mix thoroughly with a 1000uL pipet and transfer into 1 cryovial labeled "TCELLS". Cap the cryovial and freeze in the -70°C freezer IMMEDIATELY. Record time on the MESA Epi Data Collection Form.

Step 22: Storage of Negative Cells.

Centrifuge the 15 mL "CD4Neg Frac" conical vial for 10 minutes at 500 x g at 4°C. Carefully remove all buffer *without disturbing the pellet.* Leave a little buffer if necessary. Add 1000 uL of *cold* Freezing Media A to the tube containing the Negative Cell pellet. Resuspend the pellet by flushing the pipette tip repeatedly. Add 1000 uL *cold* Freezing Media B and mix until the solution is homogenous. Aliquot the Negative cells to 2 cyrovials (1mL in each vial) labeled "CD14/CD4NEG". *This work must be done quickly so the medium stays cold. If the cells warm up enough to take up DMSO, it will kill them.* Place the cryovials in a rack that can be placed inside another Nalgene "Mr. Frosty #2" for slow freezing. *Do NOT place tubes in the same "Mr. Frosty" with PBMCs that is already in the freezer.*

Record the time tubes are placed in the freezer on the Data Collection Form.

The "Mr. Frosty" must be at room temperature when the cryovials are placed inside him. The rack holding the tubes is place inside "Mr. Frosty" and then the entire container is places in a -70°C (or colder) freezer overnight. Once "Mr. Frosty" is placed in the freezer, additional tubes cannot be added. When "Mr. Frosty" is removed from the freezer in the morning, remove the cryovials and place them in their appropriate freezer box at -70°C or colder. Allow "Mr. Frosty" to warm up to room temperature before using him again. Be sure to follow the "Mr. Frosty" maintenance protocol (see 6.1).

Step 22: Shutting Down the Instruments

AutoMACs: Make sure all waste tubes are under each port (including sample) and press Sleep button. The instrument will do a thorough rinse with ethanol and rinse solutions. One the screen shows that the rinse is complete turn the instrument off.

Countess Automated Cell Counter: Be sure that the last Chamber Slide to be used has been removed. Press the Power button on the face of the instrument. Remove the Flash Drive. The cell count files from each sample have been saved to this flash drive, which will be shipped to WFU Genomics monthly. Record shutdown time on the MESA Epi Data Collection Form.

3.4 Special Circumstances

3.41 If centrifugation cannot be performed within 3 hrs of collection, process specimens *as soon as possible* after that time. Record the time of collection and centrifugation on the Data Collection Form. This is NOT recommended. Only processing times up to 3 hrs have been tested.

3.42 If cells cannot be frozen at -70°C immediately, do it *as soon as possible* after that. They may be *temporarily* placed on dry ice until transfer to -70°C freezer is possible. Record BOTH times on the Data Collection Form and make a special note.

3.43 If blood collection is incomplete, please use the following guidelines to ensure sample quality.

- If blood is greater than 6 mL (75%), then proceed as usual
- If blood is between 4 mL (50%) and 6 mL (75%), proceed as usual
- **If blood is less than 4 mL, discard the partially filled tube in biohazard**

3.44 If the Automated Cell Counter states that the concentration is above or below the recommended range.

- If the concentration is above the recommended range, use a 1:10 dilution. To do this, add 10uL sample to 90uL of PBS or Running Buffer and 100uL of 0.4% Trypan blue stain. After mixing with a pipet, add 10uL to a new chamber slide and obtain a new cell count. When you save the cell count after a dilution "DIL10" should be added to the end of the file name.
- If the concentration is below the recommended range, try counting the sample again without diluting it. If the sample was not originally diluted, make a note on the Data Collection form and continue the procedure as best as possible using the less certain cell count.

3.45 If the Automated Cell Counter states that the viability is below 90%, adjust the focus and recount the same sample. Save the new file under the same file name with _NEW added to the end. Write both counts on the processing form and make note of the reason for recounting cells. Use the NEW count for any calculations.

3.46 If there are large cell clumps visible on the Automated Cell Counter, use a 1000uL pipet to further suspend the cells in the original sample. Take a new aliquot and complete restart the cell counting procedure. Save the new file under the same file name with _NEW added to the end. Write both counts on the processing form and make note of the reason for recounting cells. Use the NEW count for any calculations.

4. Shipping Blood Samples

4.1 General Instructions: Blood samples may be shipped biweekly on Mondays to the WFU Center for Human Genomics by an overnight carrier (Federal Express is preferred). Samples must be shipped on the pre-arranged schedule, which allows the laboratory to anticipate the arrival of samples for easier processing.

4.2 Packaging - sample shipping checklist:

- Frozen MESA samples in pre-labeled sample boxes
- Styrofoam mailing container with outer cardboard sleeve
- Rubber bands for freezer boxes
- Ziploc plastic bags for freezer boxes
- Absorbent material (e.g., paper towels, newspaper)
- Packaging tape
- Dry ice (~10 pounds per mailing container)

- Ice gel packs
- Mailing labels (provided by carrier)
- Dry ice labels
- Completed MESA Epi Data Collection Form
- The Countess Flash Drive (first shipment of each month)
- Completed Shipping Form

4.3 Procedure: This shipping protocol follows procedures mandated for frozen shipment by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904.

1. Line Styrofoam mailer(s) with absorbent material.

2. Place approximately ½ the dry ice (about 5 pounds) on the bottom of the mailer.

3. Place another layer of absorbent material on top of the dry ice, so that it will be between the dry ice and the freezer boxes containing the samples.

4. Collect the freezer boxes containing samples to be shipped and check the sample ID numbers against the Data Collection Form for that shipment

5. Put a rubber band around each cardboard freezer box containing samples before enclosing each box in a Ziploc plastic bag. Carefully place these bagged boxes into the mailer. The rubber band helps prevent cryovial spill; the Ziploc bag and absorbent material are required by commercial carriers

6. Place another layer of absorbent material on top of the sample freezer boxes.

7. Place the remaining dry ice on top of this last layer of absorbent material.

8. Seal the top of the Styrofoam mailer with tape and then place the mailer in the outer cardboard sleeve.

9. Place the Data Collection Forms for all samples on top of the Styrofoam mailer before the outer sleeve is securely taped closed. If the Countess Flash drive is being included in this shipment, seal it in a small Ziploc plastic bag and place it on top of the Data Collection forms before closing the outer sleeve.

10. Fill out the shipping log, including the carrier airbill numbers, and fax to Wake Forest University at 336-716-6427.

11. Affix shipping label(s). Place the entire box in the refrigerator, if pickup will not be immediate. *Samples should not be on dry ice more than 24 hours.*

4.4 Mailing Address:

4.41 *Shipping to the Wake Forest University Center for Human Genomics*

The following samples will be shipped to the WFU Center for Human Genomics for Processing

- PLASMA (6 cryovials)
- PLATELETS (1cryovial)
- PBMC SUBSET (2 cryovials: CRYOPRE and LYSIS)
- MONOCYTES CD14+ (1 cryovial)
- TCELL CD4+ (1 cryovial)
- NEGATIVE CD14-/CD4- (2 cryovials)

Tim Howard, PhD
Center for Human Genomics
NRC Building, Room 319
Medical Center Blvd
Wake Forest University School of Medicine
Winston-Salem, NC 27157
Phone: (336) 713-7509
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5. REAGENT DESCRIPTIONS

5.1 Preparation of Reagents

5.11 Phosphate Buffered Saline (PBS): PBS will be shipped as a 10X concentrated solution. PBS shall be diluted using molecular grade water to achieve a 1X concentration. 1X PBS will be made one liter at a time by combing 100 mL of 10X PBS with 900 mL of Molecular grade water. This should be done in a 1000 mL (or 1 L) graduated cylinder to ensure the proper amounts of each solution.

5.12 Freezing Medium A:. Freezing Medium A is 100% Fetal Bovine Serum or FBS. Two mL aliquots at are stored at -20°C. Working tubes may be kept at 2 – 8 °C for up to 2 days.

5.13 Freezing Medium B: Freezing Medium B consists of FBS with 15% DMSO (dimethyl sulfoxide). This will be prepared in a 50 mL conical tube by adding 7.5 mL DMSO to 42.5 mL FBS. Invert 20 times to mix. Make 2 mL aliquots and freeze at -20°C. Working tubes may be kept at 2 – 8 °C for up to 2 days.

5.14 Running Buffer for the AutoMACs: Running buffer consists of PBS, 0.5% BSA and 2mM EDTA. To make Running buffer, add 66.7mL of 7.5% BSA and 4 mL of 0.5mM EDTA to 1L (1000mL) of 1X PBS. Feel free to make 3-5 liters at a time since the AutoMACS uses this buffer quickly. Running buffer should be stored at 2 – 8 °C for up to 2 weeks. Be sure to let this buffer come to room temperature before filling the bottle on the AutoMACs. However, cold Running buffer should be used in the processing protocol.

5.15 Rinsing Buffer for the AutoMACs: Rinsing buffer should only be made 1L at a time and can be stored at room temperature. To prepare Rinsing Solution, add 4mL of 0.5mL EDTA to 1L (1000mL) of 1X PBS.

5.2 Reagent Log Sheets

The use of every reagent will be logged on a Reagent Log Sheet. Each Sheet will include the Reagent name, manufacturer and (catalog number, size, etc), bulk storage requirements and working reagent storage requirements. Upon receiving any reagent, the LOT number, manufacturer's expiration date and date received are recorded. The data received will also be written on the label of the reagent. Once opened the data opened and expire date will also be recorded.

Storage of Reagents will be based on the information provided at the top of each Reagent Log Sheet for both Bulk Storage as well as Working Reagent Storage. Be sure to adhere strictly to these requirements.

6. EQUIPMENT MAINTENANCE

6.1 Maintaining the Mr. Frosty

The isopropanol (2-propanol) in the Mr. Frosty must be replaced after 5 uses. This is easily tracked by placing a piece of white lab tape on the side of Mr. Frosty and writing the numbers, 1-5 on the tape. Each time the unit is thawed mark through the appropriate number. When number 5 cycle has been completed, empty the alcohol and fill with fresh isopropanol. Add a new piece of tape numbered 1 – 5.

It is also recommended to use a piece of white lab tape on top of the unit to indicate when the samples can be removed from Mr. Frosty and placed into a regular storage box (overnight preferred, minimum of 4 hours). Be sure to transfer the sample to

the freezer box first thing in the morning so that the Mr Frosty can return to room temperature for that day's samples.

6.2 Maintaining the AutoMACS

6.21 End of Day: Make sure all waste tubes are under each port (including sample) and press Sleep button. The instrument will do a thorough rinse with ethanol and rinse solutions. One the screen shows that the rinse is complete turn the instrument off.

6.22 Friday Afternoon or periods of > 24 hours when not in use: After completing the End of Day sequence on Friday afternoon of if the instrument sits more than 24 hours between uses, place a 15ml tube with 5ml deionized water on the sample holder to prevent corrosion of the sample sipper.

6.23 Reagents and Buffers:

- All bottles on the instrument have level sensors. The machine will display a notification on the screen should any of the solutions become low.
- *70% ETHANOL* (FIRST BOTTLE ON RIGHT WHEN STANDING AT THE INSTRUMENT). This is used for thorough cleaning at the end of the day by the instrument when you use the SLEEP mode.
- *Running Buffer*, Blue Connection, second from right on instrument – made in the lab and should be stored at 2 – 8°C.
- *Rinsing Solution*, Yellow Connection, third from right on the instrument – made in the lab and may be stored at room temperature.
- *Column*, Miltenyi Biotech, Catalog # 140-021-101, column on the right (left column is a blank)– Store at room temperature, change every 2 weeks.
- *Air filters* (one on each of the three bottle caps, (blue - buffer, yellow - rinse, and red - waste)- Change every 6 -12 months, depending on the air quality in the room.

6.3 Maintaining the Countess Automated Cell Counter

The Invitrogen Countess Automated Cell Counter does not require any regular maintenance. The screen or body of the countess may be wiped down with a damp cloth as needed.

Brief Protocol for MESA Epigenomics Study at Exam 5 **Date:** _____

SEPARATION OF PLASMA AND PBMCS USING CPT TUBES

Blood will be collected into 4 heparin CPT tubes and 1 EDTA tube (for complete blood count) by the MESA phlebotomist. The EDTA tube collected at each site will be picked up by Lab Corp for processing.

Step 1: Centrifugation of CPT tubes.

- Copy the Blood Collection time from the Blood Collection Form onto the MESA Epi Processing Form
- Slowly invert each CPT tube 8-10 times to mix
- *Centrifuge immediately at 20°C for 30 min at 1800 x g* (be sure centrifuge brake is off)
- Tubes may sit at room temperature for up to 3 hour to allow batching (see MOP Guidelines)
- Record Time on Data Collection Form

Step 2. Instrument Start-up and Tube Labeling.

- Power On the AutoMACS
- Be sure "waste" conical tubes are on uptake port and all collection ports
- Select "Clean"
- Label all tubes necessary for Sample Processing according to Tube Labeling Guide in the MOP

Step 3. Collecting Plasma

- Transfer half of the plasma (~3mL of full 8mL tube) into 15 mL Conical Tube, Pooling each participants samples
- *Centrifuge at 20°C for 15 min at 1500 x g*
- Aliquot 2mL plasma into each "PLASMA" cryovial, without getting near pellet (leave behind some plasma)

Step 4. Storage of Plasma

- Freeze plasma by placing in -70 or -80°C Freezer IMMEDIATELY
- Record Time Placed in Freezer on Data Collection Form

PBMC PROCESSING AND STORAGE – WORK ON ICE

Step 5. Pelleting the PBMCs.

- Decant, or slowly pour, the Mononuclear Cell Layer from the CPT tube into a 50 mL "PBMC" conical tube, pooling each participant's samples and place on ice
- Fill the tube up to the 45 mL mark with cold PBS and mix gently by inverting 5 times
- *Centrifuge at 4°C for 10 min at 500 x g*
- *RECORD TIME ON DATA COLLECTION FORM*

Step 6. Washing the PBMCs.

- Transfer PBS by pipette, leaving approx 1mL behind, into a new 50 mL conical tube labeled "platelets"
- *Centrifuge "platelets" conical tube at 2,000 x g for 15 min at 4°C for Step 7.*
- Add 5 mL cold PBS to "PBMC" tube and resuspend cells by pipetting up and down with a 1000 uL pipet.
- Remove 2 aliquots of 300 uL into 2 cyrovials labeled as "PBMC-CRYO" and "PBMC-LYSIS" for Step 8
- Place the 50 mL "PBMC" conical tube in the refrigerator for Step 9

Step 7. Recovering Platelets from PBMC layer

- From 50 ml "platelet" coninical, slowly pour off 75% supernatant and pipet the rest, leaving about 1mL behind
- Using a 1000mL pipet – gently disrupt the pellet and transfer into "PLATELETS" cryovial NOTE: Pellet should remain clumpy for transfer, do NOT completely resuspend
- *Spin for 90 sec at 500 x g* and draw off any remaining supernatant.
- Freeze platelet pellet IMMEDIATELY by placing in a -70°C or colder freezer.
- RECORD TIME ON DATA COLLECTION FORM.

Step 8. Freezing small aliquot of PBMCs.

- *Centrifuge "PBMC subset" cryovials at Room Temperature for 15 minutes at 500 x g*
- Carefully remove all PBS without disturbing cell pellets
- Cryopreserve vial:
    - Add 500 uL of Freezing Media A (FBS) to the "PBMC-CRYO" cryovial and mix well by pipet
    - Add 500 uL of Freezing Media B (FBS + 15% DMSO) to each cryovial and mix by pipetting up and down
    - Place cryovial in "Mr. Frosty #1" and place in -70°C or colder freezer to slowly freeze cells
- Lysis Buffer vial
    - Add 350 uL of RLT PLUS Lysis Buffer to the "PBMC-LYSIS" tube and mix thoroughly by pipet
    - Place cryovial directly in a -70°C or colder freezer.
- Record Time Placed in Freezer on Data Collection Form

MONOCYTE ISOLATION AND STORAGE

Step 9. Determining PBMC Cell Count.

- Turn on Countess and verify that it is in Cell Mode and Sensitivity is set to 6.

- Insert USB drive and label Chamber Slide with sample number
- Mix the sample by pipetting up and down to be sure cell suspension is homogenous
- Mix 10 uL PBMC Sample + 90 uL PBS and 100 uL 0.4% Trypan Blue gently by pipetting up and down
- Add 10 uL of Sample Mixture into Chamber Port A (remainder discarded)
- Insert sample side A into Countess and press Count Cells (or Next Sample)
- Press Zoom to verify the cells are in focus
- Optimize image so Live cells have bright centers and dead cells are dark
  NOTE: only minimal adjustment should be necessary
- Press Count Cells
- Record Cell count and viability on Data Collection Form (lines A and B)and press Save filename: **SAMPLE#_PBMC_DATE_DIL10** (Date will be MMDDYY)
- *If large cell clump are visible, prepare and count a new aliquot. If the viability is below 90%, adjust the focus and recount the same sample. In either case, save the new file under the same file name with _NEW added to the end. Write both counts on the processing form and make note of the reason for recounting cells. Use the NEW count for any calculations.
- Press close and remove Chamber slide (save for monocyte count)
- Complete calculation on Data Collection Form

Step 10. Preparation of PBMCs for Monocyte Collection

- *Centrifuge PBMCs at 4°C for 10 min at 500 x g*
- Carefully remove PBS without disturbing the cell pellet – leave behind a few uL
- Resuspend in (from Line d on data collection form) uL of Cold Running Buffer and put on ice

Step 11. Adding Monocyte (CD14) MicroBeads

- Add (from Line E on data collection form) uL MicroBeads to sample
- Mix well by pipetting up and down
- Incubate at 4°C *in refrigerator* for 15 min, recording time at start of incubation on Data Collection Form
- Add 2 mL cold Running Buffer to wash
- *Centrifuge at 4°C for 10 min at 500 x g*
- Remove all buffer without disturbing cell pellet – leave behind up to 0.5 mL
- Add 1000 uL cold AutoMACS Running Buffer and mix with pipet
- Return to ice

Step 12. Collection of Monocytes (CD14 cells) using the AutoMACS

- Apply 15 mL tube labeled CD14 Positive to the Outlet Port "Pos1"
- Apply 15 mL tube labeled CD14 Negative to the Outlet Port "Neg1"
- Apply sample tube to the Uptake Port on the AutoMACS
- Select "Separation"

- Scroll down to “PosSel” for Positive Selection and press OK
-
- Record the Negative Fraction Elution Volume on the Data Collection Form (line J)Once separation is complete, removed the labeled tubes and put on ice
- Place the 50 mL “waste” tubes under all 3 collection ports and the clean 15 mL “uptake waste” tube to the uptake port
- Select “QRinse” to rinse and prepare the machine for the next sample
- Keep the Positive and Negative Fraction Tubes on ice for further processing

Step 13. Determining Monocyte Count

- Verify that Countess is in Cell Mode and the USB is inserted
- Mix 10 uL Monocyte (CD14) Sample and 10 uL 0.4% Trypan Blue gently by pipetting up and down
- Add 10 uL of Sample Mixture into Chamber Port B (remainder discarded)
- Insert sample side B into Countess and press Count Cells (or Next Sample)
- Pressing Zoom to verify that cells are in focus
- Optimize image so Live cells have bright centers and dead cells are not
- Press Count Cells
- Record Cell count and viability on Data Collection Form (lines F and G) and press Save

  filename: **SAMPLE#_MONOCYTE_DATE** (Date will be MMDDYY)
- Press close and remove Chamber Slide (discard)

Step 14. Storage of Monocytes

- *Centrifuge “CD14Pos Frac” conical vials at 4°C for 10 min at 500 x g* (can be centrifuged with CD14- cells after negative cell count)
- Carefully remove the buffer without disturbing the cell pellet (a tiny amount of buffer may be left behind if necessary, no more than 20 uL)
- Add 350uL (600uL if more than $5x10^6$ cells) of Cell Lysis RLT PLUS buffer and mix thoroughly by pipet
- Transfer into “MONOCYTE” cryovial and place IMMEDIATELY in the -70°C or colder freezer
- Record Time placed in Freezer on Data Collection Form

T-CELL ISOLATION AND STORAGE

Step 15. Count CD14 Negative Cells

- Mix 10 uL CD14 Neg Cell Sample, 90 uL Running Buffer and 100 uL 0.4% Trypan Blue
- Mix gently by pipetting up and down
- Add 10 uL of CD14 Negative Sample Mixture into Chamber Port A

- Insert sample side A into Countess and press Count Cells (or Next Sample)
- Press Zoom to verify cells are in focus
- Optimize image so Live cells have bright centers and dead cells are dark
- Press Count Cells
- Record Cell count and viability on Data Collection Form (lines H and I) and press Save filename: **sample#_CD14NEG_DATE_DIL10** (Date will be MMDDYY)
- Press close and remove Chamber slide (save for T-cell count)
- Complete calculation on Data Collection Form

Step 16. Preparation of CD14 Negative Cells for T-cell (CD4) Collection

- *Centrifuge "CD14Neg Frac" conical vials at 4°C for 10 min at 500 x g*
- Carefully remove buffer without disturbing the cell pellet – a few uL may be left behind
- Resuspend in (from line M on data collection form) uL of Cold Running Buffer and put on ice

Step 17. Adding T-Cell (CD4) MicroBeads

- Add (from line N on data collection form) uL MicroBeads to sample
- Mix well by pipetting up and down
- Incubate at 4°C *in refrigerator* for 15 min, recording the start time on the data collection form.
- Add 2 mL cold Running Buffer to wash
- *Centrifuge at 4°C for 10 min at 500 x g*
- Remove all buffer without disturbing cell pellet – leave behind up to 0.5mL
- Add 1000 uL cold AutoMACS Running Buffer and mix by pipet
- Return to ice

Step 18. Collection of T-Cells using the AutoMACS

- Apply 15 mL tube labeled T-Cell CD4 Positive to the Outlet Port "Pos1"
- Apply 15 mL tube labeled T-Cell CD4 Negative to the Outlet Port "Neg1"
- Apply sample tube to the Uptake Port on the AutoMACS
- Select "Separation"
- Scroll down to "PosSel" for Positive Selection and press OK
- Record the time on the Data Collection Form
- Once separation is complete, removed the labeled tubes and put on ice
- Place the 50 mL "waste" tubes under all 3 collection ports and the clean 15 mL "uptake waste" tube to the uptake port
- Select "QRinse" to rinse and prepare the machine for the next sample
- Keep T-Cell and CD4 Negative tubes on ice for further processing (Step 21)

Step 19. Determining T-Cell Count

- Mix 10 uL T-Cell (CD4) Sample and 10 uL 0.4% Trypan Blue gently by pipetting up and down

- Add 10 uL of Sample Mixture into Chamber Port B (discard remainder)
- Insert sample side B into Countess and press Count Cells (or Next Sample)
- Press Zoom to verify cells are in focus
- Optimize image so Live cells have bright centers and dead cells are dark
- Press Count Cells
- Record Cell count and viability on Data Collection Form (lines O and P) and press Save filename: **SAMPLE#_TCELL_DATE** (Date will be MMDDYY)
- Press close and remove Chamber Slide (discard)

Step 20. Determining Negative Cell Count

- Mix 10 uL Negative Sample 90 uL Running Buffer and 100 uL 0.4% Trypan Blue
- Mix gently by pipetting up and down
- Add 10 uL of Sample Mixture into Chamber Port B (discard remainder)
- Insert sample side B into Countess and press Count Cells (or Next Sample)
- Press Zoom to verify cells are in focus
- Optimize image so Live cells have bright centers and dead cells are dark
- Press Count Cells
- Record Cell count and viability on Data Collection Form (Lines Q and R) and press Save filename: **SAMPLE#_NEGATIVE_DATE_DIL10** (Date will be MMDDYY)
- Press close and remove Chamber Slide (discard)

Step 21. Storage of T-Cells

- *Centrifuge "CD4Pos Frac" conical vials at 4°C for 10 min at 500 x g* (May be spun with Negative cells)
- Carefully remove the buffer without disturbing the cell pellet (a tiny amount of buffer may be left behind if necessary, no more than 20 uL)
- Add 350uL (600uL if more than $5x10^6$ cells) of Cell Lysis RLT PLUS buffer to pellet and mix thoroughly by pipet
- Transfer into "TCELLS" cryovial and place IMMEDIATELY in the -70°C or colder freezer
- Record Time placed in Freezer on Data Collection Form

Step 22. Storage of Negative Cells

- Centrifuge Negative Fraction in *"CD4Neg Frac" conical vials* at 4°C for 10 min at 500 x g
- Carefully remove all buffer without disturbing the cell pellet
- Add 1000 uL of Freezing Media A and mix by pipet
- Add 1000 uL of Freezing Media B and mix by pipet
- Aliquot Negative Fraction into 2 "CD14/CD4 NEG" cryovials (1 mL per vial)

- Deposit cryovials in Mr. Frosty #2 and place Mr. Frosty #2 in - 80°C freezer overnight
- Record the time on the Data Collection Form

Step 23: Shutting Down the Instruments

AutoMACS

- ☐ Make sure all waste tubes are under each port (including sample) and press Sleep button.
- ☐ Instrument will do a thorough rinse with ethanol and rinse solutions.
- ☐ Turn instrument off.

Countess Automated Cell Counter

- ☐ Verify that the last Chamber Slide has been removed
- ☐ Press the Power button to turn the instrument off
- ☐ Record Shutdown time on the Data Collection Form.

SHIPPING OF SAMPLES

This processing sheet and each MESA Epi Data Collection Form should be shipped with the samples to WFU Center for Human Genomics on a bi-weekly basis (every other Monday). The Countess Flash Drive will also be shipped in the first shipment of each month for data recovery.

Shipping to the Wake Forest University Center for Human Genomics

The following samples will be shipped to the WFU Center for Human Genomics for Processing

- ☐ PLASMA (6 cryovials)
- ☐ PLATELETS (1cryovial)
- ☐ PBMC SUBSET (2 cryovials: CRYOPRE and LYSIS)
- ☐ MONOCYTES CD14+ (1 cryovial)
- ☐ TCELL CD4+ (1 cryovial)
- ☐ NEGATIVE CD14-/CD4- (2 cryovials)
- ☐ Place all cyrovial storage boxes in the provided well-insulated Styrofoam shipping container generously packed with dry ice for overnight shipment to the following address:

Tim Howard, PhD
Center for Human Genomics
NRC Building, Room 319
Medical Center Blvd
Wake Forest University School of Medicine
Winston-Salem, NC 27157
Phone: (336) 713-7509
Fax: (336) 713-7566

Be sure to follow the guidelines in the MOP for shipping and packaging instructions.

MESA EPIGENOMICS DATA COLLECTION FORM

Date: _____/_____/_____ (MM/DD/YY)

Phlebotomist ID:_____

Processing Lab ID:_____

Place Sample ID Label Here

SEPARATION OF PLASMA AND PBMCS USING CPT TUBES

Step 1: _____ Time at Blood Collection

_____ Time at CPT Centrifugation

Step 4: _____ Time Plasma Placed in Freezer

Notes: _____

_____

_____

PBMC PROCESSING AND STORAGE

Step 5: _____ Time at PBMC Centrifugation

Step 7: _____ Time Platelets place in Freezer

Step 8: _____ Time Mr. Frosty #1 Placed in Freezer

MONOCYTE ISOLATION AND STORAGE

Step 9: A:_____ PBMCs x10^6/ mL Diluted 1:10 B:_____ viability

C:_____ Total PBMCs (x10^6) = **(A) x 5.5mL**

D:_____ uL Running Buffer needed = **(C) x8**

E:_____ uL CD14 MicroBeads needed = **(C) x2**

Step 11: _____ Time at Start of CD14+ Incubation

Step 13: F: _____ Monocytes x10^6/ mL (CD14 + Fraction) G: _____ viability

Step 14: _____ Time CD14+ Placed in Freezer

T-CELL ISOLATION AND STORAGE

Step 15: H:_____ CD14Neg x10^6/ mL (diluted 1:10) I:_____ viability

J:_____ Total Volume from AutoMACs (typically ~3mL)

L:_____ Total CD14Neg Cells (x10^6) = **(H) X (J)**

M:_____ uL Running Buffer needed = **(L) x 8**

N:_____ uL CD4 MicroBeads needed = **(L) x 2**

Step 17: _____ Time at CD4+TCell Incubation

Step 19: O:_____ CD4+TCells x10^6/ mL P:_____ viability

Step 20: Q:_____ CD14/CD4 Negative x10^6/ mL (diluted 1:10) R:_____ viability

Step 21: _____ Time CD4+TCells Placed in Freezer

Step 22: _____ Time Mr. Frosty #2 Placed in Freezer

Step 23: _____ Time Shutdown Completed

KEY: BLUE – DATA FROM COUNTESS; RED - DATA FROM AUTOMACS; PURPLE-

CALCULATED DATA ; BLACK – TIME

3.12 Air Home Information Questionnaire

I. PURPOSE

The purpose of this questionnaire is to gather information about changes in a participant's residence and lifestyle at their primary residence since their last MESA Air Questionnaire. This information will help us estimate changes in their exposure to air pollutants. We ask questions about pollutant sources inside and outside of the home; about ventilation, heating and cooling of the home; where they spend their time; and about their daily travel. By asking participants these questions and by using the air pollution measurements taken at a relatively small number of those participants' homes during previous exams, our goal is to be able to estimate the air pollution exposures for those who have not had extensive air pollution monitoring conducted.

II. SELECTION

Mesa Classic Participants

All MESA Classic participants who consented to the MESA Air Study and completed a MESA Air Questionnaire during either Exam 3 or 4 will be selected to complete the MESA Air Questionnaire in Exam 5. Participants who only agreed to share their health and residential history data with MESA Air and did not complete a MESA Air Questionnaire previously will not be selected for the MESA Air Questionnaire during Exam 5.

Exam 5 Mesa Air Recruitment

MESA Classic participants who did not consent to the MESA Air during Exam 3 or 4 will be asked again to consent to the study during Exam 5. Participants have the option of consenting to the study in one of two ways: either sharing their previously collected health data with MESA Air, or data-sharing plus completing the Exam 5 MESA Air Questionnaire. Participants who enroll in MESA Air at Exam 5 are not eligible for the MESA Air Subclinical Cohort.

MESA Air Subclinical Cohort

MESA Air Subclinical Cohort participants are selected for the Air Questionnaire, cardiac CT and coronary ultrasound in Exam 5. MESA Air set a target to collect 3600 coronary CT scans and 3600 carotid ultrasounds during MESA Exam 5 from the subclinical cohort. Acquisition of these scans in Exam 5 has not been proceeding as quickly as anticipated, in part due to exam delays and attrition, but also in part due to under-selection. In order to meet the original targets, the selection criteria that were used in at the start of Exam 5 have being adjusted. The original selection criteria is for participants who 1) consented to mesa air during exam 3 or 4 (2005-2007) and completed the Air Questionnaire at that time; 2) had readable CT and ultrasound in exam 1; and 3) had a readable CT and ultrasound in either exam 3 or 4 (giving three data points for progression analysis). MESA Air has decided to drop this third criterion as too restrictive, given the challenges in meeting targets. This change in selection does not impact those participants who have already been into the clinic - no one will be called back in to complete these tests - this change only applies to those participants who have yet to visit the clinic. Participants newly selected as a part of this change in criteria are consented in the same way as those who were originally selected.

MESA Air New Recruits

Columbia and UCLA recruited 257 MESA Air –only "New Recruit" participants during the time frame of Exam 4. These participants will be returning for their second visit as part of MESA Exam 5, starting in February 2011 at UCLA and August 2011 at Columbia. New Recruits are part of the MESA Air Subclinical Cohort, and will receive all of the main MESA Exam 5 components, with the exception of MRI, Epigenomics, and Lung CT.

III. METHODS

All MESA Air participants will complete Section 1 of the Exam 5 MESA Air Questionnaire during their clinical exam. Sections 2-5 will be completed as directed by the answers to questions in Section 1.

In addition to the electronic or paper Air Questionnaire and this question-by-question documentation, you will need the set of 4 laminated cue cards that will assist participants in completing various questions.

There are four situations where a participant will be required to complete an Air Questionnaire regardless of their answers to the trigger questions in Section 1:

1. If a participant refused to complete a previous Air Questionnaire
2. If a previous Air Questionnaire was completed by a proxy
3. If more than one year passed since a participant indicated that a new Air Questionnaire was required during a Follow Up call.
4. QC Activities – 10% of the cohort will answer Section 3 and 10% will answer Sections 4 and 5, regardless of their answers to questions in Section 1 (only for MESA Classic, not Air New Recruits).

For all of these situations, the Air Questionnaire will appear in the list of required questionnaires in the Contact Summary screen. If a questionnaire is being repeated for one of the four reasons listed above, a note explaining the reason for the Air Questionnaire is made in the Contact Information screen.

General Instructions

1. To ensure high data quality, read the questions exactly as written and record the answers according to protocol and without discretion. Probe for clarification when a participant does not give a clear answer.

2. For most questions, possible responses are YES, NO or DON'T KNOW, and/or filling in a bubble or a blank with a number or word. Let the participants choose the appropriate responses for each question. Do not read the DON'T KNOW option. Only read PLEASE SPECIFY when the OTHER option is selected. For questions with the response option ALMOST DAILY, the participant should choose this option if they perform the activity ALMOST DAILY or DAILY.

3. Do not make interpretations or provide synonyms (unless specified in the specific instructions below). If the participant asks about the meaning of any question, re-read the statement (or question) to them.

4. Do not let participants spend too much time on any one question. Ask them to choose what seems to be the best option. If a respondent truly does not know an answer, even after a number of prompts, and it is numeric response, enter 999.

5. For numeric responses that do not use not use all of the boxes or if the response is zero, fill in unused boxes with zeroes (see below):

What is your age?	0	6	5
Number of Cigarettes smoked per day?	0	0	0

6. In general, if a participant has multiple appropriate responses for a question where only one response is required, use the one that accounts for the majority of the time. For example: Q19: What do you do at this location? A: 1) School, 2) Work, 3) Volunteer. If the respondent takes classes part-time and volunteers part time, choose the activity where the participant spends more hours per week. If there is a tie, arbitrarily use the one that is done first during the week. If a participant lives at two residences, their primary residence is the home in which they spend the most time during a given year.

7. Group Living Arrangements Rule: If the participant lives in a group setting (such as a nursing home or assisted living facility) where they spend the majority of their non-sleeping time in communal living quarters (living room, kitchen, etc.), then refer to the entire residence (private and communal) for the response (ex. In a large house that a number of people share, count all of the smokers in the house). If the participant lives in a group setting and spends the majority of their non-sleeping time in their own private quarters, then refer only to their quarters for the response (ex. If they live in an assisted living facility with their own residential area - bedroom and living room - and spend most of their time there, only count the number of smokers in their private residential area).

8. If a participant has recently moved, collect information for the new home when possible. For example, you may not be able to collect information about window usage for the past on the new home, but you will be able to record information about window type in the new residence.

Specific Instructions

Section 1: All MESA Air Participants

The purpose of this section is to gather information about changes in a participant's residence and lifestyle at a primary and/or secondary home since their last interview. This information will be used to determine whether sections 2-5 of the Home Information Questionnaire should be administered during the clinic exam.

Read aloud: The first two questions ask you about streets near your home. Please answer for the busiest street next to your home, where there is no building between your home and the street.

Q1. Are your bedroom windows facing an:

Alley
Side street with low traffic
Side street with considerable traffic
Busy road
Highway
No street

Q2. Are your living room windows facing an:

Alley
Side street with low traffic
Side street with considerable traffic
Busy road
Highway
No street

Read aloud: **The next few questions will ask about your travel time during the day.**

Q3: On average, how many hours each day do you spend doing the following during your travel time?

Walking or biking
In a private car
In a taxi
On a bus

On a train or subway
Other

If OTHER, please specify mode of transportation
Record hours and minutes spent traveling by each mode of transportation during the day. This includes BOTH commute time and recreational time using that mode of transportation.

If the participant does not use one of the modes of transportation, mark 00 in the appropriate boxes.

The overall goal is to determine how much time the participant spends traveling for work and leisure activities and the distribution of transportation types.

Q4: On average, what percent of your travel time do you spend on or next to:

Freeways, expressways, highways, toll roads, etc.
Other major, heavily traveled roads or streets
Residential or lightly traveled roads, streets, or paths

Record percentages and check if they add up to 100%. If the participant has difficulty coming up with percentages, suggest the following:

Never - 0%
Almost never - 1-25% of the time
Sometimes - 26-50% of the time
Often - 51-75%
Always - 76-100% of the time

If a participant does not leave their house during a typical week, select 'not applicable' and skip to Q6. If the participant does leave the house during a typical week, but does not travel on any of the roads described, fill in all boxes with 0%.

Q5: What traffic condition best describes the majority of your travel time during the day? (choose one):

Light traffic, moving at the speed limit
Heavy traffic, moving below the speed limit
Congested or 'stop and go"
Heavy traffic, moving at or above the speed limit
Not Applicable

Be sure to differentiate between heavy traffic, moving below the speed limit and heavy traffic, moving at or above the speed limit.

Choose 'Not Applicable' if the participant does not leave their home during a typical week, or it they do not travel on any of the roads described.

Q6: Do you spend more than four weeks per year living at another address (secondary residence)?

Q6a: How many weeks per season do you spend at your secondary residence.

Enter the number of weeks spent at the secondary residence for each season. Add up the total for all seasons and enter the number in the "Total" box. Do not read "Total" to the participant.

Q6b: Is the total weeks for all four seasons spent at the secondary residence 8 weeks or more? (do not read to participant)

If yes, complete Section 2 after asking all of the questions in Section 1.

Q7. The address used to complete your last Home Information Questionnaire is:

Street, city, state, zip

If the participant has not moved since their last Home Information Questionnaire, they will skip Section 3 on the Exam 5 Home Information Questionnaire.

If this is the first Home Information Questionnaire for the participant, do not ask Q7. Instead, choose NO for Q7 and skip to Q8b .

If the participant has moved since completing their last Air Questionnaire, complete Sections 3 and 5.

Q8a. (if addresses is pre-filled) During a previous MESA interview, you said that you spend 2 hours or more per day or 10 hours or more per week at a single location (working, school, volunteering, socializing, etc.) or doing a specific activity away from your home. Do you still spend the same amount of time at this address?

If NO, 8ai. **When did you stop going to that address?**
Enter month and year, and go to questions 8b.

If YES, 8aii. **You previously reported spending XX hours per week at this address. Has the amount of time that you spend at this address changed?**
If YES, complete Section 5

Q8b. (if address is not provided or no longer used) Do you usually spend 2 hours or more per day or 10 hours or more per week at a single location (working, school, volunteering, socializing, etc.) or doing a specific activity away from your household?

If NO go to Question 9.

If YES complete Section 4 and 5

Answer YES if participant can say yes to EITHER 2 hours or more per day OR 10 hours or more per week.

If the participant spends 2 hours or more per day or 10 hours or more per week at more than one single location (i.e. 10 hours at job #1 and two hours at job #2), or doing more than one specific activity, choose the activity or specific location that they perform or go to most often over the course of the year.

If the participant spends equal time at more than one location or performing more than one activity, have the participant arbitrarily choose the activity or location that they perform first during the day, or during the week.

Q9. Since your last MESA Interview, have your daily activities changed because,

Q9a. you have become a primary caregiver for a friend or relative?

Q9b. you have stopped acting as a primary caregiver for a friend or relative?

Q9c. someone has moved into or out of your home?'

If YES to 9a, 9b or 9c, complete Section 5.

Q9c includes persons who no longer live in the home because they have died.

For the purpose of the Air Questionnaire, Q9a and Q9b are used to identify individuals whose daily activities have changed due to their care giving status, and MESA Air is interested in activities that are performed 2 hours or more per day, or 10 hours or more per week. A caregiver is someone who spends 2 hours or more per day or 10 hours or more per week providing care or services for another person.

Section 2: Secondary Residence Characteristics

Read aloud: **You mentioned that you spend at least 8 weeks per year at your secondary residence. The next few questions ask about the structure and characteristics of your secondary residence.**

Q10. Do you use air conditioning in your secondary residence?

If NO, skip to Question 11.
If YES, answer the following:

Q10a: What type of air conditioning does your secondary residence have?

If "Central A/C" skip to Question 10b.
If "Other, please specify:" specify type used and skip to Question 10b.

If the participant has both central AC and window units, check "Other" and write "both" in the text box and specify how many window units they have.

If "Window unit(s)", ask the following:

How many of them are there?
Specify number of window units.

Q10b: How often was the air conditioning used in the past July? (choose one):

Not used at all
A few days a month
More than half of the days of the month, but less than daily
Almost daily (thermostat use also)
Other, please specify:

If A/C was used for at least one hour every day, this would be daily use. Check the "Almost Daily (thermostat use also)" category if the participant uses the air conditioning daily.

If the participant was not at the secondary residence in the past July, inquire instead about A/C use in the past June or August. If participant was not at the secondary residence in the past June – August, choose NOT USED AT ALL.

Q10c: How often was the air conditioning used in the past January? (choose one):

Not used at all
A few days a month

More than half of the days of the month, but less than daily
Almost daily (thermostat use also)
Other, please specify:

If A/C was used for at least one hour every day, this would be daily use. Check the "Almost Daily (thermostat use also)" category if the participant uses the air conditioning daily.

If the participant was not at the secondary residence in the past January, inquire instead about A/C use in the past December or February. If participant was not at the secondary residence in the past December - February, choose NOT USED AT ALL.

Q11: Please indicate the number of windows you usually had open in your secondary residence in the past summer and winter.


Note: Apply the Group Living Arrangements Rule (#7 in Section A above)

Q11a. In SUMMER (Jun. – Aug.): How many windows did you usually have open?
If the participant was not at their secondary residence during the summer months, mark NONE and skip to Q11b..

Q11b. In WINTER (Dec. – Feb.): How many windows did you usually have open?
If the participant was not at their secondary residence during the winter months, mark NONE and skip to Q11.

Q12: Is an air cleaner/filter used in your secondary residence (stand-alone or central)?


Note: Apply the Group Living Arrangements Rule (#7 in Section A above)

If NO skip to next required section.
If YES, ask the following:

Q12a: What type of air cleaner/filter is used? (please check all that apply)

HEPA filter
Electrostatic precipitator
Negative ion generator
Ozone generator
Regular or fiberglass furnace filter
Don't know
Other, please specify:

Definitions:

A central air cleaner is one that is attached to a furnace that blows air through the house (a forced air furnace). We will make the assumption that all forced air furnaces have a basic filter, but it is not standard for them to have any of the devices listed above, though they may.

Because all of these stand alone air cleaners can be packaged in a plastic, metal, or wooden case ranging in size from a loaf of bread to a two drawer filing cabinet or larger and can look identical from the outside, there are no good photos to assist with the identification. Many of the units may also have a combination of the air cleaning methods. Most of the systems have internal fans, so a quiet humming will not indicate what unit they have.

If the participant does not know the differences in the types of filters, you may read the following prompts:

High Efficiency Particulate Air (HEPA) filter: These are systems that require expensive filters (~>$40) and may also have a cheaper pre-filter. These are generally stand-alone boxes, but can be found in the furnace.

Electrostatic precipitator: These can be stand-alone units or in the furnace. These generally have metal plates that need to be washed periodically.

Negative ion generator: A negative ion generator makes negative ions which assist in reducing dust in the air. The type may be noted on the unit.

Ozone generator: A ozone generator makes ozone and pumps it out into the room air to try to remove odors, tobacco smoke, bacteria, and allergens. There may be a slightly sweet or fresh smell associated with its use. The type may be noted on the unit.

Regular or Fiberglass Furnace Filter: A regular or fiberglass filter is generally a mat of fibers (polyester or fiberglass is common) held in place by a cardboard "frame". These filters can be used in stand-alone air cleaning devices or central units.

Q12b: How often is the air cleaner/filter used? (choose one):

Never
A few days a month
More than half of the days of the month, but less than daily
Every day or nearly every day
Don't know

Section 3: Primary Residence Characteristics

Q13: What type of building do you live in?


Refer the participant to Cue Card #1 for an example of building types.

In this question, we are interested in the physical configuration of the building, rather than how they pay to live there (apartment – rent vs. condo - own). Please choose only one category.

Definitions of different types of housing:

Single family or free-standing: A house in which one family lives. A group house with multiple un-related people and one kitchen would be in this category. The house would also generally have to have all four exterior walls exposed.

Manufactured home/mobile home: Similar in function to a single family house, but has generally been built off site and transported to that location. These are also known as single- or double-wides", "mobile homes" or "trailer homes" and are often situated in trailer parks.

Row house/townhouse/brownstone: This is generally a group of housing units set down side-by-side with adjacent walls. This would differ from a side-by-side triplex because it would have more than three units.

Duplex/triplex, free-standing: A single building split either vertically or horizontally into two or three separate dwellings. Generally each will have a separate entrance that is "lockable".

High rise apartment/condo/co-op (4 floors or more): A building with multiple housing units on each floor and 4 or more floors.

Low rise apartment/condo/co-op (1-3 floors): A building with multiple housing units on each floor and 3 or fewer floors.

If "Single family or free-standing" or "Manufactured home/mobile home" skip to Question 14.

If "Row house/townhouse/brownstone"; "Low rise apartment/condo/coop (1-3 floors)"; "Duplex/triplex, free-standing"; "High rise apartment/condo/coop (4 floors or more)"; or "Other, please specify:" answer Question 13a.

Q13a: What floor do you live on? (choose one):

Basement
Ground floor
Second floor
Third floor or higher.
If a participant selects "Third floor or higher," ask: ***Which Floor?*** Indicate floor level.

Note: Refer the participant to Cue Card #2

If a participant lives on multiple floors in one of these residences, choose the floor where they spend most of their time (including sleeping).

Q14: What is the approximate age of your building?

Specify age of building in years or year built. If the exact year is not known, prompt for a decade.

To record a decade, enter the century and the decade. For example, if the participant only knows that the residence was built in the 1970's, record 0970, if 1870's record 0870. If the residence was built in the 2000's, record 0000.

If the participant's residence is built IN 1999 or 2000, you would record the year built as 1999 or 2000.

Housing built in different time periods has different characteristics that may affect the participant's air pollution exposure.

If an addition was built onto the house, choose the year of the part of the house where the participant spends the majority of their time.

Q15: Is there an attached garage or an underneath garage in your building?

Answer YES or NO.

If NO skip to Question 16.

If YES ask Question 15a:

Q15a: Is this garage used for (choose one):

Choose the closest match:
Parking one car
Parking two cars
Parking more than two cars
Storage only
Other, please specify:

Q16: Do you use air conditioning in your residence?

If NO, skip to Question 17.
If YES, answer the following:

Q16a: What type of air conditioning does your residence have?

If "Central A/C" skip to Question 16b.
If "Other, please specify:" specify type used and skip to Question 16b.

If the participant has both central AC and window units, check "Other" and write "both" in the text box and specify how many window units they have.

If "Window unit(s)", ask the following:

How many of them are there?
Specify number of window units.

Q16b: How often was the air conditioning used in the past July? (choose one):

Not used at all
A few days a month
More than half of the days of the month, but less than daily
Almost daily (thermostat use also)
Other, please specify:

If A/C was used for at least one hour every day, this would be daily use. Check the "Almost Daily (thermostat use also)" category if the participant uses the air conditioning daily.

If the participant was not home in the past July, inquire instead about A/C use in the past June or August.

Q16c: How often was the air conditioning used in the past January? (choose one):

Not used at all
A few days a month
More than half of the days of the month, but less than daily
Almost daily (thermostat use also)
Other, please specify:

If A/C was used for at least one hour every day, this would be daily use. Check the "Almost Daily (thermostat use also)" category if the participant uses the air conditioning daily.

If the participant was not home in the past January, inquire instead about A/C use in the past December or February.

Q17: Approximately how cool do you keep your residence in the summer during the day and over night?

During the day (when at home):

Specify temperature and confirm with the participant whether it is in degrees Fahrenheit or Celsius. Fill in the appropriate temperature scale button.

During the night:

Specify temperature and confirm with the participant whether it is in degrees Fahrenheit or Celsius. Fill in the appropriate temperature scale button.

Enter 888 if there is no air conditioning or they do not use it.

Enter 887 if there is air conditioning, but they cannot control it or do not have a thermostat or thermometer.

Q18: What are the heating sources used in your residence? Please tell me of any that are used at least once a month.

This question refers to heating elements used during the heating season. If a heating source is used at least once a month during the winter months, include it here.

Answer YES, NO or DON'T KNOW for all types of heating sources.

Radiators (steam or hot water)
Forced air (vents)
Electric space heater
Baseboard heat
Gas space heater
Kerosene space heater
Wood burning stove
Fireplace
Open stove
Other, please specify
Specify any other heating sources used.

Note: Refer the participant to Cue Card #3 for examples of these types of heaters.

If the participant still does not know which type of heating source they use, provide the participant with the following definitions:

Gas space heaters: include gas fireplaces and gas fireplace inserts
Wood burning stoves: include wood or pellet burning stoves
Fireplaces: wood burning fireplaces
Open stove: This category is used if you turn on your cooking stove and open it to heat the house or room

Q19: Approximately how warm do you keep your residence in the winter during the day and over night?

During the day (when at home):

Specify temperature and confirm with the participant whether that is in degrees Fahrenheit or Celsius. Fill in the appropriate temperature scale button.

During the night:

Specify temperature and confirm with the participant whether that is in degrees Fahrenheit or Celsius. Fill in the appropriate temperature scale button.

Enter 888 if there is no heating or they do not use it.

Enter 887 if there is heating, but they cannot control it or do not have a thermostat or thermometer.

Q20: Does your residence have storm windows?

Definition:
Storm windows are windows outside ordinary windows to protect against severe weather. They are sometimes removable.

If NO or DON'T KNOW skip to Question 21.
If YES, answer the following:

> **Q.21a Do you use storm windows on all, most, or a few of your windows during any season?**
> Answer ALL, MOST, or A FEW.

Q21: Does your residence have double pane windows?

Definition:
Double pane windows are windows with two panes of glass that cannot be removed and which have a space between the panes.

If the participant has triple-paned windows, mark YES.

If NO or DON'T KNOW skip to Question 22.
If YES, answer the following:

> **Q.21a Are there double pane windows on all, most, or a few of your windows?**
> Answer ALL, MOST, or A FEW.

Q22: Please indicate the number of windows you usually had open in your residence in the past summer and winter and how often you usually left the windows open.


Note: Apply the Group Living Arrangements Rule (#7 in Section A above)

Read: **In SUMMER (Jun. – Aug.):**

Q22a: How many windows did you usually have open?
If the participant was not at their primary residence during the summer months, mark NONE.
If NONE, skip to Question 22c.

Q22b: How often did you open windows? (choose one):
A few days a month
More than half of the days of the month, but less than daily
Almost daily
Other, please specify:

Read: **In WINTER (Dec. – Feb.):**

Q22c: How many windows did you usually have open?
If the participant was not at their primary residence during the winter months, mark NONE.
If NONE, skip to Question 23.

Q22d: How often did you open windows? (choose one):
A few days a month
More than half of the days of the month, but less than daily
Almost daily
Other, please specify:

Q23: Is an air cleaner/filter used in your residence (stand-alone or central)?


Note: Apply the Group Living Arrangements Rule (#7 in Section A above)

If NO or DON'T KNOW skip to Question 24.
If YES, ask the following:

Q23a: What type of air cleaner/filter is used? (please check all that apply)
HEPA filter
Electrostatic precipitator
Negative ion generator
Ozone generator
Regular or fiberglass furnace filter
Don't know
Other, please specify:

Definitions:

A central air cleaner is one that is attached to a furnace that blows air through the house (a forced air furnace). We will make the assumption that all forced air furnaces have a basic filter, but it is not standard for them to have any of the devices listed above, though they may.

Because all of these stand alone air cleaners can be packaged in a plastic, metal, or wooden case ranging in size from a loaf of bread to a two drawer filing cabinet or larger and can look identical from the outside, there are no good photos to assist with the identification. Many of the units may also have a combination

of the air cleaning methods. Most of the systems have internal fans, so a quiet humming will not indicate what unit they have.

If the participant does not know the differences in the types of filters, you may read the following prompts:

High Efficiency Particulate Air (HEPA) filter: These are systems that require expensive filters (~>$40) and may also have a cheaper pre-filter. These are generally stand-alone boxes, but can be found in the furnace.

Electrostatic precipitator: These can be stand-alone units or in the furnace. These generally have metal plates that need to be washed periodically.

Negative ion generator: A negative ion generator makes negative ions which assist in reducing dust in the air. The type may be noted on the unit.

Ozone generator: A ozone generator makes ozone and pumps it out into the room air to try to remove odors, tobacco smoke, bacteria, and allergens. There may be a slightly sweet or fresh smell associated with its use. The type may be noted on the unit.

Regular or Fiberglass Furnace Filter: A regular or fiberglass filter is generally a mat of fibers (polyester or fiberglass is common) held in place by a cardboard "frame". These filters can be used in stand-alone air cleaning devices or central units.

Q23b: How often is the air cleaner/filter used? (choose one):
Never
A few days a month
More than half of the days of the month, but less than daily
Every day or nearly every day
Don't know

Section 4: Activity

Read aloud: **The next few questions refer to the activity that you perform 2 hours or more per day, or 10 hours or more per week.**

Q24: If you go to a specific location, what is the street address? (Please give physical address; no P.O. Box)

Obtain Street, City, State and Zip Code. Try to obtain the physical address, not a P.O. box. This information will be used to map where the other location/activity is in relation to important sources of air pollution. (Addresses will be kept confidential.) If a street address is not possible, try to record the nearest intersection.

If the participant does not go to a specific location, enter “Not Applicable; I do not go to a specific location”.

The “Not Applicable” choice might apply to participants who do a specific activity, but do not go to a specific location. Some examples are:

- A retired participant who golfs 2 or more hours per day, but who goes to multiple golf courses.
- A bus driver who works more than 2 hours per day, or 10 hours per week, but who spends the work day driving around.

If "Not Applicable, I do not go to a specific location," skip to Question 26.

Q24a: Is this an indoor location or an outdoor location?

Mark the appropriate choice:
Indoor location
Outdoor location
This question enables us to better determine exposure.

Read the following statement:

Q25: What do you do at this location? (choose one):

School
Work
Volunteer
Other
If "School," skip to Question 26.

If "Other," please specify and go to Question 26.

If "Work" or "Volunteer," answer Questions 25a – 25c:

Q25a: Briefly describe the industry you work or volunteer in:

We are looking for a description of the industry where the participant works or volunteers (for example: hospital, newspaper publishing, mail order house, auto repair shop, bank). In other words, describe the type of "business" their "employer" conducts.

Q25b: Briefly describe your activities when you work or volunteer:

You can think of this as an occupation. For example: registered nurse, personnel manager, supervisor of order department, auto mechanic, accountant, receptionist at blood bank, volunteer at museum.

Q25c: While volunteering, are you regularly exposed there to vapors, gases, dusts, or fumes?

Answer YES or NO. This question refers to vapors, gases, dusts or fumes at the place where the participant volunteers or works only.

Q26: How many people smoke when they are in your immediate work/volunteer area or during your specified activity?

Include the participant in this count.

After completing Question 26, go back to question 24 and 24a to check to see if the participant gave a specific location (i.e. does the participant work/volunteer or perform an indoor activity for more than 2 hours per day or 10 hours per week at a specific location?) The electronic form will skip these questions if they are not necessary.

Note: This question is not asked of the participant. The interviewer must complete this question.

If NO, End the questionnaire or go to Section 5 if necessary.
If YES, continue to Question 27.

Read to the participant:

You previously answered that you work, volunteer, or do an activity <u>indoors.</u> The next questions ask for information on the characteristics of the building at that location.

Q27: What type of building do you go to? (choose the best one):

Small residential style building (3 floors or fewer)
Small retail style business (strip mall, neighborhood store, etc.)
Large retail style building (large mall, etc.)
Office-type building (low or high-rise)
Industrial or warehouse
Other, please specify:

Q28: Does the building have mechanical or natural ventilation? (choose one):

Mechanical (for example central heating and/or air conditioning)
Natural (for example open windows and doors)
Both
Other, please specify: If Other, specify the type of ventilation in the text box provided.
Don't know

Q29: Is there a parking garage or underground garage in your building?

Answer YES, NO or DON'T KNOW

Q30: If the building uses windows and doors for ventilation when you are there, how often are the windows or doors open during:

Mark one option for each season. Note: Do not read the percentages aloud. The percentages can be used to clarify a response with the participant.

Indicate "Never" (0%), "Almost Never" (1-25% of the time), "Sometimes" (26-50% of the time), "Often" (51-75%), "Always" (76-100% of the time).

If the building does not use windows or doors for ventilation, select this response.

Section 5: Time/Location

Q31: We are now going to talk about how you typically spend your time in the summer and in the winter. The information you describe in the next questions will be used to estimate your exposure to indoor and outdoor air pollution from different locations. While no one does exactly the same thing each and every week, try to think about the habits and routines you have, on average. With that in mind, let's start with a

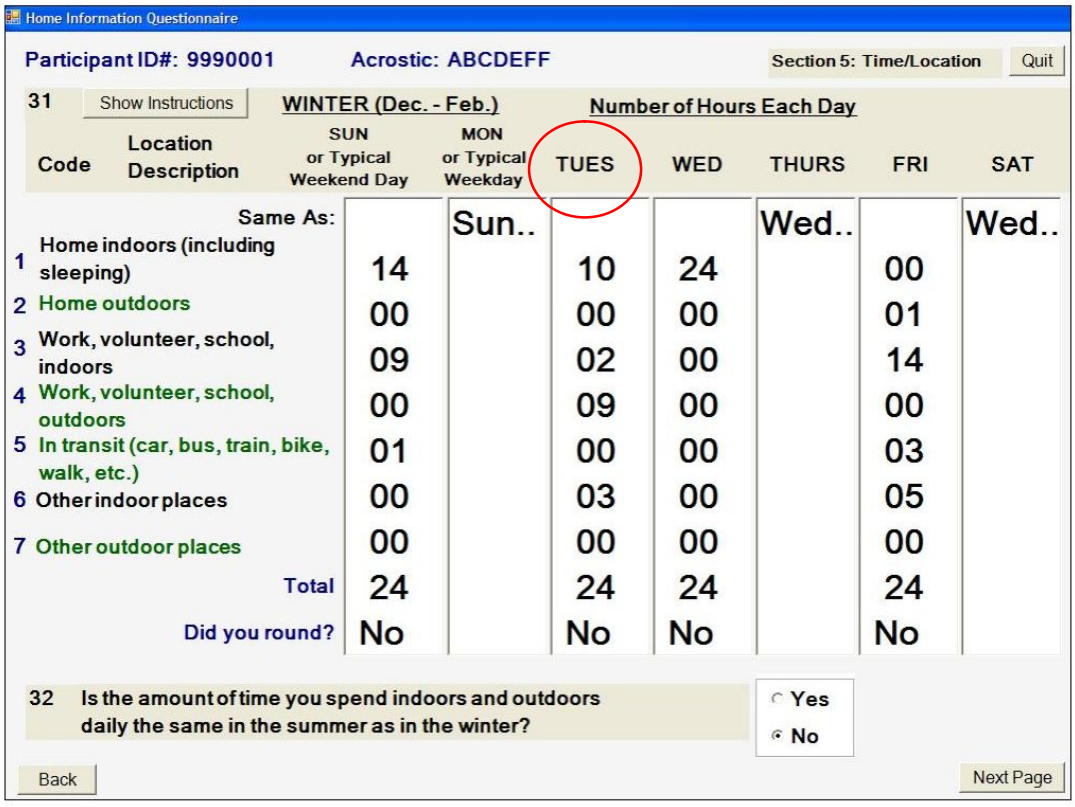
typical week in the winter, December through February. Let's begin with Sunday. On most Sundays in the winter, do you leave your house, including just going outside in your yard or patio? If so, what time do you usually leave your house on a Sunday?

Note: Refer the participant to Cue Card #4 for assistance.

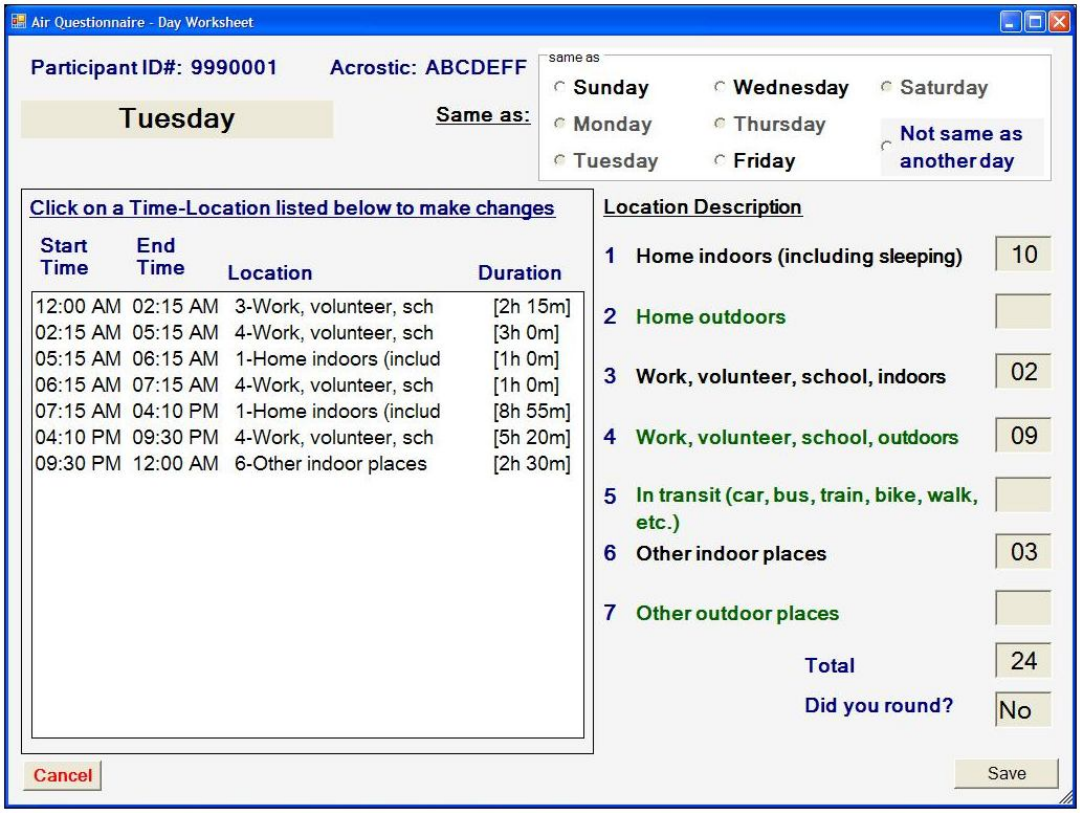
In Questions 31 and 33, we are looking for the number of hours that a participant spends in each of the 7 listed locations during the winter and summer months. This will help us predict their air pollution exposures when we do not have direct measurements. Refer the participant to Cue Card #4 (Locations) so the participant can think about the categories.

Start each day with a question such as "What time do you usually wake up on a typical Sunday?" and follow with probing questions such as "What time do you usually leave the house? Where do you go? How long does it take to get there? Is this an indoor or an outdoor location? Where do you go from here? How long does it take you to get there?" Record all of these activities and times into the electronic Time Location Worksheet. Do not simply ask a participant how many hours they spend in each location on a given day. Instead ask them where they spend their time during the day and record the time of day at each location on the electronic worksheet.

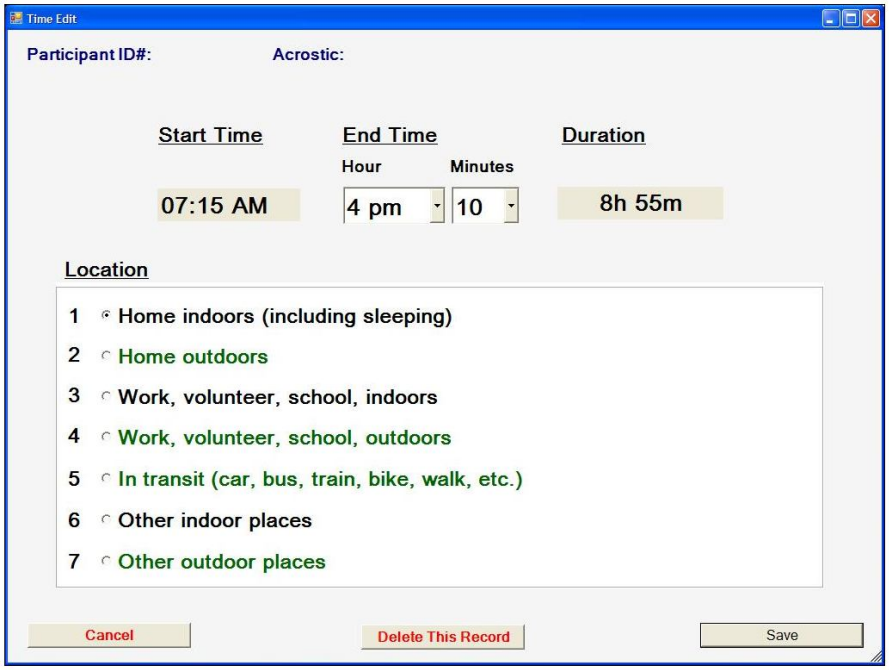
It is possible that the total hours will be greater or less than 24 hours because of rounding. If this is the case, please check "yes" in the "Did you round?" field at the bottom of that day, if either the participant estimated the hours or if the interviewer rounded totals for each category (see next paragraph regarding rounding). This will allow us to know the reason for a day having more or less than 24 hours. If you rounded and the total equals 24, check "Yes" as well. If you didn't round, please check "No". Daily totals will be between 21 and 27 hours.



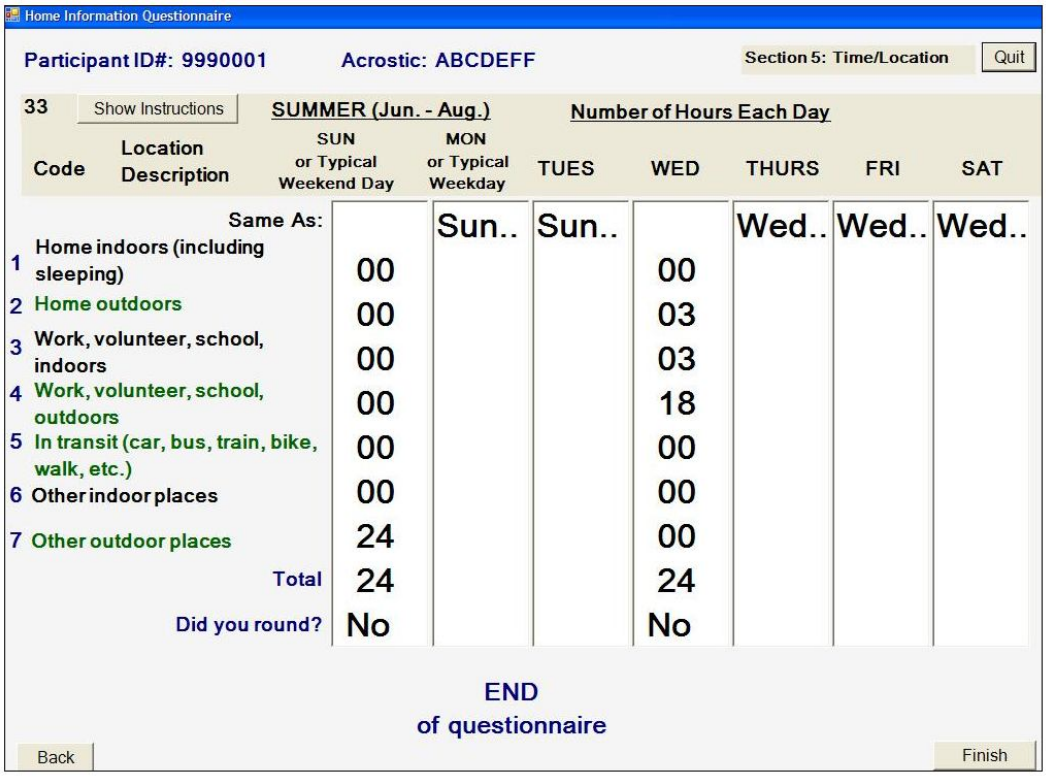
From Q31, open the Time Location Worksheet by clicking on the name of a day, then enter the times and locations of each activity for the 24 hours in the day selected.



To begin entering the information, click on ’12:00 AM’ and select the end time for the activity that begins at 12:00 AM in the pop up box, as well as the location of the activity.

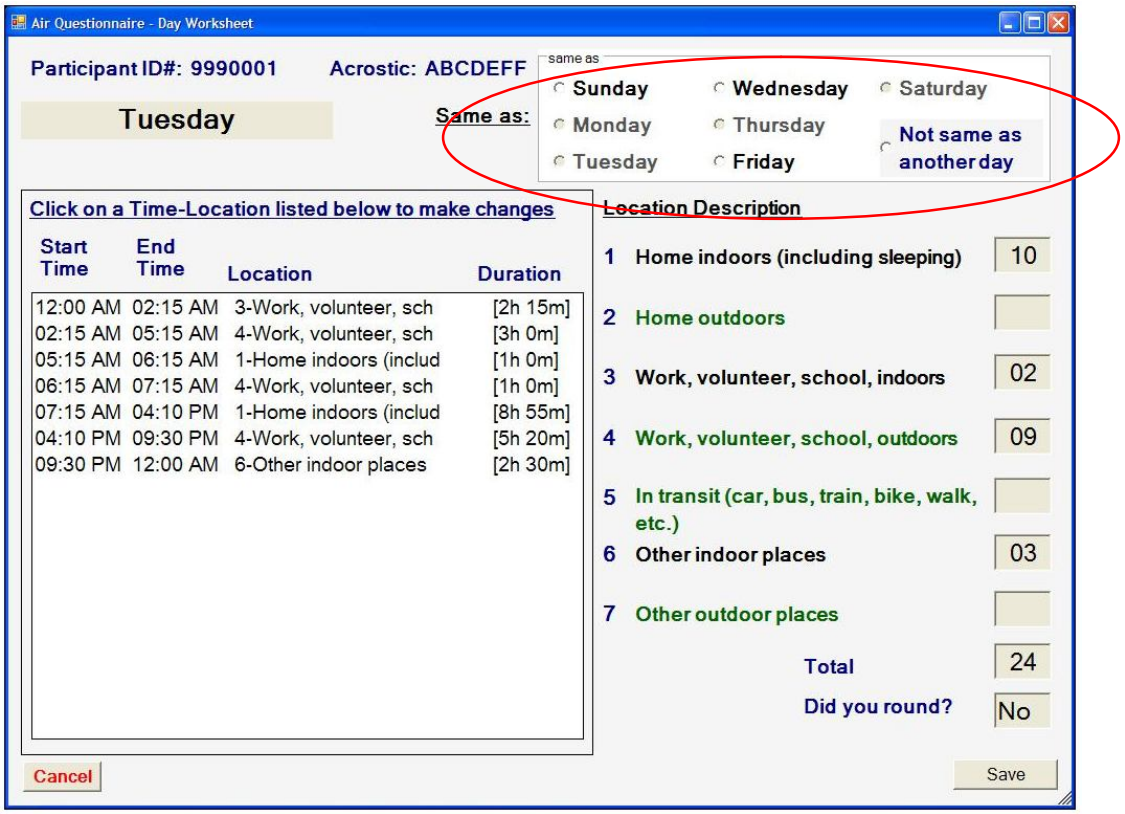


After entering the time and location, select ‘Save’ and you will see the hours for this activity recorded in the Time-Location Worksheet. Times must be entered in five-minute intervals. If a participant reports performing an activity for less than 5 minutes, do not record it in the worksheet.



Continue to fill in all the hours for the day until the last activity ends at 12:00 AM.

When all hours of the day have been recorded and the last end time reads ’12:00 AM, click ‘Save’ to return to Q31 and enter the hours for Monday through Saturday in the same manner. If a day is the same as another day that was already calculated, select the day from the ‘SAME AS’ down box in the Time-Location Worksheet.



Some possible scenarios that may arise:

The participant works indoors at home. In this case, count the work hours in the home indoor category because we want to know exposure at home verses exposure at other places.

The participant drives for part of their work day: In this case, the hours at work spent in the vehicle are coded as location 5, 'in transit.'

Q32: Is the amount of time you spend indoors and outdoors daily the same in the summer as in the winter?

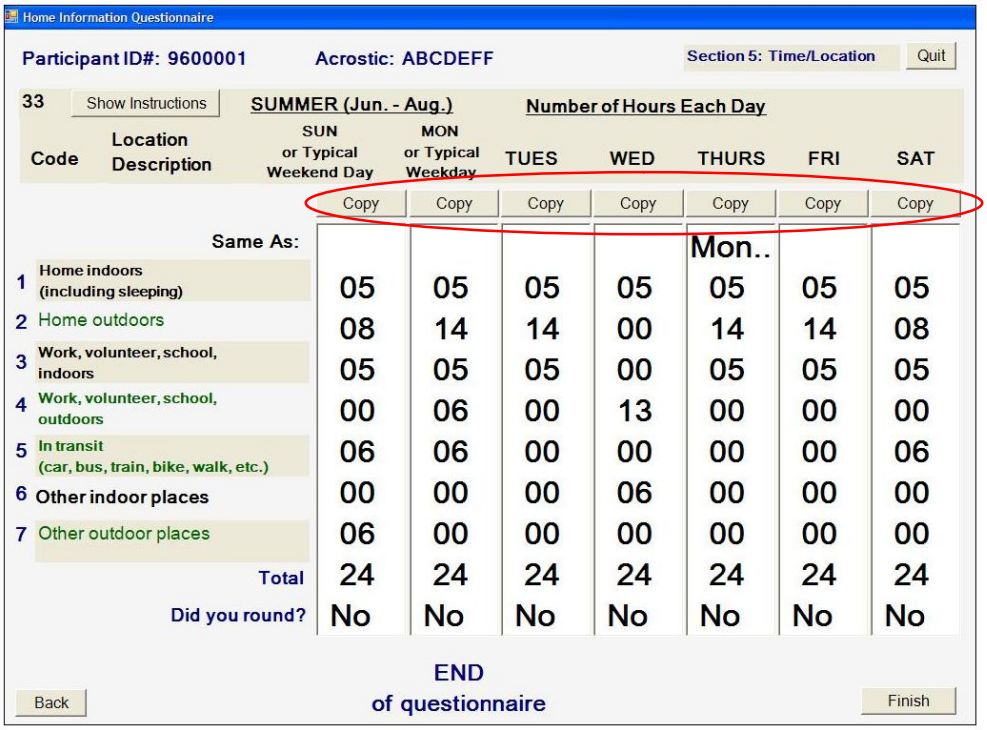
If YES, answer question, END

If NO, answer question 33.

Q33: Please indicate the number of hours you typically spend each day in the following locations in the summer (Jun – Aug) (estimate to the nearest hour).

Please see the instruction for Q31.

If a participant reports that their summer hours on a specific day are the same as their winter hours on that same day, select the "copy" option. This will import the hours from Q31 into the hours for Q33 for a given day. These hours can be edited by selecting the "edit" button.



END:

This is a suggested closing statement that may be customized depending on your Field Center requirements.

We thank you very much for the time that you've given to us. The information we've collected during this questionnaire will be kept confidential, as usual, and will be extremely helpful for us to estimate pollution exposures for you and the rest of the participants in this study.

After completing the Air Questionnaire, click ‘save and continue’.

IV. EDC BACKUP PLAN

The following plan should be followed in the event that a MESA Air Questionnaire is administered without the EDC program.

Materials

Paper Exam 5 Air Questionnaire in the appropriate language
Laminated Cue Cards (1-5)

Instructions

1. Ask all questions in section 1 of the Exam 5 Air Questionnaire. Note that the addresses will not be prefilled in questions 7 and 8, so a slight modification of the questions will be necessary. Please modify the questions as written below:

   Question 7: **Have you moved to a new primary residence since the last time you completed an Air Questionnaire?**
   Note that the last Air Questionnaire may have been completed during either a Follow Up call or at a previous MESA Air Exam. If the participant is not sure if they moved since their last Air Questionnaire, complete section 3.

   Question 8: Skip question 8a and ask question 8b to all participants.

2. After completing section 1, circle the answer to the following questions from section 1 below:

Question 6b:	Yes	No	→	If yes, complete section 2
Question 7	Yes	No	→	If yes, complete section 3 and 5
Question 8b	Yes	No	→	If yes, complete section 4 and 5
Questions 9a, b, or c	Yes	No	→	If yes, complete section 5

3. Circle the questionnaire sections below that are required:

   2 3 4 5

4. Administer the required sections of the Air Questionnaire

5. If section 5 is required, use cue card #5 to collect the times and locations for questions 31 and 33. Do not calculate the number of hour in each location manually. Instead, the totals will be calculated for you when you enter the information from cue card #5 into the EDC program (when it is available again).

V. EXAM 5 CERTIFICAITON PROCESS

Certification for the Exam 5 MESA Air Questionnaire

Certification or Re-certification for this interviewer-administered questionnaire is to be completed locally at the FC. Certification materials can be found on the MESA Air website at http://www.uwchscc.org/MESAAP/FormPacketsEng.aspx. Training and certification include the following materials and procedures:

Materials:

1. Exam 5 Questionnaire MOP
2. Exam 5 Air Training Worksheet
3. Exam 5Air Questionnaire in the EDC Software using dummy IDNOs
4. MESA Air Cue Cards
5. Taped Exam 5 Air Questionnaire Example Interview

Procedures:

1. Carefully review the Exam 5 Air Questionnaire MOP.
2. Complete Exam 5 Air Training Worksheet by referring to the Exam 5 Air Questionnaire MOP.
3. Use the recorded Exam 5 Air Questionnaire Example Interview to complete an Exam 5 Air Questionnaire using a dummy IDNO in the EDC software.
4. Print out the completed Air Questionnaire and mail, fax or email the completed Worksheet and questionnaire to Kayleen Williams for review at:

   CHSCC
   Bldg. 29, Suite 310
   6200 NE 74th Street
   Seattle WA 98115
   kmfw@u.washington.edu
   fax: (206) 616-4075
5. After reviewing the certification packet, Kayleen will contact the site Study Coordinator with a written report.
6. After reviewing the written report, the interviewer will submit tapes for 3 recorded practice Exam 5 Air Questionnaires and completed Air Questionnaire forms . The Air Questionnaire data should be entered into the FU software using a dummy IDNO and the forms printed and sent to the CC with the recordings. Please note: Interviewers who do exceptionally well on the written worksheets or have extensive knowledge of the original MESA Air Questionnaire may be required to complete fewer than 3 practice interviews, at the discretion of the Coordinating Center.
7. If the first set of tapes is not satisfactory based on the performance and quality review by the study certifier, additional tapes maybe requested.