



Manual of Operations

Study: MESA Family

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Notes:

1. MOP is expected to undergo modification as clarifications or study modifications become necessary. Modifications are expected to facilitate study recruitment needs and to accommodate introduction of ancillary studies.
2. Modifications of this document will be approved by Operations Committee and Steering Committee before implementation.

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Section 1: Overview and Clinic Equipment

1.1 MESA Family Overview

Welcome to the MESA Family Study! As an employee in a MESA Clinic, you do not have “just a job,” but a vitally important position in a national research project that will influence diagnosis and treatment of cardiovascular diseases over the next several decades. Keep this in mind as you perform the same procedures on these 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 Family is to locate and identify genes contributing to the genetic risk for cardiovascular disease (CVD) by looking at the early changes of atherosclerosis within families.

1.1.1.2 The primary objectives of MESA Family are:

- obtain nine hundred sibships, who all will undergo MESA clinical protocols to collect demographic, environmental, exposure, and behavioral data, as well as subclinical disease measures
- perform genetic linkage analysis to determine regions contributing to subclinical disease
- perform association and linkage disequilibrium studies to test previously identified candidate genes
- investigate positional candidate genes within linkage regions
- evaluate the role of candidate genes within different ethnic groups.

1.1.2 Description of the MESA study

1.1.2.1 In order to accomplish the study objectives, MESA Family has been designed using all the current organizational structures of MESA. The MESA Family Study will utilize 2700 individuals from 900 families. Half of the participants will be African-American, and half will be Hispanic-American.

1.1.2.2 The cohort will be recruited from the following six Field Centers (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 and Loyola Universities, Chicago, IL (7)
- UCLA, Los Angeles, CA (8)

1.1.2.3 In addition to the Field Centers, other centers involved in the study are:

- Harbor-UCLA (CT Reading Center)
- University of Wisconsin-Madison (Ophthalmology Reading Center)
- Tufts-New England Medical Center (Boston, Ultrasound Reading Center)
- Cedars-Sinai Medical Center (Genetics Center)
- Wake Forest University (Genetic Analysis Center)
- University of Vermont (Central Blood Lab).

1.1.2.4 The Coordinating Center at the University of Washington (Seattle) will 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 medical journals.

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

1.1.2.6 The entire MESA Family project will take 5 years to complete. The first 6 months of the study were devoted to protocol development, staff training, and pilot testing. The examinations of siblings will take place over a twenty-nine month period, beginning in February 2004. The final two years of the study will be dedicated to close out genotyping, data analysis, and publication.

1.1.3 Recruitment Phase

1.1.3.1 Between February 2004, and June 2006, each Field Center will recruit up to 150 families, meeting the sibling requirement (300 siblings) by June 2006. There are several steps in the recruitment process, which are outlined below. This section is intended to give you an overview of the process; specific instructions for each step are contained in later sections of this manual.

1.1.3.2 The first step in recruitment is to publicize the purpose, rationale, and design of the study among MESA Study participants.

1.1.3.3 In the next step, interviewers will contact MESA Study participants, who in MESA Family Study will be referred to as *probands*. If the MESA Study participant reports having at least two qualifying siblings, they will be informed of their family's eligibility to participate. They will be asked to determine whether or not their siblings as well as their parents are interested or available for MESA Family.

- 1.1.3.4 Once a MESA Study participant/Proband has indicated the willingness of at least two qualifying siblings to participate in, or hear more about, MESA Family, the siblings will be contacted by an interviewer. If two siblings meet eligibility requirements and continue to remain willing to commit to the study, clinic visits can be scheduled for the proband, the siblings, and any available parents.
- 1.1.3.5 Each afternoon, the clinic staff makes sure that enough forms are printed and blood tubes prepared for the following day's clinic visits. The data manager will print out a calendar showing who is scheduled for a visit the following day, what type of appointment (proband, sibling, or parent) what time each appointment is, and whether the person speaks English, or Spanish. (Forms are available in each language.)
- 1.1.3.6 After the person arrives and is greeted at the MESA clinic, a staff member will review the exclusion criteria to make sure that the person is eligible. If the person is still eligible, he/she will be asked to read and sign the informed consent. Once the informed consent is signed, the clinic visit can proceed. The staff member will select a set of forms for that person, and the ID printed on those forms becomes that person's MESA ID number.
- 1.1.3.7 The clinic visits consist of many procedures and interviews, which are described in detail throughout this manual. Each different type of participant (proband, sibling, parent) has different procedures. It is important that the clinic visit procedures be completed in the correct order and that those that require fasting are completed before the participant is offered a snack. During the clinic visit, the participant will also be scheduled for a CT scan (if a sibling) and a vision test (if a sibling or proband). (At some sites, one or both of these procedures will take place on the same day as the clinic visit.) In general, the CT scan and vision test should not be done until the clinic visit is completed.
- 1.1.3.8 In order to become a MESA Family participant, the person must
- sign the informed consent
 - have blood drawn
 - complete necessary interviews
 - complete the physical exam (seated blood pressure, ankle-arm blood pressure, and anthropometry) if a sibling
 - complete the CT exam (siblings only).

If a person completes all of these procedures, he/she is considered a MESA Family participant, even if the vision or ultrasound procedures are not completed.

1.1.4 What happens to all of the data?

Once the data have been entered into your local computers, they are transmitted

electronically to the Coordinating Center. There the data are read into a database, and various quality checks are performed. After all of the data for an examination have been collected and checked, investigators at all of the sites will begin working on statistical analyses of the data to see what questions MESA may have answered.

1.1.5 How to use this manual

This manual contains step-by-step instructions for completing all of the steps in recruiting people into the MESA study and for all clinic procedures. 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

Each clinic should be equipped with the following supplies and equipment:

1.2.1 Equipment Provided by the Coordinating Center

- 1 computer, 1 scanner, and 1 printer

1.2.2 Phlebotomy and Laboratory Supplies to be Provided by the Central Laboratory

- 5 mL SCAT-1 tubes
- 10 mL cryovials (#1)
- cryovials – 0.5 mL and 1.5mL with color-coded caps
- 4 mL Sarstedt clear-topped cryovials
- 10 mL cryovials (#42 and #16)

1.2.3 Equipment & Supplies to be Supplied by Field Centers

1.2.3.1 Examination Equipment

- Three Dinamap Blood Pressure Machines, Model PRO 100 (**Critikon**)
- Rapid Cuff Inflation E20 (**DE Hokanson**)
- AG 101 Airsource with BP 10D & SC 12 D and PAK5 Positioning Kit (**DE Hokanson**)
- Detecto Platform Balance, # 1915-339W Scale in lbs/kg (**Titus Home Health Care**)
- Stadiometer (Accu-Hite Measure Device w/level Bubble model # 216 catalog # 5758)

- Two Gulick II anthropometric tapes in cm, Model 67021 (**Country Technology**)
- Wall mounted mirror for verifying tape measurement (**any local vendor**)
- Wall thermometer (**local vendor**)
- Doppler machine, # EN 50 Nicolet LE 100 5 megahertz vascular probe (**Nicolet Vascular**)
- Wall mounted mercury sphygmomanometer(Baum) (**Mckesson General Medical**)
- Blood pressure cuffs (pediatric, adult, large, and thigh) (**Mckesson General Medical**)

1.2.3.2 Laboratory and Phlebotomy Equipment

- 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**

1.2.3.3 Other Equipment

- Refrigerator for storing food (i.e., a household fridge, should not be used to store blood samples or chemicals, can be ordered from Sears or a similar store)
- Ice machine, **Ice Machine Sales Service # 351-FAV-161**
- Sharps containers (wall mounted and table-top) , sharps disposal **Lab safety # 9A-25703**
- Biohazard waste containers – **Lab Safety Supply # 6** gallon container - # 9A-29190
- Emergency eye wash station – **Lab Safety Supply # 9A-2121S**
- Personal protective equipment for phlebotomy and processing face shield, **Lab safety # 9A-1402**, splash shields, other Personal Protective Equipment as needed.

1.2.3.4 Examination Supplies

- Disposable ECG electrodes (Unitrace blue heart electrode, **United Medical Supply Corp**)
- Single use thermometers, **Mckesson General Medical # 5122**
- Ultrasound transmission gel, **DE Hokanson Inc** (Ultrasonic gel – 5 gallon container)
- Gloves (latex, vinyl, or others, any vendor, e.g., **Mckesson General Medical**)
- Kim wipes (**Lab Safety 9A-22394**)
- Pregnancy test kit , **Mckesson General Medical # ICON II HCG Beckman Coulter # 4135**
- Peel-off china marker (**local book store**)

1.2.3.5 Phlebotomy and Laboratory Supplies

- Lab coats and gloves
- Phlebotomy chair
- Basin (just in case)
- Washcloths/Towels
- Smelling salts
- Lab mats and wipes
- 10% bleach solution or approved biohazard disinfectant
- Plastic cart with wheels for phlebotomy supplies (or plastic tray with compartments)
- Butterfly needles (21 G) with luer adapter (B-D # 7251)
- Vacutainer barrels
- Tourniquets
- Alcohol prep pads
- Gauze (2x2)
- Surgical tape - paper tape (easier on participants)
- Band-Aids
- Blood collection tubes (keep extras on hand):
 - 8.5mL ACD'A' tubes (B-D 364606)
 - 10 ml Serum tubes (B-D 366430)
 - 10 ml EDTA tubes (B-D 366457)
 - 4.5 ml Citrate tubes (B-D 366415)
- For Blind Duplicate Samples: 5 ml Serum (B-D 366534) & 5 ml EDTA (B-D 366452)
- Blood tube rocker
- Blood tube racks
- Ice bucket and crushed ice - filled 10 min before draw
- Stopwatches or timers (ex. Fisher Scientific # 06-662-9)
- Scissors
- Pens
- Labels

- Phlebotomy / Processing Form
- Blood Spill Kit
- Biohazardous waste container
- Needle/sharps container
- Material for urine collection

1.2.3.6 Shipping Supplies

- Styrofoam/insulated boxes (Polyfoam Packer # 355-CS for frozen shipments, #33- 12KD for refrigerated shipments)
- Ziplock bags for freezer boxes, **Health care logistics**
- Dry ice
- Ice (gel) packs (U-Tek Refrigerant VWR# 15715-105)
- Absorbent material (old newspaper or paper towels) for layering between dry ice
- elastic bands for freezer boxes (**local vendor**)
- Packing tape

1.2.4 Vendors

CRITIKON

4502 Woodland Corporate Blvd
Tampa, FL 33614
1-877-274-8456, extension 2235

HEALTH CARE LOGISTICS

P.O. Box 25
Circleville, OH 43113-0025
1-800-848-1633
1-800-447-2923 (FAX)

LAB SAFETY

P.O. Box 1368
Jamesville , WI 53547-1368
1-800-356-0783
1-608-754-2345
1-800-393-2287 (FAX)

NICOLET VASCULAR

6355 Joyce Dr.
Golden, CO 80403
1-800-982-6525
1-303-431-9400

VWR

3745 Bayshore Blvd
Brisbane, CA 94005
1-800-999-4897

MAILING:

P.O. Box 7900
San Francisco, CA 94120

FISCHER

(Customer Service)
2761 Walnut Ave.
Tustin, CA 92781
1-800-766-7000

MAIN OFFICE

2000 Park Lane
Pittsburgh, CA 15275
1-412-490-8300

SARSTEDT

P.O. Box 468
1025 St. James Church Rd
Newton, NC 28658
1-800-257-5101

MC KESSON GENERAL

8741 Landmark Ave.
Richmond, VA 23228
1-804-264-7500

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Section 2: Recruitment

2.1 Overview of Recruitment

2.1.1 Selection of Samples

Up to 150 families will be recruited at each site from MESA rosters. These listings will be supplemented to include information concerning family size and availability (siblings residing within 50-100 miles of a MESA Center), and verification of individual consent to be re-contacted by MESA for MESA follow-up and possible inclusion of family members into the MESA Family Study. MESA participants will be recruited by letter, provided with explanatory brochures, and asked to respond to the Field Center with respect to interest of their relatives (siblings and parents, when available).

Proposed MESA Family Recruitment by Ethnic Group
and Field Center

Center	Recruitment for MESA Parent Study (# of Subjects)		MESA Family Study Goals (# Families)	
	African- American	Hispanic	African- American	Hispanic
Wake Forest	503	2	150	
Columbia	382	494		150
Johns Hopkins	562		150	
Minnesota		456		150
Northwestern	302		150	
UCLA	155	536		150

Given the unique logistics of recruitment at each Field Center, some tracking will be done locally. A study-wide tracking form will be used to record recruitment status including outcome of contact efforts, scheduled appointments, and completion status for the various components of the baseline exam. Recruitment tracking information will be recorded on a paper or computerized form and entered into a database at the field center, allowing for review of recruitment status of a given person as well as database queries for groups (e.g., “pending scheduled visit”). An updated database will be sent periodically to the Coordinating Center for centralized tracking.

The Coordinating Center will report, on a monthly basis, Field Center-specific recruitment counts by gender, ethnicity, and family strata. Recommendations for modification of recruitment strategies will be made to individual Field Centers every four months on the basis of cumulative counts.

Recruitment will begin February 1, 2004 and end June 30, 2006 with a total of 1800

siblings to be phenotyped (the 900 MESA index subjects are being phenotyped as part of MESA); thus each Field Center will, on average, be responsible for enrolling and studying 21 subjects in the first quarter of 2004 and 31 subjects in every quarter thereafter. Although each Field Center has a successful recruitment record for MESA, the family recruitment progress will be given priority attention by the MESA Family Study Steering Committee. Monthly conference calls among recruiters will facilitate family collection. A Family Recruitment Subcommittee (a subcommittee of the Operations Committee) will be established to assist in problem-solving and strategic planning. The Coordinating Center will assist tracking by providing monthly summaries to each Field Center and quarterly reports to the Executive Committee, the Family Recruitment Subcommittee and NHLBI representatives. Given the experience of MESA in weekend and evening clinic hours and travel coordination, there are few additional barriers to family member recruitment. If another MESA center is located more conveniently for family members, the family members will be assisted in scheduling their clinical evaluations at the alternative MESA site. We anticipate focusing recruitment efforts on families in which the requisite numbers of siblings reside close to the MESA Field Center where the index case is enrolled. However, recruitment will also be maximized by arranging to study out of town relatives during planned visits, or by the option of a visit to another more conveniently located Field Center. Travel funds will also be available to assist with transportation for non-local siblings.

2.1.2 Recruitment Letters and Brochures

The recruitment strategy for the MESA Family study will have a number of steps, including publicizing the purpose, rationale, and design of the study among MESA Study participants. A letter will be sent to each of these participants explaining the purposes of the MESA Family Study and requesting they discuss the study with their siblings and parents.

Initial Contact

The initial personal contact will usually be made by telephone. In some cases (unlisted phone, not reached by phone) the interviewer will visit the home, to try to screen and recruit in-person. The goal of the initial contact is to complete the Initial Pedigree Screening Form, and determine if the MESA participant is eligible for MESA Family. It is important for the interviewers to recognize the multi-purpose nature of the initial contact:

- To introduce or re-introduce MESA Family and raise the respondent's level of interest in the study
- To enumerate age-eligible siblings in the proband's family
- To recruit eligible respondents
- To obtain some information about non-respondents.

2.1.3 Recruiting Families

Recruiting families for research participation is somewhat different than recruiting individuals, in that it is necessary to ascertain that several members of the family are willing to participate, not just a single person. Therefore, it may be necessary to talk to several members of the family prior to scheduling any family member to come in to the clinic. For MESA Family, it will be necessary to confirm that two siblings, in addition to the MESA participant, are agreeable to being in the study. Other relatives, including additional siblings and the parents (if alive) can be contacted about participation later on, once the minimum sibling trio has been recruited.

The first contact with potential family members must occur through the proband (index case; in this study, the MESA participant). Remember that it is no longer allowable to collect names, addresses, or phone numbers for relatives from the proband unless, at a minimum, the relatives have verbally given consent to the MESA participant to give the study team this information. Be sure and note in the research record that such consent was given to the MESA participant for *each* relative on whom you collect direct identifiers. While we anticipate that most IRB's will allow you to contact family members with such verbal consent, each IRB is different and you must follow your own IRB's requirements.

When a MESA participant has sufficient siblings (a minimum of two) to qualify for the MESA Family Study, explain the study to the MESA participant and ask for their assistance in involving their siblings in the study. Also provide copies of the MESA Family Study brochure, so that they can be distributed to siblings and living parents. In general, we find that it is best to call the proband about 10 days-two weeks later to find out if they have spoken with their relatives and if the relatives are interested in learning more. When the proband indicates that his/her sisters have indicated interest in participating in the study, administer the Pedigree Construction form, and utilize this information to draw a pedigree on the Pedigree Form including parents and siblings. Record any additional family members as they are mentioned throughout the course of the study. Be sure to note the gender and current age of all individuals. This will help you determine exactly who in the family qualifies as potential study participants.

When relatives are willing to be contacted, the study coordinator will make initial direct contact by telephone. Be sure and start the phone call by confirming that the sibling/parent has heard about the study from his sibling/offspring and did indeed agree to be contacted by the study team. Briefly explain the study and answer any questions. If the individual has not received a copy of the MESA Family Study brochure, offer to mail a copy. Some family members will definitely want to participate and may be ready to schedule a clinic visit at the time of the first call. Others may want to wait to receive additional information in the mail, or may just need more time to think about whether they want to participate or not. In such cases, offer to call again in about 1 1/2-2 weeks.

Once you have identified who in the family is willing to participate, note who you

intend to study on the Pedigree Form and fax it to the attention of Kaye Roll RN and Cyndy Hernandez-Walker, RN at Cedars-Sinai Medical Center Division of Medical Genetics (fax # 310-423-0237) for preliminary approval. The approved pedigree will be faxed back within 24 hours. If there are questions or concerns about the pedigree, you will receive a phone call or email for clarification. For consistency, all centers will initially be required to do this. This is expected to be a requirement only for an initial, short term training period to assure that everyone is confident they know who is which families are appropriate. You can always continue submitting pedigrees for pre-approval, even after the training period is over. In particular, any questionable pedigrees can and should be faxed – it is always better to discuss problematic families before bringing anyone in for examination, rather than realizing after the fact that you have enrolled someone whose data cannot be used.

2.2 Recruitment Questionnaires

2.2.1 Initial Pedigree Screening Form

2.2.2.1 PURPOSE

This form is administered to potential MESA Family probands. In most cases the form will be administered over the phone, but in some cases it will be administered in person, at the subject's home. Please note that the respondent might not have any prior knowledge of the study or, in some cases, may confuse MESA Family the main MESA study. The purpose of the Initial Pedigree Screening Form is to determine MESA participants' eligibility for MESA Family, but please remember that at the same time you will be introducing the study and trying to raise the respondent's interest, while maintaining their interest in the main MESA study..

2.2.2.2 MATERIALS/EQUIPMENT

No special equipment/materials needed.

2.2.2.3 DEFINITIONS

- A full brother/sister is one sharing both biological parents as the proband.

2.2.2.4 METHODS

General Instructions

1. The Initial Pedigree Screening Form Questionnaire is printed in English and Spanish. Language forms will differ from center to center.
2. Complete form using a black ink pen. In order to facilitate scanning, please write legibly and make sure that all circles are completely filled in.
3. Try to adhere to the scripts included on the questionnaire, but feel free to use common sense. (For example, don't repeat information that the participant obviously already knows.) If the participant asks questions or requests a clarification, try to answer to the best of your ability. If you don't know the answer, tell the person that you don't know the answer and that you will check with your supervisor and get back to him/her. Remember: you are the first MESA Family representative to interact with a potential participant. The person's decision about whether or not to join MESA Family is dependent, in part, on his/her interaction with you.
4. Unless instructions on the form specifically direct you to end the interview or to

skip a question, complete the entire form for each participant.

5. Questions people might ask about why you are calling, and some sample responses:
 - *I don't want to buy anything!*
“We are not selling anything. We are calling about a new medical research study associated with the Multi-Ethnic Study of Atherosclerosis, of which you are already a participant.”
 - *How did you get my name?*
“Your name was selected from a list of MESA participants who are eligible for this new study.”
 - *I'm too busy!*
“I realize that people are very busy these days. Are there other days and times that it would be better to tell you about the study?”
 - *How long will this take?*
“It will only take about 5 minutes of your time today. I'll move through the questions as quickly as I can.” Immediately ask the first question

Specific Instructions

Header Information.

- For ***date*** enter date of the call or visit. For example, May 25, 2004 would be entered 05/25/2004.
- For ***phone number called*** enter the three-digit area code followed by the seven-digit phone number.
- Record the MESA ID number in the box provided.

Record the date(s) and time(s) of attempted phone calls on a phone call log. Follow your Field Center rules for the maximal number of unsuccessful phone calls.

Item Instructions

Introductory Section

“Hello, my name is <insert name> and I am calling from <your MESA Field Center>. Thank you very much for your continued participation in MESA. I am calling to tell you about a new study which is an extension of MESA. This new study is called the MESA Family Study and is funded by the National Institutes of Health. The new study aims at examining genetic factors that contribute to heart disease. In this study we want to include brothers and sisters of current MESA participants. The brothers and sisters will have an examination similar to your first MESA exam. To qualify, we would like to include at least two

of your siblings in the study. Your brothers should be 40 years or older and sisters should be 50 years or older. I would like to ask you some questions to see if your family qualifies.” If the person is not available, try again later unless another household member is also a MESA participant.

1. **“How many full brothers above the age of 40 do you have?”**
Record number of brothers in space provided.

2. **“How many full sisters above the age of 50 do you have?”**
Record number of sisters in space provided.

If the total number of brothers and sisters is 2 or more, go to question #3.

If the total number of brothers and sisters is less than 2, say “I am sorry. Your family does not qualify for the study. Thank you very much for your time.”
Go to Number 8.

3. **“How many of these brothers and sisters live in this state or neighboring states?”**
Record number in space provided.

4. **“How many of these brothers and sisters live in the United States?”**
Record number in space provided.

If the participant does not have at least 2 siblings living in the US, say “I am sorry. Your family does not qualify for the study. Thank you very much for your time.” Go to Number 8.

If the participant has 2 or more siblings living in the US, say “Your family qualifies for the study. We would appreciate your help in telling your brothers and sisters about the study. As I mentioned earlier, your brothers and sisters will have an examination similar to the one you had. The examination will require about 4-5 hours and may be done on one or two days. You will have the MESA 3rd exam and possibly an additional visit. We would like also to include your parents if possible. Parents will have only a brief examination that includes an interview and collection of a blood sample.”

5. **“Is your father alive?”**
Record answer in appropriate bubble.
6. **“Is your mother alive?”**
Record answer in appropriate bubble.

If Yes to either 5 or 6 or both, go to Number 7. If no, go to Number 8.

7. **“Do/does your parents/father/mother live in the U.S.?”**

Record answer in appropriate bubble.

8. Result of Interview: Record the result of the interview in the appropriate bubble.

Fill in your interviewer or review ID number.

2.2.2 Pedigree Construction Form

2.2.2.1 PURPOSE

This form is administered to potential MESA Family probands, after they have indicated that they have spoken with their siblings and that their siblings are interested in participating in MESA Family. In most cases the form will be administered over the phone, but in some cases it will be administered in person, at the subject's home. The purpose of the Pedigree Construction Form is to facilitate collection of the MESA Family Pedigree, and determine the size of the family and the number of eligible participants in the family.

2.2.2.2 MATERIALS/EQUIPMENT

No special equipment/materials needed.

2.2.2.3 DEFINITIONS

- A full brother/sister is one sharing both biological parents as the proband.

2.2.2.4 METHODS

General Instructions

1. The Pedigree Construction Form is printed in English and Spanish. Language forms will differ from center to center.
2. Complete form using a black ink pen. In order to facilitate scanning, please write legibly and make sure that all circles are completely filled in.
3. Try to adhere to the scripts included on the questionnaire, but feel free to use common sense. (For example, don't repeat information that the participant obviously already knows.) If the participant asks questions or requests a clarification, try to answer to the best of your ability. If you don't know the answer, tell the person that you don't know the answer and that you will check with your supervisor and get back to him/her. Remember: you are the first MESA representative to interact with a potential participant. The person's decision about whether or not to join MESA is dependent, in part, on his/her interaction with you.
4. Unless instructions on the form specifically direct you to end the interview or to skip a question, complete the entire form for each participant.

Specific Instructions

Header Information.

- For *date* enter date of the call. For example, May 25, 2004 would be entered 05/25/2004.

Identifying Information.

- Refer to your Field Center's IRB approvals for how much identifying information to collect on this form, and what constitutes permission to contact.
- If no permission to contact has been obtained, do not collect identifying information.
- As the proband indicates that parents or siblings have given permission to contact them (as defined by your IRB approved recruitment procedures), check the box next to this participant's name. This recording of permission will determine whether or not you are permitted to directly contact that individual

Item Instructions

Q1. **“Could you tell me the name of your biological father?”**

Record appropriate information in space provided.

“Is your father alive?”

Record appropriate answer in bubble provided.

Q2. **“Could you tell me the name of your biological mother?”**

Record appropriate information in space provided.

“Is your mother alive?”

Record appropriate answer in bubble provided.

Q3. **“How many brothers and sisters do you have? Do not include step brothers and sisters. Step brothers or step sisters do not share either a mother or a father.”**

Record number of siblings in space provided.

a. **“Are any of your brothers and sisters half brothers or sisters? Half brothers and sisters share a mother or a father, but not both.”**

Record appropriate answer in bubble provided. If the proband indicates that any siblings are half brothers or sisters, probe to determine which of each sibling in Question 4 is a half sibling. At this time, half siblings are NOT eligible for MESA Family participation.

b. **“Are any of your brothers and sisters adopted?”**

Record appropriate answer in bubble provided. If the proband indicates that any siblings are adopted brothers or sisters, probe to determine which of each sibling in Question 4 is adopted. Adopted siblings are NOT eligible for

MESA Family participation.

c. **“Are any of your brothers and sisters part of a multiple birth (such as twins or triplets)?”**

Record appropriate answer in bubble provided. If the proband indicates that any siblings are part of a multiple birth, probe to determine which of each sibling in Question 4 is a part of a multiple birth. Only ONE of a set of identical twins can be used as a MESA Family proband or sibling, as identical twins share identical genes and provide no new genetic information.

Q3. **“Could you tell me their names and ages, starting with the one who was born first and continuing in order until you reach the one born last?”**

Record information in spaces provided for all siblings identified by the proband. Indicate if each is adopted or part of a multiple birth, and whether they are half siblings. For each multiple birth, please be sure to assess identical (I) or fraternal (F) status of each multiple birth sibling. If the participant is unsure of identical/fraternal status, probe with the question “When you/they were young, were you/they considered to be as similar as peas in a pod?”. If yes, bubble I (Identical), if no, bubble F (Fraternal). If the status is still uncertain, bubble DK (Don’t Know).

Once all sibling and parent information has been collected, utilize this information to complete the Pedigree

2.2.3 Pedigree

2.2.3.1 PURPOSE

This form is administered to MESA Family probands, and is a by-product of the Pedigree Construction Form. In most cases the form will be administered over the phone, but in some cases it will be administered in person, at the subject's home. The purpose of the Pedigree is to have a visual depiction of the family unit.

2.2.3.2 MATERIALS/EQUIPMENT

No special equipment/materials needed.

2.2.3.3 METHODS

General Instructions

1. Complete form using a dark pencil. In order to facilitate faxing, please write legibly.
2. Because copies of the Pedigree will be sent to the central Genetics Center at Cedars-Sinai and Wake Forest, do not put direct identifying information (full name, birthdate, social security number, etc.) on the pedigree. Such identifiers should be kept in the separate research forms and files that will be retained at the Field Center or the Coordinating center.
3. A pedigree for this project should contain a minimum of two generations (i.e. MESA participant and siblings and parents), however any information that can be collected on offspring should be included.
4. Use the symbols listed in Appendix A to indicate male, female, relationships (proband, marriage, offspring), deceased or still living, studied or not, to construct a family pedigree.
5. The Pedigree will be a constantly evolving and changing structure as new family members provide information. When a sibling is recruited, and fills out the family history questionnaire, you will be able to identify the number of offspring and illnesses in that segment of the pedigree.

Specific Instructions

Header Information.

- For **date** enter date of the call. For example, May 25, 2004 would be entered 05/25/2004.

Constructing a Pedigree

Appendix A shows the most common symbols used in constructing a family pedigree. It is wise to start by sketching a rough draft of the pedigree in pencil until you know the extent of the family size, and the amount of information to be noted on each individual.

The amount of information you will collect and place on the pedigree for each individual will depend upon whether you are obtaining information directly from the individual after he/she has consented to participate in the MESA Family Study, or whether you are collecting information about a non-participating (or not yet participating) family member from another relative. When obtaining information about a non-consented individual, obtain the minimum amount of information required to determine if that individual would potentially qualify for the study (age, sex, age, relationship to MESA participant, and any serious medical problems that would prevent participation in MESA Family) and record the information without direct identifiers.

The index case is the MESA participant who brought the family into the project. To draw the pedigree, start with the index case in the center of the page, and work laterally to show siblings, and then vertically to show parents, aunts, uncles, and grandparents, and offspring (where applicable). Draw siblings from left to right in birth order. Show all matings and their offspring (number matings in chronological order). Always remember to ask if siblings are full or half and note accordingly.

Males are usually drawn on the left, and females on the right. Generations are labeled with Roman numerals.

Using the symbols below, indicate the sex of each individual and whether that individual is still living or deceased. Make a notation above each symbol to show if that individual has actually been studied (had blood drawn, etc.). Beneath each individual symbol, indicate the following:

1. Individual's ID#
2. Age, or if deceased age at death
3. If affected with **CAD-related diagnosis**, indicate diagnosis and age of onset.
4. Other major medical problems (except routine hospitalizations and surgeries).
5. If deceased, cause of death

Indicate index case (MESA participant who led to identification of the family) with an arrow.

After obtaining information from the index case, interview all other family members

present regarding their own nuclear family, in order to obtain the most accurate information.

Family members who choose to participate may opt to come into clinic together. However, when interviewing individual family members, it is wise to choose a private place, as they may be reluctant to disclose sensitive information in the presence of other family members. While it is unlikely that highly sensitive information will be collected as part of MESA Family, on occasion you may find that someone may wish to divulge information (for example, a case of non-paternity) critical to analysis of study data. Ensure that all family members are provided with your telephone number, so that they will be able to call you at a later time with new or sensitive information.

You may find it useful to develop a series of symbols to denote the presence of CAD, and related conditions. The disease in question (**CAD**) and other associated diseases/findings can then be depicted by shading in the symbol of that individual. To depict various manifestations of the disease or associated conditions, simply divide the symbol into two or more segments, and shade differently or use vertical or horizontal lines. Be sure to provide an accompanying key. Another way to note CAD and related conditions is to write the various conditions under the symbol for the individual. If you use this approach, be sure and write legibly!

Be sure to make a note of the date that the information was obtained, and from whom information was obtained. Label the pedigree with the ID code of the index case.

2.2.4 Screening/Recruitment Questionnaire

2.2.4.1 PURPOSE

This form is administered to potential MESA Family Sibling participants. In most cases the form will be administered over the phone, but in some cases it will be administered in person, at the subject's home or in the clinic after the third MESA exam. Fill out a separate form for each eligible sibling. The purpose of the Screening/Recruitment Questionnaire is to determine eligibility for MESA Family, but please remember that at the same time you will be introducing the study and trying to raise the respondent's interest.

2.2.4.2 MATERIALS/EQUIPMENT

No special equipment/materials needed.

2.2.4.3 DEFINITIONS

2.2.4.4 METHODS

General Instructions

1. The Screening/Recruitment Questionnaire is printed in English and Spanish. (Language forms will differ from center to center.)
2. Complete form using a black ink pen. In order to facilitate scanning, please write legibly and make sure that all circles are completely filled in.
3. Try to adhere to the scripts included on the questionnaire, but feel free to use common sense. (For example, don't repeat information that the participant obviously already knows.) If the participant asks questions or requests a clarification, try to answer to the best of your ability. If you don't know the answer, tell the person that you don't know the answer and that you will check with your supervisor and get back to him/her. Remember: you are the first MESA Family representative to interact with a potential participant. The person's decision about whether or not to join MESA Family is dependent, in part, on his/her interaction with you.
4. Unless instructions on the form specifically direct you to end the interview or to skip a question, complete the entire form for each participant.

Specific Instructions

Header Information.

- For **date** enter date of the call. For example, May 25, 2004 would be entered 05/25/2004.
- For **phone number called** enter the three-digit area code followed by the seven-digit phone number.
- Record the Recruitment ID number in the box provided.

Record the date(s) and time(s) of attempted phone calls on a phone call log. Follow your Field Center rules for the maximal number of unsuccessful phone calls.

Item Instructions. Please note that responses marked in **bold** with an asterisk (*) indicate INELIGIBILITY.

Introductory Section

- A. **“Hello, my name is <insert name> and I am calling from <your MESA Field Center> about the MESA Family Study. <Name of proband> indicated that you had expressed interest in this study. “**
If the person is not available, try again later.
1. **“Are you comfortable answering questions in English or Spanish?”**
Ask only in Hispanic Centers. Fill in appropriate bubble.
“Please feel free at any time to interrupt me if you have questions or comments”

Screening

- B. **“The study we’re doing is called the MESA Family...”**
2. **“The study includes only men 40 years and older and women 50 years and older. Are you in that age range?”**
If in the appropriate age range, ask respondent for his/her birthdate, and then continue to section 3.
If a male younger than 40 or a woman older than 50, respondent is INELIGIBLE. “I’m sorry, but our study includes men over 40 and women over 50” Skip to question 21 on page 4 and check “ineligible.”
3. **“Great! You are in the age range we need. The MESA Family study is funded by.....”**
- After reading this script, ask the respondent if he/she has any questions. Continue with the script, if the respondent agrees, asking again at the conclusion if the respondent has any questions. (Please note that *you are not obligated to use the recruitment script if it seems redundant.* For example, there is no need to describe the study to an enthusiastic respondent who obviously knows enough about MESA.) If the respondent is agreeable continue with Question 4.

Questions people might ask about the clinic examination, and some sample responses:

- *I can't help you because I've never had heart problems.*
 "Because we need to look more closely at risk factors for heart disease, we are very interested in people who do not have heart disease."
- *What do I get out of the study?*
 "There are several ways in which you might benefit from this study. You will receive, at absolutely no cost to you, a state-of-the-art medical examination. This exam would be very expensive if it were not part of this study. It is possible that we could find a medical condition that you were not aware of. If we did, you would be able to get treatment from your own doctor, if you so desired. You will also have the opportunity to participate in an important national heart study."
- *What is involved in the clinic examination?*
 "We will be doing a number of tests, some of which you may have had before. We will check your height, weight, and blood pressure. We will also ask you a series of questions about your life and health and about your family history. We will do an "EKG," which is a routine test of your heart function, and an ultrasound examination of the arteries in your head and neck. Ultrasound involves putting a microphone-like device on your skin—one that uses sound waves to detect whether there is hardening of the arteries. We will also draw some blood to measure the levels of substances such as cholesterol and blood sugar."
- *I don't like to have blood drawn!*
 "I can understand that. Many people do not like to have their blood drawn. But we have very specially trained personnel and we use small needles to make it easier. The blood tests are very important parts of the study and are needed to compare with other study results."

4. "Do you think you might be interested in participating in this research if we find out that you are eligible for the MESA Family study?"
If yes or maybe, continue to question 5.
If no, skip to question 21 on page 4 and select "Uncertain Eligibility."

(Please note that there is no need to complete the screener once it becomes clear that the person is ineligible. Recruiters should use their discretion, however, to determine if it is appropriate to terminate the interview at that point or better to ask a few additional questions.)

5. "Would you say, in general, your health is..." (Read the list and fill in the appropriate circle)
6. "Has a doctor ever told you that you have/had any of the following conditions?" (List each condition and wait for a response).

- a. Heart failure
- b. Atrial fibrillation or a fibrillating heart
If yes, ask, “Do you still have it?”
- c. Renal Failure

If respondent answers yes to any item, except atrial fibrillation, he/she is INELIGIBLE. For atrial fibrillation, respondent is ineligible only if he/she *currently* has the condition.

If no or don't know, continue to question 7.

7. **“Are you undergoing dialysis or have you had a kidney transplant?”** If respondent answers yes to any item, he/she is INELIGIBLE.”
If no or don't know, continue to question 8.
8. **“Have you ever had cancer?”**
If yes, ask:
“Have you received radiation or chemotherapy in the last year?”
If yes, respondent is INELIGIBLE.
If no or don't know, continue to question 9.
9. **“Do you have any other serious medical conditions that would prevent long-term participation in this study?”** If the respondent asks for your advice about whether or not a particular condition would prevent his/her long-term participation, say, **“We leave this to your judgment; but, if you want, I can consult with our physicians and get back to you.”** Be sure to follow-up on this question with your supervisor. And double-check the list of conditions/procedures on pages 2–3 that make the respondent ineligible.
If yes, respondent is INELIGIBLE.
If no or don't know, continue to question 10.
10. **“Have you ever had a CAT scan of your chest in the past year?”**
If yes, respondent is temporarily INELIGIBLE. They can remain on an eligibility list and become a participant after one year has elapsed from the time of this or any cat scan.
If no or don't know, continue to question 11.
11. For women aged 50–55 only: **“Do you think you may be pregnant or are you currently nursing?”**
If yes, respondent is temporarily INELIGIBLE. They can remain on an eligibility list and become a participant after they either give birth or finish nursing.
If no or don't know, continue to question 12.

12. **“How much do you weigh?”** *Record weight in pounds.*
If weight is over 300 pounds, respondent is INELIGIBLE. Regardless of weight, continue to question 13.
13. **“Are you Spanish, Hispanic, or Latino?”**
If no, continue to question 16.
If yes, ask, “Which of the following best describes your ethnicity?” Read the list, fill in the appropriate circle, and continue to question 14.
14. **“Which of the following best describes your race?”** (Or, if the respondent selected one of the categories in question 14, ask, “Do you also identify yourself with one of these other groups?”) Respondent may select more than one. Read the list, fill in the appropriate circle (s), and then continue to sidebar question in lower right.

SIDEBAR Did respondent answer “yes” to question 13 or choose one of the underlined groups in question 14?
If yes, continue to question 15.
If no, respondent is INELIGIBLE. Continue to question 15.

Conclusion

15. Interviewer: In your opinion, is the respondent eligible to participate in the study?
If yes, skip to ELIGIBLE.
If no or unsure, skip to INELIGIBLE
- **INELIGIBLE.** Choose the most appropriate script.
 - **ELIGIBLE.** Read script and then continue to question 16.
16. **“Would you like to participate in the MESA Family study?”**
If yes, exclaim “great!” one more time. “We are excited to include you in this important research. Now, I just need to get some contact information, and see if you have other siblings willing to participate.”
If no, ask, “Perhaps you would like to think this over and have me call you back in a day or two?”
If yes, arrange to call back and then skip to question 21.
If no, thank respondent for his/her time and skip to question 21.
17. **“Please let me check the spelling of your name.”** Ask respondent to spell out full name and *record in spaces provided.*
18. **“What is your street address?”** This is the address to which MESA Family

correspondence will be sent. *Record address, including apartment or room number, if applicable, and zip code, in spaces provided.*

19. **“Could I verify your home phone number?”** *Record home phone number, including area code, in spaces provided.*

20. **“Are there other phone numbers that I could use to reach you, if necessary, such as a cellular phone or business phone?”** *Record alternate phone number(s), including area code, in spaces provided.*

D. “Thanks very much for all of this information. It will make it easier for us to get in touch with you in the future.... Now we need to schedule your clinic visit....” Read remaining script and schedule visit. *Do not*, however, schedule a clinic visit for an acutely ill (e.g. “flu” or bronchitis) participant. Arrange to contact him/her again to schedule an appointment when he/she has recovered. *Record date and time of visit in boxes provided.*

21 Final disposition of interview. Fill in the appropriate circle.

Record any comments in space provided at the bottom of the form (COMMENTS). Fill in your interviewer or review ID number and record the date the form was completed.

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Section 3: Examination Guidelines and Interviewing Techniques

3.1 Pre-Exam Activities

Make the following preparations prior to clinic visits:

3.1.1 Forms and Calendars

- 3.1.1.1 The data manager should print out the Daily Calendar showing clinic visits scheduled for the following day. The Calendar lists the preferred language of each person scheduled for a clinic visit, which will help you to determine how many sets of each language-specific forms you will need for the day.
- 3.1.1.2 Print several sets of the forms that will be completed for interviews and procedures. These will be pre-printed with participant IDs, which will be matched to an individual when he/she begins the exam. Forms can be printed as far in advance as you would like, and it is advisable to have enough printed forms on hand for at least several days of clinic visits. Forms will be printed in English or Spanish. For each participant, gather all the forms required for a visit, including the informed consent, medical release, and screening (telephone interview) form, and place into a binder labeled with his/her ID.

3.1.2 Supplies and Equipment

- 3.1.2.2 Set up vacutainer and aliquoting tubes on the racks and attach the pre-printed labels to the tubes. Place all phlebotomy supplies on the blood drawing table (21 g, luer adaptor, vacutainer barrel, tourniquet, alcohol pad, gauze 2x2, surgical tape, Band-Aid).
- 3.1.2.2 Make sure that the examination rooms are clean and have clean linen (sheets, pillow cases, wash cloth, blankets).
- 3.1.2.3 Prepare the participants' gowns or (scrubs) and slippers.
- 3.1.2.4 Prepare the examination room for the anthropometry measurement, Doppler, and seated BP. Check all instruments that will be used for the examination.
- 3.1.2.5 Make sure breakfast will be available for the participants.

3.1.3 Staffing

- 3.1.3.1 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.

- 3.1.3.2 If participants have been scheduled for ultrasound or CT, make sure that the respective technicians have the participant schedule and forms.

3.2 Examination Guidelines

3.2.1 MESA Family Examination Guidelines

The MESA Family examinations will be scheduled over a twenty-six month period, beginning March 2004. The examination will include several questionnaires, anthropometry, blood pressure measurement, ankle-arm ratio by Doppler, fasting blood and urine collections, carotid ultrasound, and coronary calcium determination with EBCT or helical CT. We estimate that the complete examination will require about three hours. The examination may be performed on one day or over two 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 to some degree, if needed; but the requirements listed below may not be altered.

- 3.2.1.1 Questionnaires, anthropometry, blood pressure measurement, ankle-arm ratio, and fasting blood and urine collection should be performed before the cardiac CT. This allows excluding subjects who are not eligible for participation. If a subject is found ineligible during the clinic examination, the examinations should be completed. At the conclusion of the exam, give the participant the results of the clinic examination and inform him/her (in a nice way) that he/she will not have the cardiac CT.
- 3.2.1.2 Anthropometry, and blood collection should be performed while the participant is fasting. Blood pressure measurement should be done before venipuncture. Coronary CT, carotid ultrasound, ankle-arm ratio, questionnaires, and urine collection do not require fasting.
- 3.2.1.3 Coronary CT and carotid ultrasound may be done any time of the day and do not require fasting.
- 3.2.1.4 Blood drawing should be done after a 12-hour fast and before 10:30 am.
- 3.2.1.5 A pregnancy test should be performed in all women of child-bearing potential within 48 hours of the CT for coronary calcium. This could be done in the clinic or in the Radiology Department.

3.2.2 Examination Order

The following guidelines identify elements of clinic order. Many elements are left to the discretion of the individual center.

- 3.2.2.1 Anthropometry, blood pressure, and urine collection could be done immediately following the greeting and informed consent, and *before* venipuncture. These components may be done in any order, but the resting blood pressures should be obtained after the subject has been in the seated position for at least five minutes.
- 3.2.2.2 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.
- 3.2.2.3 Questionnaires may be administered at any time during the examination. During the interviews make every effort to avoid distractions, ensure privacy, and maintain confidentiality. Do not conduct interviews during the snack or in the waiting area in the clinic.
- 3.2.2.4 Provide a snack after completing the blood draws.

3.2.3 Instructions to Participants Before the Clinic Visit

Mail these instructions to the participant 10–14 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.

- 3.2.3.1 Participants must fast for at least 12 hours before the examination. This restriction applies to all food and beverages (except water), including alcohol. Instruct them to consume dinner at least 12 hours before their scheduled appointment at the clinic. Only water and prescription medications are allowed from dinner until the start of the examination the next morning. Diabetic patients should *not* take their hypoglycemic medications the morning of the clinic visit; they should *bring the morning dose to the clinic* to be taken after venipuncture.
- 3.2.3.2 Participants should avoid heavy exercise during the 12 hours before the visit.
- 3.2.3.3 Participants should not smoke on the morning of the visit.
- 3.2.3.4 Participants should bring all current medications, both prescription and over-the-

counter, including vitamin preparations and dietary supplements, 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.

- 3.2.3.5 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.
- 3.2.3.6 Participants should wear or bring loose-fitting clothes, preferably t-shirt, sweat pants, and slip-on shoes or sneakers.

3.2.4 Guidelines for Examination of Diabetic Participants

- 3.2.4.1 Diet-controlled diabetics must fast overnight and are treated the same as non-diabetics.
- 3.2.4.2 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 Interviewing Guidelines and Techniques

3.3.1 General Interview Information

3.3.1.1 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

3.3.1.2 Characteristics of a good interview

1. The interviewer creates a friendly, but businesslike atmosphere.
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.
3. The interviewer obtains the answer to the question that is asked by:
 - Proper use of probes.
 - Repeating a question rather than interpreting it.
4. The interviewer obtains clarification of confusing answers.
5. The interviewer gives only neutral responses to the respondent's answers.
6. The interviewer accurately records responses.

3.3.1.3 Specific skills required for interviewers

1. The ability to ask questions at the correct pace and in a conversational tone.
2. A thorough knowledge of the questions and response categories (this will keep the interview flowing smoothly).
3. Knowledge of how and when to use probes.
4. The ability to think as an interviewer and to temporarily put aside other roles (e.g., researcher, health care provider).
5. The ability to maintain a positive attitude about the interview (this lets the respondent know that the interview is important).
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).

7. Additional interviewer attributes:
 - Mobility (for personal interviews)
 - Flexibility over schedule
 - Neat, pleasant, professional appearance
 - Not too timid, not too aggressive

3.3.1.4 Interviewer training

1. Training must cover all aspects of the interview, including:
 - Introducing yourself
 - Handling people who are reluctant "at the door"
 - Following instructions for selection of respondent
 - Obtaining consent
 - Answering questions
 - Obtaining privacy for the interview
 - Setting respondent at ease
 - Administering the interview
 - Ending the interview
2. Role playing, using both standard and problematic situations, is an important aspect of training and allows trainees to discuss and solve problem that could arise in an interview with a participant.

3.3.2 Interviewing Techniques

3.3.2.1 Standardized Interviewing Technique

1. MESA Family is a collaborative study being conducted through six field centers located throughout the United States. The goal of this collaboration is to produce a study that represents 2700 people from 900 different families of ethnic minority status.
2. In order to produce data that can be considered collaborative, MESA Family 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.
3. Trainers will use scripts to teach probing techniques and to determine if interviewers are following skip patterns in the forms and adhering to the protocol. All *clinic* interviews will be taped. Interviewers will be trained to introduce taping to the study respondent and to secure his/her agreement. The Interviewer Supervisor will systematically review tapes to determine if questions are asked as

written. Interviewers will be trained to avoid leading or providing answers for the study respondent.

4. The study is further standardized by using centralized training for supervisors and, where possible, for interviewers. We will initially train local interviewer supervisors; they, in turn, will train new, on-site personnel as needed. Supervisors will be in touch with each other and will share tapes to determine protocol adherence.

3.3.2.2. Recruiting the Study Respondent

MESA Family participants represent a variety of people, ranging in age from 40 and up. The first contact with the study respondent will be by telephone. Because telephone contact can make it easy for the respondent to decline, interviewers will be trained in effective telephone technique. They will also be taught to overcome respondents' objections and deal with difficult situations, some of which are described below.

1. Suspicion about the project. Many people will not be aware that NIH is doing a study in the community and will seem suspicious. Your thorough understanding of the study will help to allay the respondent's fears or suspicions. You must learn to put the respondent at ease and to establish the legitimacy of your call.
2. Handicaps. If a respondent has a disability, you must determine its severity and if it will prevent the respondent from participating in the study. If the respondent has a person who can act as a proxy, you will need to secure their agreement to participate with the study respondent. If no such person exists, thank the study respondent and terminate the interview. Code the result appropriately and provide notes so that your supervisor can evaluate the case.
3. Difficulty in understanding the questions. Some of your calls will be with persons who have difficulty understanding your questions. Read questions slowly and distinctly and allow the respondent adequate time to answer. Repeat questions, if necessary, but be careful not to insult the respondent by suggesting that he/she does not understand.
4. Focusing the interview. Some respondents will welcome the opportunity to talk to a neutral person about their health and family problems. In doing so, they may stray from the questions asked. You must know when to allow a respondent time to elaborate and when to re-focus him/her on the question. Control the interview, but do not alienate the respondent.
5. Leading the respondent. Some respondents will give answers that they believe you and/or the government *expect*; and they may expect you to help them with answers rather than give their own opinions or information. We are trying to gather objective data. Reassure the study respondents that there are no wrong or

right answers. Encourage them to respond out of their experience and their knowledge.

6. Diffusing sensitive questions. Some respondents may hesitate or decline to answer questions they consider intrusive or sensitive (e.g., questions about alcohol consumption or income). Your professional handling of a sensitive issue can help to alleviate their fears. The more secure you feel about the confidentiality of the study, the more apt you will be to give a sense of security to the study respondent. However, if all else fails, you may simply offer them the option to decline answering a specific question.
7. Getting permission from study respondent's family. It is important that the family of the respondent be clear about what is involved in the process of recruitment. Encourage both family members and respondent to raise questions or concerns about the study and the respondent's participation in it. Encouraging them to ask questions, and your thorough and thoughtful responses to those questions, will help to alleviate their concerns.

3.3.3 The Interview

The following procedures are recommended for a successful interview:

- 3.3.3.1 Prior to the visit prepare all materials (e.g., appropriate forms, identification, stamped self-addressed envelopes) that will be necessary for the interview.
- 3.3.3.2 The interviewer should confirm the appointment with the participant to avoid confusion
- 3.3.3.3 Find an area where both you and the participant can talk and write comfortably with minimal distractions.
- 3.3.3.4 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 or asking him/her to restate the question you are asking. At the same time, be careful not to impose your interpretations on the interview questions or the participant's comments.
- 3.3.3.5 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.
- 3.3.3.6 Participants may try to convince you to answer certain questions for them. Let the participants know that you are interested in *their* answers.

- 3.3.3.7 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.
- 3.3.3.8 Communicate with other interviewers and the project director to share ideas about how to deal with difficult situations and to agree on consistent explanations for questions that are frequently misunderstood by participants.
- 3.3.3.9 Encourage, but do not force, participants to answer to all questions.
- 3.3.3.10 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.
- 3.3.3.11 Be able to adapt to interruptions. Let the participant know that you are willing to continue the interview after the interruptions are completed.
- 3.3.3.12 Make the interview a positive experience for the participant. React favorably to answers and give compliments, when appropriate.
- 3.3.3.13 Give the participant clear information about when the next clinic visit will be conducted and follow through with the plans that you make. If a change is necessary, be sure the participant is informed and understands the reason for the change.

3.3.4 Overcoming Difficult Questions

The following are examples of, and suggested responses to, questions you may encounter.

- ***"How do I know you and the research are legitimate?"*** If the respondent is concerned about the legitimacy of the research study, repeat your introduction, remind him/her about the introductory letter and brochure, and offer to mail another one if necessary. Suggest that he/she call the number on the brochure for information and verification. Also point out that local health officials are aware of the survey, and offer to mail to the respondent a reproduction of newspaper clippings and/or endorsements. *If you make a home visit, always wear your identification badge.*
- ***"What's this survey about?"*** Explain that we are interviewing approximately 2700 family members in six communities in the United States to collect data about their health and discover genetic health links. The data we collect will help the U.S. National Institutes of Health and local area health professionals to better understand the factors associated with heart and blood vessel diseases.
- ***"I don't want to buy anything."*** Explain that we are not selling anything. We are doing an important research study and all the tests will be done free of charge.

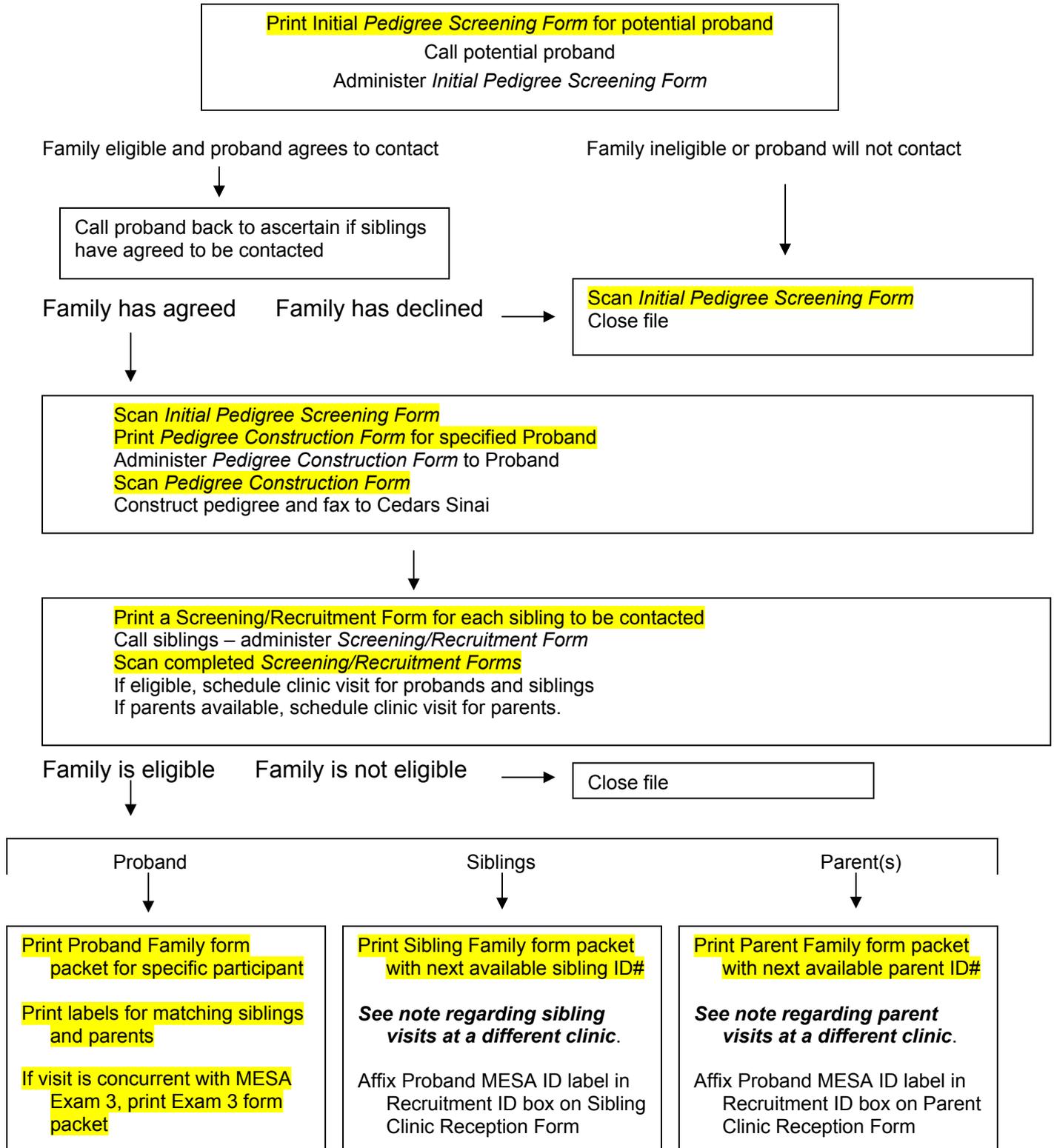
- ***"I can't help you because I have never had heart disease."*** Explain that the study is enrolling people who do not already have heart disease and that you would like to talk to the respondent about his/her health.
- ***"Will this affect my medical care?"*** Explain that we are doing research and that the study will not affect any medical care the respondent now receives. The clinic is only gathering data, not doing diagnostic work. All results of the medical examination will be forwarded to his/her doctor, if so desired. Also explain that *all information* is held in strict confidence and that public reporting of the findings of this study will contain only statistical information.
- ***"My doctor may not want me to be in the study."*** Explain that all area doctors support MESA Family. Suggest that the respondent contact his/her physician to discuss the study.
- ***"How long will it take to complete?"*** Explain that the interview this day will take about 30 minutes and that questionnaires and a physical examination at the clinic will take about 6–8 hours.
- ***"I don't drive and do not have a friend who can bring me."*** Offer free transportation. Explain that we will send a taxi to take the him/her to and from the clinic appointment(s).
- ***"Where did you get my name?"*** Explain that we selected the respondent's name at random from a list of people living in the area. If applicable, remind that respondent that he/she was sent a letter about this selection process.
- ***"I want to participate in the study, but you say I'm not eligible."*** Explain that, although we are talking with a great many people, not everyone can be invited to participate in the random sample. You may also want to suggest that they volunteer in the study when we have special tests to run on equipment or special certifications to do for the technicians.
- ***"I already have a doctor and s/he examines me regularly."*** Explain that all information gathered in the study will be made available to his/her physician. Restate that many of the study examinations are not done in a doctor's office. These examinations may or may not turn up important information about his/her health; if they do, we will share it with the respondent and his/her physician.
- ***"I do not have the stamina to go through a day of tests."*** Explain that all of the tests do not have to be done in one day.
- ***"My veins are not good and I had a very bad experience at the hospital when they tried to draw blood. I do not want to have that done."*** Explain that you understand that having such an experience is not pleasant and that you can see why he/she does not

want to have blood drawn. You may add that MESA Family has only very skilled phlebotomists who will take great care to make sure the procedure is as painless as possible. Also mention that drinking water prior to the exam will make the blood draw quicker and easier.

- ***“I do not have the time”*** or ***“I am going to be in Florida for the next few months”*** or ***“I cannot take time off from work.”*** Explain that the recruitment period will last about two years and that an appointment can be scheduled when he/she has time. Explain that we also have weekend appointments for people who cannot come to the clinic during the week.

An Overview of Clinic Activities for MESA Family

(See last page for information about forms and form packets.)



GO TO NEXT PAGE

Proband



- Affix Proband ID label to appropriate position on approved pedigree; fax to Cedars.
- Obtain MESA Family blood samples from proband.
- Perform eye procedures.
- Ship Red Cell sample to Vermont.
- Ship ACD'A' samples to Cedars.



Siblings



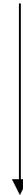
- Affix Sibling ID label to appropriate position on approved pedigree; fax to Cedars.
- Obtain full set of MESA Family Sibling blood and urine samples.
- Perform MESA Family clinic procedures.
- Administer MESA Family interviews.
- Perform eye procedures.
- Perform or schedule CT and ultrasound.
- Ship EDTA, Red Cell, Citrate, SCAT-I, Serum, and Urine samples to Vermont.
- Ship ACD'A' samples to Cedars.



Parent(s)



- Affix Parent ID label to appropriate position on approved pedigree; fax to Cedars.
- Obtain MESA Family Parent blood samples.
- Administer Family History Form
- Ship Red Cell sample to Vermont.
- Ship ACD'A' samples to Cedars.



Scan and verify completed sets of forms.
Transmit data to Coordinating Center.
Transmit Completion Form data to Reading Centers.

- Initial Pedigree Screening form should be completed and scanned before any additional forms are scanned for proband Family visit, sibling, or parent. This step creates the Family and begins the tracking process.
- The Family ID is the proband's MESA ID number
- A full pedigree should be constructed after interviewing the potential proband. This pedigree should be faxed to Cedars Sinai as soon as it is completed and approval from Cedars is required. As the proband, siblings, and parents come to the clinic, the appropriate ID number is added to the pedigree and it is re-faxed to Cedars.
- Information about siblings and parents should be entered into the appropriate fields on the Pedigree Construction Form only as specific permission to contact is granted. Each line of sibling information is identified by a unique letter.
- Each sibling or parent is assigned a "Recruitment" ID number, which is the proband's MESA ID number plus a suffix. For siblings, the suffix is the unique letter on the Pedigree Construction Form preceding that person's information. For parents, the suffix is a "1" for fathers and a "2" for mothers.
- Sibling and parent form packets may be printed in advance, with unassigned ID numbers pre-printed on each form. As parents or siblings come to the clinic, a prepared packet is assigned to each one and the Clinic Reception Form should be immediately labeled with the Recruitment ID#. This will consist of a printed label with the proband ID# and the appropriate suffix hand-written in the provided box.
- As Clinic Reception Forms are scanned, the participant is linked to the Family by the proband's MESA ID number, which is on a label that is affixed to the Clinic Reception Form.
- ***If a sibling or parent is to be seen at a different clinic than the one where the proband is enrolled, clinic staff should print a sibling or parent form set with the next available sibling or parent ID number and mail the paperwork to the site where the person will be seen. When the exam has been performed and the forms completed, they should be mailed back to the "home" clinic for scanning into the database. This will allow the inclusion of the sibling or parent into the system where the rest of the family is recorded and facilitate the tracking and reporting of family participation.***

MESA Family Forms and Forms Packets**Proband Forms**

Recruitment and Screening:

- *Initial Pedigree Screening*
- *Pedigree Construction*
- *Pedigree*

Clinic Exam Packet:

- *Proband Clinic Reception*
- *Phlebotomy Form*
- *Processing Form*
- *Eye History*
- *Retina Completion Form*
- *Vision Completion Form*

Sibling Forms

Recruitment and Screening:

- *Screening/Recruitment*

Clinic Exam Packet:

- *Clinic Reception*
- *Check-off Sheet*
- *Phlebotomy Form*
- *Urine Collection Form*
- *Processing Form*
- *Anthropometry*
- *Seated Blood Pressure*
- *Ankle-Arm BP*
- *Eye History*
- *Retina Completion Form*
- *Vision Completion Form*
- *CT Exam Form*
- *Ultrasound IMT Form*
- *Medical History*
- *Personal History*
- *Physical Activity*
- *Health & Life*
- *Medications*
- *Family History*
- *Participant Contact*

Parent Forms

Clinic Exam Packet:

- *Parent Clinic Reception*
- *Phlebotomy Form*
- *Processing Form*
- *Family History*

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Section 4: Proband Clinic Exam

4.1 Examination Components

4.1.1 Overview

The MESA Family Proband Exam will be brief in comparison with the proband's prior two MESA examinations, consisting only of a blood draw and vision examination. In some cases, the proband's MESA Family Examination will be done in conjunction with their Exam 3 visit. In these instances, it is extremely important that all MESA Family paperwork be filled out separately from Exam 3, and the three tubes of blood collected be processed separately from the blood collected for the regular examination.

4.1.2 Forms

The forms associated with a MESA Family Proband Examination are:

- Clinic Reception
- Phlebotomy (See Section 7 on Laboratory Procedures)
- Processing (See Section 7 on Laboratory Procedures)
- Eye History (See Section 9 on Vision)
- Retina Completion (See Section 9 on Vision)
- Vision Completion (See Section 9 on Vision)

4.2 Clinic Reception Form

4.2.1 Purpose

The Clinic Reception Form is the means by which important information collected during the reception process is recorded for scanning into the clinic database. This form must be completed accurately to allow for the tracking of the participant's progress through the study and to provide a link to the sampling and recruitment data.

4.2.2 Materials/Equipment

The Clinic Reception Form is generated by a computer with scanning software installed, so access to this computer is necessary to obtain the form. In addition, the following materials are required:

- A black ink pen (for best results when form is scanned)
- The list of participants scheduled for the day (produced by the MESA Recruitment system)
- Informed consent documents and pen
- Gown (or robe) and slippers

4.2.3 Definitions

- Daily calendar: The list of participants scheduled for a given day. It lists the name, acrostic, recruitment/household ID, preferred language, and time of appointment. There is also a field to record participant status.
- Acrostic: A code used instead of the name for confidentiality reasons. It is composed of the first four letters of the last name, the first two letters of the first name, and gender indicator (F/M).

4.2.4 Methods

4.2.4.1 General Instructions

1. The clinic reception process is the participant's introduction to the MESA Family clinic staff and procedures. 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. Give the proband the informed consent document. Instruct the participant to read it carefully, answer the questions at the end, and sign it. Once the participant has completed and signed the informed consent, the clinic visit will begin.
3. 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 if necessary. 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.

4.2.4.2 Specific Instructions for Completing the Clinic Reception Form

- 1 Because the Clinic Reception Form serves several important functions, a second staff member must review it before scanning. This reviewer should be absolutely sure that the recruitment ID and acrostic have been correctly transcribed into the appropriate fields.

2. The ***participant ID***, the ***QC ID***, and the ***language*** are preprinted on the form in the upper right corner.
3. In the ***visit date*** fields record a two-digit month and date and a four-digit year (e.g. 05/25/2004). Whenever possible the second appointment date, if any, should be entered on the form before scanning. If the second appointment cannot be scheduled before the form is scanned, the data manager should update the database as soon as the information is available.
4. In the ***acrostic*** field record the acrostic that is printed on the daily calendar. Ask the participant for his/her birthday and record it in the appropriate box.
5. In the ***medical identification number*** field record the participant's local hospital or medical record number. This number is not required, however.
6. ***Informed Consent Responses***. Transcribe the responses from the participant's consent form by filling the appropriate bubble.
7. In the ***Reception Interviewer ID*** field, record the ID number of the person who administered the Clinic Reception Form.
8. In the ***Reviewer ID*** field, record the ID number of the person who reviewed the Clinic Reception Form.

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Section 5: Sibling Clinic Exam

5.1 Examination Components

5.1.1 Overview

The MESA Family Sibling Exam will be comparable with the proband's first MESA Examination. Pay careful attention to the order of procedures for clinic exams outlined in Section 3.

5.1.2 Forms

The forms associated with a MESA Family Proband Examination are:

- Clinic Reception
- Check-off Sheet
- Phlebotomy (See Section 7 on Laboratory Procedures)
- Urine Collection
- Processing (See Section 7 on Laboratory Procedures)
- Anthropometry
- Seated Blood Pressure
- Ankle-Arm BP
- Eye History (See Section 9 on Vision)
- Retina Completion (See Section 9 on Vision)
- Vision Completion (See Section 9 on Vision)
- CT Exam Form (See Section 9 on Computed Tomography)
- Ultrasound IMT Form (See Section 10 on Ultrasound IMT)
- Medical History
- Personal History
- Physical Activity
- Health & Life
- Medications
- Family History
- Participant Contact

5.2 Clinic Reception Form

5.2.1 Purpose

The Clinic Reception Form is the means by which important information collected during the reception process is recorded for scanning into the clinic database. This form must be completed accurately to allow for the tracking of the participant's progress through the study and to provide a link to the sampling and recruitment data.

5.2.2 Materials/Equipment

The Clinic Reception Form is generated by a computer with scanning software installed, so access to this computer is necessary to obtain the form. In addition, the following materials are required:

- A black ink pen (for best results when form is scanned)
- The list of participants scheduled for the day (produced by the MESA Recruitment system)
- Informed consent documents and pen
- Gown (or robe) and slippers

5.2.3 Definitions

- Daily calendar: The list of participants scheduled for a given day. It lists the name, acronym, recruitment/household ID, preferred language, and time of appointment. There is also a field to record participant status.
- Acrostic: A code used instead of the name for confidentiality reasons. It is composed of the first four letters of the last name, the first two letters of the first name, and gender indicator (F/M).

5.2.4 Methods

5.2.4.1 General Instructions

1. The clinic reception process is the participant's introduction to the MESA Family clinic staff and procedures. 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. Give the proband the informed consent document. Instruct the participant to read it carefully, answer the questions at the end, and sign it. Once the participant has completed and signed the informed consent, the clinic visit will begin.
3. 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 if necessary. 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.

5.2.4.2 Specific Instructions for Completing the Clinic Reception Form

1. Because the Clinic Reception Form serves several important functions, a second staff member must review it before scanning. This reviewer should be absolutely sure that the recruitment ID and acrostic have been correctly transcribed into the appropriate fields.
2. The **participant ID**, the **QC ID**, and the **language** are preprinted on the form in the upper right corner.
3. In the **visit date** fields record a two-digit month and date and a four-digit year (e.g. 05/25/2004). Whenever possible the second appointment date, if any, should be entered on the form before scanning. If the second appointment cannot be scheduled before the form is scanned, the data manager should update the database as soon as the information is available.
4. In the **acrostic** field record the acrostic that is printed on the daily calendar. Ask the participant for his/her birthday and record it in the appropriate box.
5. In the **medical identification number** field record the participant's local hospital or medical record number. This number is not required, however.
6. **Informed Consent Responses.** Transcribe the responses from the participant's consent form by filling the appropriate bubble.
7. In the **Reception Interviewer ID** field, record the ID number of the person who administered the Clinic Reception Form.
8. In the **Reviewer ID** field, record the ID number of the person who reviewed the Clinic Reception Form.

5.3 *Clinic Check Off Sheet*

5.3.1 Purpose

The *Clinic Check Off Sheet* is the means to ensure that all parts of the MESA Family Exam are completed or scheduled. This form must be completed accurately to allow for the tracking of the participant's progress through the exam.

5.3.2 Methods

5.3.2.1 General Instructions

1. The form should be filled out as completely as possible during the exam visit day. Make sure to schedule dates for the participant's CT and/or Ultrasound examination.
2. Provide the participant with appointment reminders for the CT and/or Ultrasound exams, if appropriate.
3. 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 sheet and the clinic visit day for the participant.

5.3.2.1 Specific Instructions for Completing the Clinic Check Off Sheet

1. For each step/procedure, make certain to fill out the start and end time in the appropriate fields. Note any comments and record the Tech ID.
2. Visit day progress order should be as recommended for the first five steps/procedures. The remainder of the procedures could be performed in any order except the MESA eye component. The Eye history questionnaire should always precede the eye examinations. MESA Vision (refraction) should be completed before MESA Retina (retinal photograph).
3. Call and schedule CT and/or Ultrasound 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.
4. At the end of the form, ask the participant for his/her "preference in method of future MESA contacts". Choices include: Telephone call, Letter, E-mail. Fill in the box that applies. If the participant prefers e-mail, ask for the e-mail address and transcribe on the line provided.
5. **Do Not Scan this form!**

5.4 Anthropometry

5.4.1 Purpose

Anthropometry was obtained in the MESA “Baseline Exam” or Exam 1 and Exam 2. The purpose is the same as for these exams and is obtained in MESA Exam 3 for longitudinal studies.

5.4.2 Materials and equipment

- Stadiometer (Accu-Hite Measure Device with level bubble)
- Detecto Platform Balance Scale in lbs/kg
- Gulick II 150 cm anthropometric tape
- Full length mirror
- Four 50-pound weights (certified prior to first MESA visit) to calibrate scale

5.4.3 Definitions

5.4.4 Methods

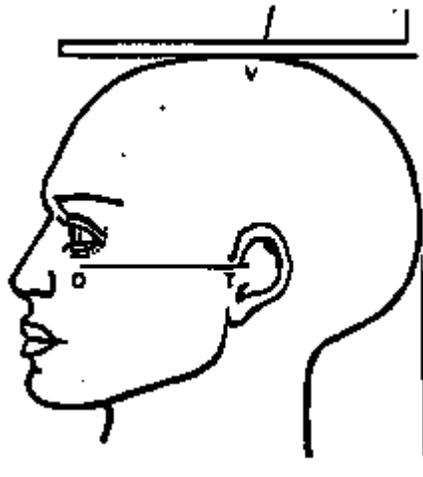
5.4.4.1 General Instructions for Body Size Measurements

1. For all measurements, participants should wear light clothing but no shoes (thin socks or “pillow slippers” OK). Keep a supply of surgical scrubs at the clinical center for participants who forget to wear or bring the appropriate clothes. Have participants completely empty their pockets and remove excessive amounts of jewelry that could affect the weight measurement. Provide lockers with locks for valuables.
2. 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.
3. Take a single measurement at each body site and record using specific rounding rules. Record any modifications in measurement techniques (e.g., height decreased from a hunched posture or weight that exceeds the capacity of the scale) on the Anthropometry Form.

5.4.4.2 Standing Body Height

1. Equipment
 - Stadiometer (Accu-Hite Measure Device with level bubble)

2. Before measuring height, check to 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.
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

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.
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.*
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?"*

5.4.4.3 Body Weight

1. Equipment

- Detecto Platform Balance Scale in lbs/kg
2. 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 pound, in Box 2a on the Anthropometry Form.*
 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.
 4. If a participant has a prosthetic limb or breast prosthesis, measure weight with the limb *on*.
 5. If a participant is frail or unsteady, measure weight while participant is lightly steadied by you or an assistant.
 6. If a participant is unable to stand on the scale for a weight measurement, do not attempt a weight measurement. *Answer "yes" to the question, "Was there a modification in protocol?"*

5.4.4.4 Girth Measurements

1. Equipment
 - Gulick II 150 cm anthropometric tape
 - Full length mirror
2. Technique
 - Do *not* take abdominal and hip girth measurements over clothing.
 - If a participant is very large, you may have him/her hold one end of the tape while walking the tape around to obtain the measurement. If any circumference exceeds 150 cm, use the 250 cm tape measure. (Take care not to offend the person being measured, and change to the larger tape as discretely as possible.)

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 cm and record in Box 3a on the Anthropometry Form. If the circumference exceeds 150 cm, record “yes” for the question, “Was there a modification in protocol?”

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 cm and record in Box 3b on the Anthropometry Form. If the circumference exceeds 150 cm, record “yes” for the question, “Was there a modification in protocol?”*

5.4.4.5 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 4 on the Anthropometry Form and record comments in the space provided. If there are no comments or modifications, answer “no” to question 4.

5.4.4.6 Calibration Check of Scales and Equipment Check

1. Equipment:

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

2. Check scales for accuracy on a monthly basis.

- Place two weights on the 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.
- The values obtained 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.
- When the scale is not in use, keep it balanced at 300 pounds. This keeps the tension off the internal spring mechanism.

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

5.5 Seated Blood Pressure

5.5.1 Background and Purpose

Blood pressure (BP) level is a major risk factor for coronary heart disease, congestive heart failure, and stroke. Heart rate reflects autonomic nervous system function and cardiovascular fitness. The measured BP level is subject to biological and observer variability. 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.

The main advantages of the Dinamap[®] automated device are accuracy comparable to manual mercury sphygmomanometry, with reduced potential for observer biases and less demand on research assistants in terms of training and effort in data collection. The available data that describes the accuracy of the Dinamap[®] and other oscillometric BP devices are reviewed in Appendix A.

5.5.2 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).
- Hand calculator (to average 2nd and 3rd BP readings).
- 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).
- Resting Heart Rate/Blood Pressure Form.

5.5.3 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.

5.5.4 Classification of the Participant's Blood Pressure within the JNC VI Categories and criteria for alerts and referrals

The 1993 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:
 - Diastolic BP >120 mm Hg
 - Systolic BP >210 mm Hg
2. Alert levels requiring urgent referral (within one week) are:
 - Diastolic BP 110–119 mm Hg
 - Systolic BP 180–209 mm Hg
3. BP >140/90 mm Hg requires follow-up within two months time, and, therefore, we recommend physician notification for systolic or diastolic BP above these levels.
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 will include fields for these averaged values and for any actions taken.

5.5.5 Methods

5.5.5.1 Preparation

1. Record the date of the procedure and the Dinamap[®] number on the Seated Blood

Pressure Form during the five-minute rest period.

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.”

3. Make sure the room temperature is between 70° and 76° Fahrenheit.

5.5.5.2 Cuff Size Selection

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. Measure the right arm circumference as follows:
 - Ask the participant to bare the upper arm.
 - Instruct 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. Tape should not indent the skin. *Record the arm circumference in 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
17-25	Small Adult	10
23-33	Adult	13
31-40	Large Adult	17

38-50	Thigh	
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* These circumferences are printed on the corresponding cuff for verification.

** Critikon Dura-cuf® nomenclature, also printed on the cuff.

5.5.5.3. Setting up the Dinamap® BP Machine

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

5.5.5.4 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, 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.
3. The participant should not talk, eat, or drink during the procedure.
4. Ideally, the Dinamap output will not be visible to the participant during the measurement, as this may cause anxiety.

5.5.5.5 Application of the Blood Pressure Cuff

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.”

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.
3. Wrap the cuff snugly around the arm, with the palm of the participant's hand turned upward.
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. 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.
6. Be sure all air is squeezed out of the cuff before each inflation.

5.5.5.6 Rest Period

1. The participant should rest for five minutes (timed using a watch or stop watch) prior to the heart rate and blood pressure measurement.
2. When the five-minute rest period is over, but before the first blood pressure measurement is started, *record the time of day in Field 4 on the Seated Blood Pressure Form* (examples: 04:25 P [p.m.] or 11:38 A [a.m.]).
3. *Record the room temperature in Field 7 on the Seated Blood Pressure Form.*

5.5.5.7 Blood Pressure Measurement

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.

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.)

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:
 - 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.
 - 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”).
 - 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.
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.
5. *Record the three sequential blood pressure readings in Fields 4, 5, and 6 on the Seated Blood Pressure Form.*
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. Paste or staple the printout in the ad-hoc page.
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.

5.5.5.8 BP Measurement Instructions for Participants With Short, Thick Arms

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*.
2. Measure the forearm circumference at the midpoint between the olecranon and the ulnar styloid (wrist bone on pinkie side). Select the proper size cuff based on the forearm measurement. The blood pressure procedure is otherwise the same.
3. You must document on the Seated Blood Pressure Form that you have measured the *forearm blood pressure*.

5.5.5.9 Reporting Blood Pressure Results to Participants

1. 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.
2. Alternatively, if the blood pressure is normal (<140/90), the technician may say that it is normal, particularly if asked.
3. 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.
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.)

5.5.6 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.

- * Calibration will be performed by Critikon. Contact the Critikon sales representative. A loan device will be provided while the study device is being calibrated. In addition, Critikon will perform yearly calibration on all study devices.

5.5.7 Certification for Resting Blood pressure Measurement in mesa

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

5.6 *Ankle/Arm Blood Pressure*

5.6.1 Background and Rationale

The presence of peripheral vascular disease will be assessed with the ankle/arm blood pressure index (ABI). A low ABI is highly correlated with lower extremity arterial disease and has been shown to predict all-cause and cardiovascular mortality in several studies. A normal ABI is 1.0 to 1.5, with progressively lower values corresponding to worsening arterial disease. This method may underestimate, however, the occurrence of the disease in patients with long-standing diabetes, due to medial arterial calcification and incompressibility of blood vessels.

5.6.2 Equipment and Supplies

- Nicolet Doppler apparatus (EN50 LE 100, Nicolet vascular, Golden, CO) with an earpiece.
- gV batteries.
- Two full tubes of ultrasound transmission gel. (Gel can be purchased in bulk and tubes refilled.)
- A mercury sphygmomanometer with a male quick release coupler. The mercury column manometer should be mounted at “eye level.”
- Blood pressure cuffs: adult, large adult, and thigh
- Arm cuffs: Two 16 cm wide arm blood pressure cuffs (large arm), two 12 cm wide arm blood pressure cuffs (regular arm or adult), two 10 cm wide arm blood pressure cuffs (pediatric or child), and two thigh blood pressure cuffs.
- Tissue or wash cloth to remove the ultrasound contact gel.
- Black ball point pen and a marker.
- Ankle-Arm Blood Pressure Form.

5.6.3 definitions

1. Peripheral vascular disease, peripheral atherosclerosis, and peripheral arterial obstructive disease are synonyms. Peripheral vascular disease does not refer to venous disease, small-artery obstructive disease, vasospastic disease, cold sensitivity, or capillary disease.
2. The ABI is a ratio of ankle to arm pressure and is computed separately for each leg. The numerator for the right leg is the higher of the two systolic ankle pressures (posterior tibial or dorsalis pedis) in the right leg. The numerator for the left leg is the higher of the two systolic ankle pressures in the left leg. The denominator for both legs is the average of the right and left brachial systolic blood pressures. If the two arm pressures differ by 10 mm Hg or more, take the higher arm pressure instead as the denominator.
3. An ABI of 0.90 or less is considered peripheral arterial disease, although peripheral

arterial disease could exist if the ABI is 0.95 or less.

4. Moderate to severe lower extremity arterial obstructive disease is considered to be present if the ABI is less than 0.80.
5. Medial calcification could exist in an artery, if the ankle systolic blood pressure exceeds 290 mm Hg in any participant or 240 mm Hg in a participant with brachial systolic pressure less than 160 mm Hg; or if the ABI index is >1.5 .

5.6.4 Methods

5.6.4.1 Preparation

1. Thoroughly explain the procedure to the participant and allow him/her to ask questions.
2. 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 measurement is being made. Have the participant lie supine, with arms and legs (to mid-calf) bared, on a comfortable horizontal examination table.
3. Do not place blood pressure cuffs over any open lesion that could be a potential source of contamination.
4. Have the participant rest quietly for at least 5 minutes before beginning the measurement procedure. *Record the date of the examination on the Ankle-Arm Blood Pressure Form.* 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.
5. Place an appropriate blood pressure cuff around the arm, based on arm circumference at midpoint:
 - Adult (12 cm width) for arm circumference of >32 cm
 - Large adult (17 cm width) for arm circumference of 32–42 cm
 - Thigh (20 cm width) for are circumference of ≥ 43 cm

For the ankle, use the adult (12 cm) cuff size. Place the cuff so that the lower portion rests 3 cm above the medial malleolus (ankle bone).

5.6.4.2 Arterial Blood Pressure Measurement

1. *These first two steps are optional:* Locate the brachial artery on both arms by palpation in the antecubital fossa. Similarly, locate the dorsalis pedis (dorsum of

the foot) and posterior tibial (medial ankle) arteries on both legs. Mark the location of each artery with a black marker. Sometimes the arterial location in the feet will not be palpable but can be determined with the Doppler.

2. Using the procedure below, measure systolic arterial pressures in the following order:
 - right brachial artery
 - right dorsalis pedis
 - right posterior tibial
 - left dorsalis pedis
 - left posterior tibial
 - left brachial
 - Place a *liberal* amount of ultrasound conducting gel over pulse location.
 - Place the Doppler probe over the artery and then turn on the Doppler. Angle 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.
 - In a small percentage (< 10%) of participants, you will not be able to find the 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 here, enter "999" in Field 1 on the Ankle-Arm Blood Pressure Form.
 3. Inflate the cuff slowly until the pulse is no longer audible. Inflate to 20 mm Hg above the level at which pulse sound disappeared. (If the pulse cannot be obliterated, you may raise pressure to a maximum 300 MMHg. If not obliterated at that point, record "unable to occlude.") 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 systolic pressure at this location. Deflate the cuff completely. *Record the measurement in Field 1 on the Ankle-Arm Blood Pressure Form.* Wait for 20 seconds and then repeat the process to obtain a pressure measurement at each of the remaining sites.
 4. If the signal remains faint as more pressure is released or if the probe moves off the artery, deflate the cuff completely, wait for 20 seconds, and then repeat the measurement.
- 5.6.4.3 *Record the outcome (completed/not complete) in Item 2.* If not completed, continue to Item 3 and record the reason(s) that the procedure was not completed.

5.7 *Medical History*

5.7.1 Purpose

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

- classify persons who are not candidates for certain incident non-CVD disease endpoints (such as diabetes or cancer);
- be used to adjust for co-morbidity;
- characterize the participant's access to medical care; and
- characterize family history of CVD.

5.7.2 Materials/Equipment

None.

5.7.3 Definitions

None.

5.7.4 Methods

5.7.4.1 General instructions

This is an interviewer-administered form. For each question, possible responses are "Yes", "No," "Don't Know," and/or "Not Applicable" or "N/A" (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.*

5.7.4.2. Specific instructions

Questions 1-21 pertain to conditions the participant has been told he or she ever had by a doctor, or medical procedures performed. 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 health care practitioner 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.

For any question that asks about the age at diagnosis or when treatment was begun, assist the participant in converting the year to age, if necessary.

"Has a doctor ever told you that you had any of the following?"

1. **Emphysema.** Include "chronic bronchitis" or "chronic obstructive pulmonary

disease" or "COPD."

2. **Asthma.**

3. **Arthritis.** Include "osteoarthritis," "wear-and-tear arthritis," "rheumatoid arthritis," and forms of arthritis associated with other diseases.

4. **Cancer**

If yes, select type(s):

- a. Prostate cancer
- b. Breast cancer
- c. Lung cancer
- d. Colon cancer
- e. Non-melanoma skin cancer (i.e., basal cell carcinoma or squamous cell carcinoma)
- f. Blood cancer (e.g., leukemia, lymphoma, multiple myeloma)
- g. Other cancer (specify)

5. **Rheumatic heart disease or heart valve problems.**

6. **Blood clots** in the lungs or leg veins. Include "phlebitis," "deep vein thrombosis" (a blood clot in a leg vein that obstructs blood flow), "thrombophlebitis," or "pulmonary embolus" (a blood clot that has traveled from a leg vein to the lung: this is serious, often requires hospitalization, and can occur after surgery and prolonged bed rest). Clots are often treated with Coumadin and other blood thinners.

7. **Liver disease.**

If yes, select type:

- a. Cirrhosis (can also be called "liver failure") is a *chronic* condition (can develop and progress over many years) and may be associated with jaundice (yellow eyes and skin), weight loss, abdominal and leg swelling, and skin changes.
- b. Hepatitis is an *acute* condition (occurs shortly after the infection) and usually accompanied by jaundice and sometimes abdominal pain, which may be the only signs of infection.

If yes, select type(s):

- Hepatitis A (sometimes called "infectious hepatitis")
- Hepatitis B (sometimes called "serum hepatitis")
- Hepatitis C, D and E (may also be transmitted by blood products)

8. **Heart attack.**

9. **Angina.**
10. **Stroke.**
11. **TIA or small stroke.**

“Have you ever had any of the following medical procedures?”

12. **Coronary bypass surgery.**
13. **Balloon angioplasty.**
14. **Heart valve replacement.**
15. **Pacemaker implant.**
16. **Defibrillator implant.**
17. **Other surgery on your heart or arteries in your neck.**

“Has a doctor ever told you that you had any of the following?”

18. **Kidney disease.**
19. **High blood pressure or hypertension.** If the participant asks about "borderline" or "mild" hypertension, try to determine if the readings were greater than or equal to 140/90. If not, tell the participant to indicate "No." This level of blood pressure would generally not be treated with medication and, therefore, in order to avoid classifying some persons with mild hypertension as having hypertension and others as not, these persons should be recorded as *not* having hypertension. The MESA examination's blood pressure readings will generally help in distinguishing between these types of hypertension.)

If no, skip to question 10

If yes, answer the following:

- a. **Are you taking medicine for this?**
If yes, answer the following:
- b. **At what age did you begin taking medications?**
Provide age or answer “don’t know.”

20. **High cholesterol level.**
If yes, answer the following:

- a. **Are you taking medicine for this?**
If yes, answer the following:

- b. **At what age was this first treated?**
Provide age or answer “don’t know.”
21. **Diabetes.** (sugar in the blood)
If yes, answer the following:
- a. **Are you taking medicine for this?**
If yes, choose insulin or pills or both
- b. **At what age was the diabetes first treated?**
- c. **Was insulin your first diabetes medicine?**
- d. For women only: **Did diabetes occur ONLY during pregnancy?** (This would have been discovered by “spilling sugar in the urine,” by a glucose test administered during pregnancy, or by a history of taking insulin during pregnancy.)

Additional questions about the participant’s current health and health history. If the participant has difficulty remembering, encourage him/her to provide a best guess.

22. **What was your highest weight in the last 3 years?**
Give answer in pounds (lbs).
- a. **What did you weigh at age 20*?** (Give answer in pounds [lbs])
- b. **What did you weigh at age 40*?** (Give answer in pounds [lbs])
*Women: **If you were pregnant at either of these ages, give your weight just BEFORE your pregnancy started.**

Reproductive History—for women only—men skip to question 28

23. **Have you ever been pregnant?**
If yes, answer the following:
- a. **Number of pregnancies?** Give number.
- b. **Number of live births?** Give number.
- c. **Age at first live birth?** Provide age.
24. **Have you had a hysterectomy (surgery to remove your uterus/womb)?**
If yes, answer the following:
- a. **At what age?** Provide age.
25. **Have you had surgery to remove your ovaries?** Removal of the ovaries might have been in conjunction with a hysterectomy.
If yes, answer the following:

- a. **At what age?** Provide age.
 - b. **How many ovaries were removed?** Select 1 or 2. (If both ovaries have been removed, but at different times, record age at which second ovary was removed.)
26. **Have you ever taken birth control pills?** (Y/N/DK)
If yes, answer the following:
- a. **Please estimate the total number of years that you took birth control pills, keeping in mind that you may have started and stopped several times. Give estimate rounded to the nearest whole year.**
27. **Have you gone through menopause (“change of life”)?**
If yes, skip to questions 17d, e, and f.
If no or don't know, continue with question 17a.
- a. **Are you currently going through menopause?** (This would generally be associated with signs such as irregular menstrual periods, hot flashes, insomnia, vaginal dryness.)
If no, skip to question 18.
If yes or don't know, answer the following, if applicable:
 - b. **Date of last menstrual period** (if less than 12 months ago)? Give two-digit month and four-digit year.
 - c. **How many periods have you had in the last 12 months?** Give number.
 - d. **At what age did you go through menopause?** Provide age; provide current age if currently going through menopause.
 - e. **Have you ever taken hormone replacement therapy?**
If no, skip to question 28
If yes, answer the following:
 - f. **Are you currently using hormone replacement therapy?**
If yes, at what age did you begin?
If no, answer the following:
 - **At what ages did you start and stop taking hormones?** Provide ages.
 - **What type of therapy were you on?** (Common estrogen-only preparations are Premarin or Estratab; common estrogen+progestin regimen is Premarin plus Provera, Estratab plus Provera, Prempro, or Premphase.) Select “estrogen-only” or “estrogen+progestin.”
28. **Do you ever get pain in either leg or buttock while walking?**
If yes, answer the following:
- a. **Does this pain ever begin when you are standing still or sitting?**

- b. **In what part of your leg or buttock do you feel the pain?** Select “includes calf” or “does not include calf.”
 - c. **Do you have pain if you walk uphill or hurry?**
 - d. **Do you have pain if you walk at an ordinary pace on the level?**
 - e. **Does the pain ever disappear while you are walking?**
 - f. **What do you do if you have pain when you are walking?** Select “stop or slow down” or “continue on.”
 - g. **What happens to the pain if you stand still?** Select “relieved” or “not relieved.” **If relieved, 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.
29. **Have you ever had swelling (“edema”) of your feet or ankles?** For women, exclude edema during pregnancy. When the swollen extremity is pressed with a finger, an imprint or pit remains temporarily.
If yes, answer the following:
- a. **Did it tend to come on during the day and go down overnight?**
30. **Have you had to sleep on two or more pillows to help you breathe?** Some people may have to sleep in a chair to assist breathing; count this instance as “yes.”
31. **Have you been awakened at night by trouble breathing?**
32. **In the past two weeks, have you had any of the following:** This question is asked to determine if the participant has had some type of inflammatory condition. Select all that apply.
- a. Fever
 - b. Cold, flu, or sore throat
 - c. Urinary infection (also called “bladder infection”)
 - d. Seasonal allergy, such as hayfever
 - e. Bronchitis
 - f. Sinus infection or sinusitis
 - g. Pneumonia
 - h. Gums bleeding while brushing or flossing (include “periodontal disease” and “gingivitis”)
 - i. Tooth infection requiring antibiotics and/or root canal
 - j. Flare-up of gout
 - k. Flare-up of arthritis

33. **Approximately how many times have you been treated with antibiotics in the past year?** Estimate number of times or select “none.” If the participant has been on daily antibiotics for an extended period, record “99” and make comment.
34. **Approximately how many times have you been treated with antibiotics in the last 5 years?** Estimate the number of times or select “don’t know.” If the participant has been on daily antibiotics for an extended period, record “99” and make comment.
35. **Have you ever used aspirin on a regular basis?** Examples of "regular" are daily, every other day, and weekly. If the participant says he/she takes it less than once a week, indicate zero.
If no or don’t know, skip to question 26
If yes, answer the following:
- a. **At what age did you start?** Provide age.
- b. **Are you taking aspirin now on a regular basis?**
If yes, how many days a week are you taking aspirin? Give number of days/week.
If no, at what age did you stop taking it? Provide age.
36. **Has a dentist ever told you that you have periodontitis or gum disease?** (“Gingivitis” is another term.)
37. **Have you lost any of your teeth due to gum disease?** (Do not count tooth loss due to trauma.)
If yes, how many teeth have you lost?

5.8 *Personal History*

5.8.1 Purpose

The Personal History form 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.

5.8.2 Materials/Equipment

None.

5.8.3 Definitions

None.

5.8.4 Methods

This form is usually self-administered. Instruct the participant to fill out all questions, except those that are skipped due to skip patterns. 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.

Participant Information (questions 1–17)

1. **What is your gender?** Fill in the appropriate circle.
2. **Where were you born?**
Select one of the following
 - One of the 50 US states (please specify state). Provide two-letter abbreviation (e.g., WA, MI, AK, TX).
 - Puerto Rico
 - Another country (please specify country). Write in space provided.If the born in Puerto Rico or another country, how many years have you lived in the United States? Record number of years in box provided.
3. **Where were your parents and grandparents born?** If born in the US, please select “one of the 50 US states” and specify the state (specific state not required for grandparents). If born in Puerto Rico, select “Puerto Rico.” If born in another country, please select “another country” and write in the name of the country
4. **What language is generally spoken in your home?** Fill in the appropriate circle. If “other,” specify language.
5. **What is your marital status?** Fill in the appropriate circle. (“Separated” includes both legal and informal separations but not cases in which spouses live in separate geographic locations for professional reasons.)
6. **What is the highest degree or level of school you have completed?** Fill in the appropriate circle. If currently enrolled, mark the highest grade completed or highest degree received.

“We are asking for your Social Security number because data from this study will be linked with data supplied by the National Center for Health Statistics. Your number will be kept confidential, in accordance with the Privacy Act of 1974, and will be used only for research purposes. Providing this information to MESA is extremely important for the purposes of the study but is entirely voluntary on your part.”

- 7a. **What is your Social Security number?** Write the number in the spaces provided.

- 7b. **Sometimes dependents or spouses can apply for Medicare benefits using the Social Security number of another family member. Did you ever get Medicare benefits using a Social Security number other than your own?**
If no, skip to question 9.
If yes, write that Social Security number in the spaces provided.
(When necessary, reassure the person that this is entirely legal and is not a “trick” question intended to get him/her into trouble.)
8. **Please choose one of the following that best describes your current occupation.** Fill in the appropriate circle.
If homemaker, did you previously work outside the home?
If no, skip to question 13.
If yes, continue to next question.

Questions 9–12 are used to compute industry and occupation codes. The actual coding will be done at a later time. The interviewer may or may not be the person who does the coding, so it is important that job information is complete, accurate, and legible. (Note: Questions 9–12 will not be scanned. Only the final codes will be included in the database.)

9. **For whom do/did you work?** (Name of company, business, organization, or other employer. If you are not working now, please respond regarding your main occupation before you stopped working.) Write answer in space provided.
10. **What type of business or industry is/was this?** (e.g., hospital, newspaper publishing, mail order house, auto repair shop, bank, etc.) Write answer in space provided.
11. **What kind of work do/did you do or what was your job title?** (e.g., registered nurse, personnel manager, auto mechanic, accountant, grinder operator, etc.) Give answer in space provided.
12. **What were your most important activities or duties?** (e.g., patient care, directing hiring policies, repairing automobiles, reviewing financial records, operating grinding mill, etc.) Give answer in space provided.

“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.”

13. **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. Fill in the appropriate circle.

- 14a. **Including yourself, how many people are supported by the income listed above?** Write a number in the box provided.
- 14b. **How many of these are:**
- **Children under 18?**
 - **Adults 65 and over?**
- Write a number in each box. Enter 00 if no one in that age category is supported by the given income.
15. **This question is about the house or apartment where you live. Do you...?** Choose one and fill in the appropriate circle. (“Rent” includes renting a room in an owner-occupied dwelling or renting a room in a rooming house. “Other living arrangements” include, for example, living for free with parents or friends or receiving free rent in exchange for apartment management, yard work, etc.)
16. **Where do you usually go for medical care?** Choose one and fill in the appropriate circle. If “other,” specify. 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.
17. **To help pay for your medical care, do you now have...?** Choose as many that apply and fill in the appropriate circles. If “other,” specify. Mark “other” only if the response clearly does not fit one of the given responses.

Smoking (questions 18–44)

The following script introduces the participant to questions on smoking. The goal of these questions is to identify non-smokers, former smokers, and current smokers of any tobacco product and to document participants’ smoking habits. These questions were developed from NHANES III, the National Health Interview Survey, and ARIC. If the participant feels uncomfortable with these questions, please reassure him/her that all collected data is strictly confidential. Questions are in boldface type; explanations, if needed, follow in standard type.

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

18. **Have you smoked at least 100 cigarettes in your lifetime?**
The goal of this question is to measure lifetime cigarette smoking habits. Most US cigarettes, and cigarettes sold abroad, are and have been sold in packages

containing 20 cigarettes. Therefore, 100 cigarettes will usually be equivalent to 5 packs.

If no, skip to question 23.

19. **How old were you when you first started smoking cigarettes?**
Provide age. If participants do not remember the exact age they first smoked cigarettes, ask them to give their best estimate.
This question applies to both current and former smokers—those who answered “Yes” to question 18.
20. **Have you smoked cigarettes during the last 30 days?**
If yes, skip to question 22.
21. (For former smokers) **How old were you when you quit smoking cigarettes?**
Provide age. If participants do not remember the exact age they quit smoking cigarettes, ask them to give their best estimate.
This question applies only to former smokers—those who answered “No” to question 20.
22. **On average, about how many cigarettes a day do/did you smoke?** Provide number of cigarettes per day. Record 00 if the average number of cigarettes per day is/was less than one. This question applies to both current and former smokers. Make sure participants record the information in number of cigarettes per day. If participant answers in number of packs per day, calculate the number cigarettes per day (1 pack = 20 cigarettes).
23. **Have you ever used any other tobacco products (e.g., cigars, pipes, snuff, chewing tobacco)?** The goal of this question is to obtain information about the use of other tobacco products. This question should be answered by all participants.
If no, skip to question 44
24. **Have you smoked more than 20 cigars in your lifetime?**
If no, skip to question 29
25. **How old were you when you first started smoking cigars?**
Provide age. If participants do not remember the exact age they first smoked cigars, ask them to give their best estimate. (Provide age)
26. **Have you smoked cigars during the last 30 days?**
If yes, skip to question 28
27. **How old were you when you quit smoking cigars?**
Provide age. If participants do not remember the exact age they quit smoking cigars, ask them to give their best estimate. (Provide age)

28. **On average, about how many cigars a day do/did you smoke?**
Provide number of cigars per day. This question applies to both current and former cigar smokers. Record 00 if the average number of cigars per day is/was less than one.
29. **Have you smoked at least 20 pipefuls of tobacco in your lifetime?**
If no, skip to question 34
30. **How old were you when you first smoked a pipe?**
Provide age. If participants do not remember the exact age they first smoked a pipe, ask them to give their best estimate.
31. **Have you smoked a pipe during the last 30 days?**
If yes, skip to question 33
32. **How old were you when you quit smoking a pipe?**
Provide age. If participants do not remember the exact age they quit smoking a pipe, ask them to give their best estimate.
33. **On average, about how many pipefuls a day do/did you smoke?**
Provide number of pipefuls per day. This question applies to both current and former pipe smokers. Record 00 if the average number of pipefuls per day is/was less than one.
34. **Have you used chewing tobacco, such as Redman, Levi Garret, or Beechnut, at least 20 times in your lifetime?**
If no, skip to question 39
35. **How old were you when you first chewed tobacco?**
Provide age. If participants do not remember the exact age they first chewed tobacco, ask them to give their best estimate.
36. **Have you chewed tobacco during the last 30 days?**
If yes, skip to question 38
37. **How old were you when you quit chewing tobacco?**
Provide age. If participants do not remember the exact age they quit chewing tobacco, ask them to give their best estimate.
38. **On average, about how many times a day do/did you chew tobacco?** Provide number of times per day participant chewed tobacco.
This question applies to both current and former tobacco chewers. Record 00 if the average number of uses per day is/was less than one.
39. **Have you used snuff, such as Skoal, Skoal Bandits, or Copenhagen, at least 20 times in your lifetime?** Snuff is a finely chopped tobacco product that is not

chewed. A small amount is held in the mouth between the cheek and gum, where nicotine is rapidly absorbed into the bloodstream.

If no, skip to question 44

40. **How old were you when you first used snuff?**
Provide age. If participants do not remember the exact age they first used snuff, ask them to give their best estimate.
41. **Have you used snuff during the last 30 days?**
If yes, skip to question 43
42. **How old were you when you quit using snuff?**
Provide age. If participants do not remember the exact age they quit using snuff, ask them to give their best estimate.
43. **On average, about how many times a day do/did you use snuff?**
Provide number of uses per day.
This question applies to both current and former snuff users. Record 00 if the average number of uses per day is/was less than one.
44. (Current non-smokers only) **During the past year about how many hours per week were you in close contact (in your home, at work, in a car, etc.) with people when they were smoking?**
Provide number of hours per week. This question applies to current non-smokers and former users of any kind of tobacco product. 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 per week.

Alcohol Use (questions 45–54)

The goal of these questions is to identify current, previous, and non-drinkers, and to document current and past consumption patterns. Frequency of alcohol intake is determined by usual weekly intake. The serving sizes are different for beer (a 12 oz. glass, can, or bottle), wine (4 oz.), and hard liquor (1.5 oz). Mixed drinks or cocktails (e.g. martini, margarita) are prepared with hard liquor and other beverages, such as soda, seltzer, syrup, etc. Questions are in boldface type; explanations, if needed, follow in standard type; possible answers follow in parentheses.

45. **Have you ever consumed alcoholic beverages?** The goal of this question is to determine if a participant has ever consumed alcoholic beverages during his/her life. Participants who report no alcohol consumption are asked no more questions in this form.

If no, you have completed this form

46. **How old were you when you first started drinking alcoholic beverages?**
Provide age. If participants do not remember the exact age they first used alcohol, ask them to give their best estimate.
47. **Do you presently drink alcoholic beverages?**
If yes, skip to question 51
48. **For how many years did you drink alcoholic beverages?** (*Do not count times when you did not drink alcohol.*)
Provide number of years. If necessary, explain that if there were periods of times during which they didn't drink any alcohol they should not take those years in account. If participants do not remember the exact number of years, ask them to give their best estimate. Record 00 if participant drank alcoholic beverages for less than one year.
49. **In the past, what types of alcoholic beverages did you ordinarily drink?**
Select all that apply. Participants may choose more than one type of beverage.
50. **What was the usual number of drinks you had per week before you stopped drinking alcoholic beverages?** (One drink means one beer or one glass of wine or one shot of hard liquor or one mixed drink. Record 0 if you had less than one drink per week.) Provide number of drinks per week. "Usual" means "on average." "Per week" includes weekends. If participants do not remember the exact number of drinks per week, ask them to give their best estimate. If they give number of drinks per month, divide by four to derive weekly intake. Record 00 if participant had less than one drink per week.
51. IF YOU CURRENTLY DRINK ALCOHOL: **For how many years have you been drinking alcoholic beverages?** (*Do not count times you did not drink alcohol.*) Provide number of years. If necessary, explain that if there were periods of times during which they didn't drink any alcohol they should not take those years in account. If participants do not remember the exact number of years, ask them to give their best estimate. Record 00 if participant has been drinking alcoholic beverages for less than one year.
52. **What is the usual number of drinks you have per week?** (One drink means one beer or one glass of wine or one shot of hard liquor or one mixed drink. Record 0 if you have less than one drink per week.) Provide number of drinks per week. "Usual" means "on average." "Per week" includes weekends. If participants do not remember the exact number of drinks per week, ask them to give their best estimate. If they give number of drinks per month, divide by four to derive weekly intake. Record 00 if participant has less than one drink per week.
53. **During the past 24 hours how many drinks have you had?** Provide number of

drinks in past 24 hours. The number of drinks includes all the wine, beer, and hard liquor consumed within the 24 hours prior to the interview. Record 00 if the participant has had less than one drink.

54. **In the past month what is the largest number of drinks you had in one day?** Provide the largest number of drinks per day. The largest number of drinks includes all the wine, beer, and hard liquor consumed within a single 24-hour period in the past month. Record 00 if the participant had less than one drink on any given day in the past month.

At this point the form is completed. If the form was self-administered, check for completeness. Fill in the box labeled, "For MESA Field center Use Only." Mark if form was self-administered or interviewer-administered. Record Interviewer or Reviewer ID (your ID number) and the date the form was completed.

5.9 Physical Activity

MESA Typical Week Physical Activity Survey (MESA-TWPAS) Administration Guidelines

5.9.1 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 28 questionnaire 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 and volunteer activities.

5.9.2 materials/equipment

None.

5.9.3 Definitions

None.

5.9.4 Methods

5.9.4.1. General Instructions

1. Read the following instructions to the participant.

“Think about the types of activities you did in a typical week in the past month. For each activity, note which you did in a typical week by filling in the circle for ‘yes’ (Y) or ‘no’ (N). For each item that you mark as ‘yes,’ fill in the circle for the number of days in a typical week you did these activities and the average time per day in hours and minutes you did these activities.”

2. 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.”

3. Explain the example to the participant. To orient the him/her to the past month, you will identify that period for the participant. In the text below, if we assume, for instance, that the current date is March 15, the past month would start on Feb 15.

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.”

“Let me show you an example of how you will fill out the survey.”

4. Provide the example 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.

5. 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.
6. 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 2 hours/day 1 day/week, ask him/her to add about 15 minutes extra to each day, to take into account a single day that has a prolonged bout of activity compared to the usual.

7. At this point allow the participant to complete the form him-/herself, but be available to answer questions and provide clarification.

5.9.4.2 Specific Instructions

Items to be completed by the interviewer:

- For **date** enter date form was completed. For example, July 7, 2000 would be entered 07/07/2000.
- For **ID #** enter the participant's study identification number.

5.9.4.3 Item-by-Item Clarification

“In a typical week in the past month, did you do...”

Household chores

1. Light effort: 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: 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

3. Moderate Effort: 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: 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

5. Light Effort: 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: 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.

Transportation

7. These are all light activities and do not include walking. Have the respondent include all time spent in transportation where they are sitting.

Walking (not at work)

8. 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.
9. Walking for exercise, pleasure, social reasons, walking during work breaks, and walking the dog is classified as intentional walking. 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 8.

Dancing/Sport Activities

10. 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.
11. 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.
12. 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.
13. Individual activities. These may be sports activities, such as golf and bowling, or more individual relaxation/mediation 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.

14. Moderate Effort. 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.
15. Heavy Effort. 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.

16. 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.

17. Read, knit, sew, visit, do nothing, non-work recreational computer. These are markers of sedentary activities that exclude watching TV. If people report knitting while watching TV, make sure they select one category or the other and avoid duplicate category reporting.

Occupational Activities.

18. **Do you work to earn money?**

If no, skip to question 24.

If yes, continue to question 19.

Respondents should report activities only if they work to earn money. Volunteer activities will be asked about next. Unpaid household activities and caring for others were included in questions 1–6.

19. Days/week and hours/day worked. If the respondent works at more than one job, record the total days worked per week in all jobs. To estimate the average hours worked per week, ask the respondent to consider the hours they work in the second job (or third, if applicable) and then add them to the average time they work per day. See the example about averaging time per day when activities are done longer on some days of the week than on others.

Respondent should fill in the circles for the time spent in each activity at work. The hours per day for all activities should equal the total hours per day worked. Recommend that the respondent keep a cumulative total of the hours recorded doing occupational activities, in order to avoid estimating more time in occupational tasks than time they work on an average day. They can also subtract the hours from the total time worked per day as they identify how the time is spent in different types of activities.

“At work, did you do....”

20. Light Effort/Sitting Activities. For most respondents, this will be the most hours in the work day.
21. 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.
22. 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).

23. Heavy Effort: 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.

Volunteer Activities

24. **Did you work as a volunteer doing activities you have not yet mentioned on this survey?**

If no, skip to question 28.

If yes, continue to question 25.

This question is designed to identify time spent in unpaid, work-like activities outside the home.

“Did your volunteer work include....”

25. Light Effort. The respondent should identify light activities that required little effort and were done predominantly while sitting or standing (e.g., cooking at church banquet).
26. Moderate Effort: The respondent should identify activities that required moderate effort and were done predominantly while standing and walking (e.g., participating in a neighborhood clean-up project).
27. Heavy Effort. The respondent should identify activities that required heavy effort, such as pushing, lifting, carrying, and climbing (e.g., building a house for Habitat for Humanity).

Walking Pace

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

Fill in appropriate circle. 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).

Offer the following guidelines:

- 2 mph = 30 minutes per mile (slow or casual)
- 2-3 mph = 20-30 minutes per mile (typical or average)
- 4-5 mph = 12-15 minutes per mile (very fast or almost a slow jog)
- More than 5 mph = 10 minutes per mile (race-walking)

5.10 Health and Life

5.10.1 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 anger, anxiety, depression, chronic burden (stress), and emotional social support. A section on perceptions of unfair treatment or discrimination is also included, because it has been suggested that the perception of discrimination may be an important source of stress for some social groups.

5.10.2 Materials/Equipment

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

5.10.3 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 every day life.

5.10.4 Methods

5.10.4.1 General Instructions

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.
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.

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 through, 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.

5.10.4.2 Specific Instructions

1. Questions 1A–1T correspond to the Spielberger trait anger and trait anxiety scales. The instruction on the form reads, "**For each of the following statements, please choose the one response that best describes you.**" Possible answers are "almost never," "sometimes," "often," "almost always." Answers are selected by filling in the appropriate circle.

Question 1:

- A. I am quick tempered
- B. I have a fiery temper
- C. I am a hotheaded person
- D. I get angry when I'm slowed down by others' mistakes
- E. I fly off the handle
- G. When I get mad, I say nasty things
- H. It makes me furious when I am criticized in front of others
- I. When I get frustrated, I feel like hitting someone
- J. I feel infuriated when I do a good job and get a poor evaluation
- K. I am a steady person

- L. I feel satisfied with myself
- M. I feel nervous and restless
- N. I wish I could be as happy as others seem to be
- O. I feel like a failure
- P. I get in a state of turmoil or tension as I think over my recent concerns and interests
- Q. I feel secure
- R. I lack self-confidence
- S. I feel inadequate
- T. I worry too much over something that does not matter

2. Questions 2–6 were designed to measure chronic burden or stress. (There is no strict definition of "ongoing". If asked, say "something that has been going on for a while." If the question arises, financial strain can be clarified as "economic or money problems.") The instruction on the form reads, "**Many people experience ongoing problems in their everyday lives. Please tell us whether any of the following has been a problem for you.**" Possible answers are "yes" or "no." Answers are selected by filling in the appropriate circle.

Question 2:

- a. Serious ongoing health problem (yourself)
If no, skip to question 3
If yes, answer the following:
- b. **Has this been a problem for six months or more?**
- c. **Would you say this problem has been... (select one):**
 - Not very stressful
 - Moderately stressful
 - Very stressful

Question 3:

- a. Serious ongoing health problem (someone close to you)
If no, skip to question 4
If yes, answer the following:
- b. **Has this been a problem for six months or more?**
- c. **Would you say this problem has been... (select one):**
 - Not very stressful
 - Moderately stressful
 - Very stressful

Question 4:

- a. Ongoing work difficulties with your job or ability to work
If no, skip to question 5
If yes, answer the following:

- b. **Has this been a problem for six months or more?**
- c. **Would you say this problem has been...** (*select one*):
 - Not very stressful
 - Moderately stressful
 - Very stressful

Question 5:

- a. Ongoing financial strain
If no, skip to question 6
If yes, answer the following:
- b. **Has this been a problem for six months or more?**
- c. **Would you say this problem has been...** (*select one*):
 - Not very stressful
 - Moderately stressful
 - Very stressful

Question 6:

- a. Ongoing difficulties in a relationship with someone close to you
If no, skip to question 7
If yes, answer the following:
 - b. **Has this been a problem for six months or more?**
 - c. **Would you say this problem has been...** (*select one*):
 - Not very stressful
 - Moderately stressful
 - Very stressful
3. Questions 7A–7Q 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”

4. Questions 8–13 are designed to measure emotional social support. The instruction on the form reads, “**Please read the following questions and mark the answer that best describes your life now.**” Possible answers are “none of the time,” “a little of the time,” “some of the time,” “most of the time,” or “all of the time.” Answers are selected by filling in the appropriate circle.

Question 8: Is there someone available to you whom you can count on to listen to you when you need to talk?

Question 9: Is there someone available to give you good advice about a problem?

Question 10: Is there someone available to you who shows you love and affection?

Question 11: Is there someone available to help you with daily chores?

Question 12: Can you count on anyone to provide you with emotional support (talking over problems or helping you make a difficult decision)?

Question 13: Do you have as much contact as you would like with someone you feel close to or someone in whom you can trust and confide?

5. Questions 14–21 are intended to assess perceptions of having been treated unfairly. The perception of unfair treatment (as a result for example of discrimination) may be an important source of stress, and could be related to health and cardiovascular disease. (Gender can be clarified as sex. Sexual orientation can be clarified as “whether you are gay, lesbian or straight.”) The instruction on the form reads, “**Now we would like to ask you a few questions about situations where you might have felt that you had been treated unfairly**” Possible answers for 14–19 are “yes” or “no.” Answers are selected by filling in the appropriate circle.

Question 14:

- a. **Do you think you have ever been unfairly fired or denied a promotion?**
If no, skip to question 15
If yes, answer the following:
- b. **What was the main reason? (select one)**
 - Race or ethnicity
 - Gender
 - Age
 - Religion
 - Physical appearance
 - Sexual orientation
 - Income level or social class
 - Other
- c. **Did this happen in the last 12 months?**

Question 15:

- a. **For unfair reasons, do you think you have ever not been hired for a job?**
If no, skip to question 16
If yes, answer the following:
- b. **What was the main reason? (select one)**
 - Race or ethnicity
 - Gender
 - Age
 - Religion
 - Physical appearance
 - Sexual orientation
 - Income level or social class
 - Other
- c. **Did this happen in the last 12 months?**

Question 16:

- a. **Have you ever been unfairly stopped, searched, questioned, physically threatened or abused by the police?**
If no, skip to question 17
If yes, answer the following:
- b. **What was the main reason? (select one)**
 - Race or ethnicity
 - Gender
 - Age
 - Religion
 - Physical appearance
 - Sexual orientation

- Income level or social class
 - Other
- c. **Did this happen in the last 12 months?**

Question 17:

- a. **Have you ever been unfairly discouraged by an advisor or teacher from continuing your education?**
If no, skip to question 18
If yes, answer the following:
- b. **What was the main reason? (select one)**
- Race or ethnicity
 - Gender
 - Age
 - Religion
 - Physical appearance
 - Sexual orientation
 - Income level or social class
 - Other
- c. **Did this happen in the last 12 months?**

Question 18:

- a. **Have you ever been unfairly prevented from moving into a neighborhood because the landlord or a realtor refused to sell or rent you a house or apartment?**
If no, skip to question 19
If yes, answer the following:
- b. **What was the main reason? (select one)**
- Race or ethnicity
 - Gender
 - Age
 - Religion
 - Physical appearance
 - Sexual orientation
 - Income level or social class
 - Other
- c. **Did this happen in the last 12 months?**

Question 19:

- a. **Have you ever moved into a neighborhood where neighbors made life difficult for you or your family?**
If no, skip to question 20
If yes, answer the following:
- b. **What was the main reason? (select one)**

- Race or ethnicity
- Gender
- Age
- Religion
- Physical appearance
- Sexual orientation
- Income level or social class
- Other

c. **Did this happen in the last 12 months?**

Possible answers for 20 are “almost every day,” “at least once a week,” “a few times a month,” “a few times a year,” “less than once a year,” or “at least once a week.” Answers are selected by filling in the appropriate circle.

Question 20: In your day-to-day life how often have any of the following things happened to you?

- A. You are treated with less courtesy than other people
- B. You are treated with less respect than other people
- C. You receive poorer service than other people at restaurants or stores
- D. People act as if they think you are not smart
- E. People act as if they are afraid of you
- F. People act as if they think you are dishonest
- G. People act as if they’re better than you
- H. You are called names or insulted
- I. You are threatened or harassed

Question 21: For the next two questions, please choose the one best answer:

- a. **If you feel you’ve been treated unfairly, do you usually:**
 - accept it as a fact of life?
 - try to do something about it?
- b. **And if you feel you’ve been treated unfairly, do you usually:**
 - talk to other people about it?
 - keep it to yourself?

6.6 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) ID number.

5.11 Medications

5.11.1 Background and Rationale

1. The MESA Medications Form and this section of the Manual of Operations were adapted from those used for the Cardiovascular Health Study. 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 transcribed, 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.

5.11.2 Materials and Equipment

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

5.11.3 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 or physician assistant 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 will 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.

5.11.4 Methods

- 5.11.4.1 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 and prescription and non-prescription medications taken during the two weeks prior to the clinic visit.

- 5.11.4.2 Medication use interview. Prior to beginning the interview, place all medications in front of the participant. 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:

A. Medication Reception

“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, 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?”

If yes, ask to see the medications and continue with Section B

If no, make arrangements to obtain medications

If refused, record reason for refusal in Comments Section

If took no medicines, *form is completed*

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 a 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.

B. Prescription Medications

1. The interviewer transcribes the name and dosage information from each medication container onto the Medications Form using the following guidelines:
2. Medication name. Print complete medication name using block capital letters. Record all characters and numbers referring to strength. 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 20 spaces available on the form, transcribe as much as possible and then record the complete medication name in the Comments Section. If it is not possible to transcribe the drug name, insert an asterisk (*) and explain in the Comments Section.
3. 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 a strength. Record the name in the "Medication Name" space and leave the "Strength" column blank.

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 propranolol 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.

4. Strength.
 - 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 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) \div 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.
5. 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). Circle 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).

6. 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: 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).

7. PRN (“as needed”) Medication is generally used for allergy, pain, or sleep; sublingual nitroglycerin is also used PRN.
 - Record in the “PRN Medicine?” column whether the medication is prescribed to be taken on an “as needed” basis.
 - Circle “Y” only when the prescription instructions state “as needed,” “when needed,” “if needed,” etc.
 - Circle “N” when the prescription instructions do *not* use the words “as needed,” “when needed,” “if needed,” etc.
 - The words “as directed” do *not* mean the same as “as needed.”
8. **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 average during the last two weeks, how many of these pills (or other medication items) did you take per 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.
 - Code “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.

C. Over-the-Counter Medications

Complete this section following instructions for Section B, above, but disregarding the instructions pertaining to “Number Prescribed” and “PRN Medication.”

D. Chinese and Other Traditional Medicines

Whenever possible, record traditional medicines in the usual way, according to

MOP protocol. If this is not possible, record the *purpose* of the medicine.

5.12 Family History

5.12.1 Purpose.

The Family History is designed to gather information regarding the health history of the participant's biological (natural) parents and biological (natural) siblings (including all blood related siblings e.g. half-siblings). Detailed family histories provide important information regarding familial clustering of disease. Participant reports of family histories have been employed in several family studies (i.e. the NHLBI Family Heart Study (FHS), the Family Blood Pressure Program) with good success. The MESA study is particularly interested in family history of medical conditions that are related to atherosclerotic (plaque) formation and diseases as well as cancer and asthma. Specifically, we are interested in family history of heart attack, stroke, high blood pressure, diabetes, and other heart diseases, i.e. valvular heart diseases, septal heart defects etc.

5.12.2 Definitions

In this questionnaire, an attempt has been made to use common terms that require no explanation, because they are used in the way they tend to be used by most people in every day life. However, some terms are more scientific and may require clarification. In such cases, please refer to the Definition and Synonyms for Medical Terminology at the end of the instruction.

However, it is critical that the participant understand that we are interested in gathering information only about his/her biological or natural parents and biological siblings. It should be explained that full biological siblings are brothers and sisters who have the same biological or natural parents as the participant. If indicated, it can additionally be explained that biological family members differ from adopted, step, or foster family members in that they have a genetic or "blood" relationship to the participant.

5.12.3 Methods

5.12.3.1 General Instructions

This is an **interviewer-administered form**. 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 Family History may be important in understanding their health. The information may, in turn, help us to better understand the causes of heart disease.

5.12.3.2 Specific Instructions for Completing the Family History Questionnaire

The interviewer should take time to become familiar with the Family History form. Note that the form is arranged with the disease of interest in the columns along the top (x-axis) and the “blood” relation of interest is in rows down the page (y-axis). Proceed with the first “relation” and ask about the diseases of interest pertaining to that particular “relation” along the row and across the page. Response choices are: Yes (Y), No (No), or Don’t know (D).

For example: First begin with “Spouse”, skip blacked out boxes and proceed to the next column. In this case, under the column “Age at last birthday or age at death,” fill in the digits for age of last birthday or at death. Then, under the next column “Heart Attack or M.I.” fill in “Y, N, or D”. If yes, ask for the age of first heart attack; fill in the digits for the age then continue to the next column. For each column of disease or condition, if the response is “Yes”, ask for the age of when this *first* occurred. For the column asking about cancer, in addition to age, ask the participant for the type of cancer. If the participant responded with more than one cancer, record the cancer that was diagnosed first.

Begin the questionnaire with the first column and the first row – regarding “Spouse” by asking:

Do you currently have a spouse?

If “no”, skip to the next “relation-person”, mother.

If “yes”, the interviewer should say, “*the next series of questions are about your spouse.*”

Then proceed with the next column:

Definition: *Spouse* is defined as a married person by state or common law. For the purpose of this questionnaire, same sex domestic partnership will not be considered as spouse.

What was your spouse’s age at the last birthday? If the spouse is deceased, then ask, ***“What was your spouse’s age at death, (when he passed away)?”***

Round off the age to the nearest whole number and enter the digits into the boxes then proceed to the next column of disease of interest. If there are more than one deceased spouse, only collect information on the most recent spouse.

Has your spouse ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*at what age did your spouse have the first heart attack?*”

Enter the age at first heart attack into the boxes then proceed to the next column.

Definition: To aid the participant, a *heart attack* can be defined as damage to, or death

of, part of the heart muscle due to insufficient blood supply.

The intent is to record whether the first occurrence of one of these events indicates that coronary heart disease was before or after a certain age. If the participant cannot answer this question confidently, the response should be coded as “Don’t Know.”

Has your spouse ever had heart surgery or a cardiac (heart) procedure, such as coronary bypass surgery, balloon angioplasty, or stent placement because arteries of the heart were blocked?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “at what age did your spouse have this procedure?”

Enter the age when the first procedure was done into the boxes then go to the next column.

Definition: *Coronary bypass* can be defined as surgery on the heart to improve blood supply/flow to the heart muscle. This surgery is most often performed when narrowed coronary arteries reduce the flow of oxygen-containing blood to the heart. *Balloon angioplasty* can be defined as a procedure to dilate (widen) narrowed coronary arteries. A catheter with a deflated balloon on its tip is passed into the narrowed artery segment, the balloon is inflated, and the narrowed segment is widened. Acceptable symptoms for heart attack, coronary bypass surgery, or balloon dilation are listed in the Definition and Synonyms for Medical Terminology at the end of these instructions.

The intent is to record whether the first occurrence of one of these procedures indicating coronary heart disease was before or after a certain age. If the participant cannot answer this question confidently, the response should be coded as “Don’t Know.”

Has your spouse ever had a stroke?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “at what age did your spouse have the first stroke”.

Enter the age at first stroke into the boxes then proceed to the next column.

Definition: To aid the participant, a *stroke* can be defined as the sudden loss of muscle function, vision, sensation, or speech resulting from brain cell damage caused by insufficient supply of blood to a part of the brain. A stroke may result from blockage of the arteries by blood clots, atherosclerotic plaques, or rupture of an artery. *Cerebral hemorrhage* can be defined as bleeding into or around the brain caused by a rupture of an artery or aneurysm in the brain. Cerebral hemorrhage usually causes sudden onset of very severe headache and is associated with other symptoms described above for stroke or with loss of consciousness. **DO NOT include TIAs or ‘mini-strokes’.** Acceptable synonyms for stroke and cerebral hemorrhage are listed in the Definition and Synonyms for Medical Terminology at the end of this instruction.

The intent is to determine whether the first time one of these events occurred prior to 60 years of age. If the participant cannot answer this question confidently, the response should be coded as "Don't Know."

Does/Did your spouse have high blood pressure or hypertension diagnosed by a physician?

If "no" or "don't know", fill in the appropriate circle then skip to the next column.

If "yes", ask, "at what age was your spouse first diagnosed with high blood pressure?"

Enter the age at first diagnosis of high blood pressure into the boxes then proceed to the next column.

A "Yes" response means that, to the best of the participant's knowledge, a physician specifically told his/her spouse of the diagnosis of high blood pressure or hypertension. Other conditions, such as being anxious, tense, or "hyper" should not be confused with high blood pressure.

Definition: To aid the participant, the interviewer can explain that blood pressure is measured using a device similar to the one used in this study. Based on those measurements, a physician may have diagnosed the person as having *high blood pressure or hypertension*. A spouse who may have made a self-diagnosis based on readings obtained using an automatic device in a mall or other location should not be considered as having high blood pressure, unless a doctor also verified the condition. In most cases, unless a doctor has prescribed anti-hypertensive medication or arranged a plan for follow-up blood pressure readings, a diagnosis of high blood pressure is in doubt.

Does/did your spouse have Diabetes or high blood sugar diagnosed by a physician?

If "no" or "don't know", fill in the appropriate circle then skip to the next column.

If "yes", ask, "at what age was your spouse first diagnosed with Diabetes?"

Enter the age at first diagnosed of diabetes into the boxes then proceed to the next column.

A "Yes" response to the question means that a physician has specifically told the participant's spouse that he/she has diabetes or high blood sugar. Other blood sugar conditions, such as "hypoglycemia", should not be considered a "Yes" response to this question. If a physician had told the participant's spouse that he/she has "borderline" or "early" diabetes (or high blood sugar), code the response as "yes".

Does/did your spouse have Cancer diagnosed by a physician?

If "no" or "don't know", fill in the appropriated circle then skip to the next column.

If “yes”, ask, ***“was this a cancer other than skin cancer such as basal cell or squamous cell skin cancer?”***

A “Yes” response to the question means that a physician has specifically told the participant’s spouse that he/she had cancer. An attempt should be made to encourage the participant to specify the type of cancer. If this is not possible, leave the fill in box blank.

If this cancer was a type other than the two mentioned then continue below; otherwise skip to the next column of disease/condition:

“Do you know the specific type of the cancer your spouse had?”

“At what age was your spouse diagnosed with this cancer (or the very first cancer if more the participant gives more than one cancer)?”

Enter the age when first diagnosed and the specific type of cancer into the appropriate boxes then proceed to the next column of disease of interest.

Does/did your spouse have any other heart diseases/problems diagnosed by a physician i.e. heart valve problems, heart defects, heart enlargements, heart conduction disorders or irregular rhythm disorders?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, ***“At what age was your spouse first diagnosed with this heart disease/problem?”*** Enter the age at first diagnosed into the boxes then proceed to the next column.

A “Yes” response to the question means that a physician has specifically told the participant’s spouse that he/she had some other heart disease/problems other than those specifically asked previously. An attempt should be made to encourage the participant to specify type of heart disease/problem. This will ensure that indeed some other heart disease exists.

Does/did your spouse have Asthma diagnosed by a physician?

If “no” or “don’t know”, fill in the appropriated circle then skip to the next column.

If “yes”, ask, ***“At what age was your spouse first diagnosed with Asthma?”***

Enter the age at first diagnosed with asthma into the boxes.

A “Yes” response to the question means that a physician has specifically told the participant’s spouse that he/she had Asthma. It can be explained to the participant that a person with asthma usually has an inhaler that is used to improve breathing.

The interviewer has now completed all questions in the first row regarding “Spouse”. The interviewer should now proceed to the second row.

Questions regarding the Biological/Birth Mother:

The following questions are regarding your Biological or Birth Mother, do you have any comments before we begin?

If the participant was adopted or for some reason does not have information about his/her biological/birth mother, the interviewer should confirm whether the participant responded “no,” or “don’t know” and fill in the appropriate circle.

If “no”, skip to the next row with question about the biological father.

If “yes” or “don’t know”, the interviewer will continue with the questions across the column i.e.

What was your mother’s age at the last birthday (if deceased, age at death)?

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column.

Has/Did your mother ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “at what age did your mother have the first heart attack?”

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next “Relation” column.

Questions regarding the Biological/Blood Father.

The interviewer should begin with:

The following questions are regarding your Biological Father; do you have any comments before we begin?

If the participant was adopted or for some reason does not have information about his/her biological/birth father, the interviewer should confirm whether the participant responded “no,” or “don’t know” and fill in the appropriate circle.

If “no”, skip to the next row with question about the biological siblings.

If “yes” or “don’t know”, the interviewer will continue with the questions across the column i.e.

What was your Father’s age at the last birthday (if deceased, age at death).

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column of disease of interest.

Has/Did your Father ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “at what age did your Father have the first heart attack?”

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next "Relation" column.

Questions regarding the Biological/Blood Siblings

The interviewer should begin with:

The following questions are regarding your Biological Siblings; we will begin with the oldest sibling and work our way to the youngest. Do you have any comments before we begin?

If the participant has *no siblings*, skip this and proceed to the next "Relation" category – Child.

If the participant has *more siblings than available on the form*, use a second form to obtain complete information on all siblings.

If the participant for some reason does not have information about his/her biological/blood siblings, the interviewer should confirm whether the participant said "no," or "don't know" and fill in the appropriate circle.

If "no", skip to the next row with question about the biological Children.

If "yes" or "don't know", the interviewer will continue with the questions across the column i.e.

What was your Sibling's age at the last birthday (if deceased, age at death).

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column.

Has/Did your Sibling ever had a heart attack or Myocardial Infarction?

If "no" or "don't know", fill in the appropriate circle then skip to the next column.

If "yes", ask, "***at what age did your Sibling have the first heart attack?***"

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next sibling or "Relation" column.

Questions regarding the Biological/Blood Children

The interviewer should begin with:

The following questions are regarding your Biological Children. We will begin with the oldest child and work our way to the youngest. Do you have any comments before we begin?

If the participant has *no children*, skip this and the interview is now completed.

If the participant has *more children than available on the form*, use a second form to obtain complete information on all children.

If the participant for some reason does not have information about his/her biological/birth children, the interviewer should confirm whether the participant said “no,” or “don’t know” and fill in the appropriate circle.

If “no”, skip this section and the interview is now complete. Thank the participant.

If “yes” or “don’t know”, the interviewer will continue with the questions across the column i.e.

What was your child’s age at the last birthday (if deceased, age at death).

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column.

Has/Did your child ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “at what age did your child have the first heart attack?”

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next child.

Once all the information has been obtained for the last child, the interview is 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,” select whether the form was self-administered or interviewer-administered. Record reviewer (if form self-administered) or interviewer (if form interviewer-administered) ID number.

Table of Medical Terms

MEDICAL CONDITION OR PROCEDURES	DEFINITIONS & SYNONYMS
Hypertension or high blood pressure	A chronic increase in blood pressure above its normal range that does not include being anxious, tense or "hyper".
Diabetes or high blood sugar	A chronic disease where the body doesn't produce or properly use insulin that occurs at times other than during pregnancy. Also called diabetes mellitus.
Heart attack	Death of, or damage to, part of the heart muscle due to an insufficient blood supply. Also called myocardial infarction or MI.
Coronary bypass surgery	Surgery to improve blood supply to the heart muscle. This surgery is most often performed when narrowed coronary arteries reduce the flow of oxygen-containing blood to the heart itself. Also called CABG, "cabbage

Hypertension or high blood pressure	A chronic increase in blood pressure above its normal range that does not include being anxious, tense or "hyper".
	operation"; by-pass graft or operation; vein by-pass operation; open heart surgery.
Balloon Angioplasty	A procedure used to dilate (widen) narrowed arteries. A catheter with a deflated balloon on its tip is passed into the narrowed artery segment, the balloon inflated, and the narrow segment widened. To keep arteries from collapsing, stents (stainless steel supports) can be inserted into the artery during angioplasty. Also called percutaneous angioplasty, balloon dilation, PTCA, stents, etc.
Stroke	Loss of muscle function, vision, sensation or speech resulting from brain cell damage caused by an insufficient supply of blood to part of the brain. Also called apoplexy, cerebrovascular accident, or cerebral vascular accident.
Transient ischemic attack, or TIA	A temporary stroke-like event that lasts for only a short time and is caused by temporarily blocked blood vessels to part of the brain.

5.13 Participant Contact Form

5.13.1 Purpose

The Tracking Form 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.

5.13.2 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 on the Tracking Form.

5.13.3 Definitions

- 5.13.3.1 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.

- 5.13.3.2 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.
- 5.13.3.3 Email address. A computer address at which electronic mail is received (e.g., bozo@clownmail.com).
- 5.13.3.4 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.

5.13.4 Methods

5.13.4.1 General instructions

1. 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 be filled out 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.
2. Participants should clearly print, in ink and in capital letters, all information in the appropriate boxes. 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 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.

5.13.4.2 Participant Information

- A. ***“Please PRINT your name, address, telephone number (s), and email address (if you have one).”***

After completing the name, address, telephone number, and email address section, the participant will need to answer the following question:

1. **Will there be a change in your local address within the next three months?**
If yes, what will your new address be? (provide street address, city, state, and ZIP code)

5.13.4.3 Contact Information

- B. *“Please provide the following information on two 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.

5.13.4.4 Health Care Provider Information

- C. *“Please provide the following information about your health care provider.”***

1. **Do you have a clinic, doctor, nurse, or physician’s assistant who provides your usual medical care?**
If no, the form is completed.
If yes, please provide the following information for this clinic or person (provide name, title, address, telephone number) and then continue to the next question.
2. **When were you last seen by this person?** (provide month/year)
3. **Would you like us to send your MESA results to this person?**
If yes, arrange to do so.

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Section 6: Parent Clinic Exam

6.1 Examination Components

6.1.1 Overview

The MESA Family Parent Exam consist only of a blood draw and vision examination. In some cases, the proband's MESA Family Examination will be done in conjunction with their Exam 3 visit. In these instances, it is extremely important that all MESA Family paperwork be filled out separately from Exam 3, and the three tubes of blood collected be processed separately from the blood collected for the regular examination.

6.1.2 Forms

The forms associated with a MESA Family Proband Examination are:

- Clinic Reception
- Phlebotomy (See Section 7 on Laboratory Procedures)
- Processing (See Section 7 on Laboratory Procedures)
- Family History

6.2 Clinic Reception Form

6.2.1 Purpose

The Clinic Reception Form is the means by which important information collected during the reception process is recorded for scanning into the clinic database. This form must be completed accurately to allow for the tracking of the participant's progress through the study and to provide a link to the sampling and recruitment data.

6.2.2 Materials/Equipment

The Clinic Reception Form is generated by a computer with scanning software installed, so access to this computer is necessary to obtain the form. In addition, the following materials are required:

- A black ink pen (for best results when form is scanned)
- The list of participants scheduled for the day (produced by the MESA Recruitment system)
- Informed consent documents and pen
- Gown (or robe) and slippers

6.2.3 Definitions

- **Daily calendar:** The list of participants scheduled for a given day. It lists the name, acronym, recruitment/household ID, preferred language, and time of appointment. There is also a field to record participant status.
- **Acrostic:** A code used instead of the name for confidentiality reasons. It is composed of the first four letters of the last name, the first two letters of the first name, and gender indicator (F/M).

6.2.4. Methods

6.2.4.1 General Instructions

1. The clinic reception process is the participant's introduction to the MESA Family clinic staff and procedures. 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. Give the parent the informed consent document. Instruct the participant to read it carefully, answer the questions at the end, and sign it. Once the participant has completed and signed the informed consent, the clinic visit will begin.
3. 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 if necessary. 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.

6.2.4.2 Specific Instructions for Completing the Clinic Reception Form

1. Because the Clinic Reception Form serves several important functions, a second staff member must review it before scanning. This reviewer should be absolutely sure that the recruitment ID and acronym have been correctly transcribed into the appropriate fields.
2. The **parent ID** is preprinted on the form in the upper right corner.
3. In the **acrostic** field record the acronym that is printed on the daily calendar. Ask the participant for his/her birthday and record it in the appropriate box.
4. Affix the Recruitment Label ID below the acronym.
5. In the **visit date** fields record a two-digit month and date and a four-digit year (e.g.

05/25/2004). Whenever possible the second appointment date, if any, should be entered on the form before scanning. If the second appointment cannot be scheduled before the form is scanned, the data manager should update the database as soon as the information is available.

6. *Parent Demographic Information.* In the appropriate fields, please record the parent birth date as a two-digit month and date and a four-digit year (e.g. 01/25/1944), gender, place of birth, and parent place of birth.
7. *Informed Consent Responses.* Transcribe the responses from the participant's consent form by filling the appropriate bubble.
8. In the *Reception Interviewer ID* field, record the ID number of the person who administered the Clinic Reception Form.
9. In the *Reviewer ID* field, record the ID number of the person who reviewed the Clinic Reception Form.
10. In the *Data Entry ID* field, record the ID number of the person who entered the Clinic Reception Form.

6.3 Family History

6.3.1 Purpose.

The Family History is designed to gather information regarding the health history of the participant's biological (natural) parents and biological (natural) siblings (including all blood related siblings e.g. half-siblings). Detailed family histories provide important information regarding familial clustering of disease. Participant reports of family histories have been employed in several family studies (i.e. the NHLBI Family Heart Study (FHS), the Family Blood Pressure Program) with good success. The MESA study is particularly interested in family history of medical conditions that are related to atherosclerotic (plaque) formation and diseases as well as cancer and asthma. Specifically, we are interested in family history of heart attack, stroke, high blood pressure, diabetes, and other heart diseases, i.e. valvular heart diseases, septal heart defects etc.

6.3.2 Definitions

In this questionnaire, an attempt has been made to use common terms that require no explanation, because they are used in the way they tend to be used by most people in every day life. However, some terms are more scientific and may require clarification. In such cases, please refer to the Definition and Synonyms for Medical Terminology at the

end of the instruction.

However, it is critical that the participant understand that we are interested in gathering information only about his/her biological or natural parents and biological siblings. It should be explained that full biological siblings are brothers and sisters who have the same biological or natural parents as the participant. If indicated, it can additionally be explained that biological family members differ from adopted, step, or foster family members in that they have a genetic or “blood” relationship to the participant.

6.3.3 Methods

6.3.3.1 General Instructions

This is an **interviewer-administered form**. 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 Family History may be important in understanding their health. The information may, in turn, help us to better understand the causes of heart disease.

6.3.3.2 Specific Instructions for Completing the Family History Questionnaire

The interviewer should take time to become familiar with the Family History form. Note that the form is arranged with the disease of interest in the columns along the top (x-axis) and the “blood” relation of interest is in rows down the page (y-axis). Proceed with the first “relation” and ask about the diseases of interest pertaining to that particular “relation” along the row and across the page. Response choices are: Yes (Y), No (No), or Don’t know (D).

For example: First begin with “Spouse”, skip blacked out boxes and proceed to the next column. In this case, under the column “Age at last birthday or age at death,” fill in the digits for age of last birthday or at death. Then, under the next column “Heart Attack or M.I.” fill in “Y, N, or D”. If yes, ask for the age of first heart attack; fill in the digits for the age then continue to the next column. For each column of disease or condition, if the response is “Yes”, ask for the age of when this *first* occurred. For the column asking about cancer, in addition to age, ask the participant for the type of cancer. If the participant responded with more than one cancer, record the cancer that was diagnosed first.

Begin the questionnaire with the first column and the first row – regarding “Spouse” by asking:

Do you currently have a spouse?

If “no”, skip to the next “relation-person”, mother.

If “yes”, the interviewer should say, “*the next series of questions are about your spouse.*”

Then proceed with the next column:

Definition: *Spouse* is defined as a married person by state or common law. For the purpose of this questionnaire, same sex domestic partnership will not be considered as spouse.

What was your spouse’s age at the last birthday? If the spouse is deceased, then ask, ***“What was your spouse’s age at death, (when he passed away)?”***

Round off the age to the nearest whole number and enter the digits into the boxes then proceed to the next column of disease of interest. If there are more than one deceased spouse, only collect information on the most recent spouse.

Has your spouse ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*at what age did your spouse have the first heart attack?”*

Enter the age at first heart attack into the boxes then proceed to the next column.

Definition: To aid the participant, a *heart attack* can be defined as damage to, or death of, part of the heart muscle due to insufficient blood supply.

The intent is to record whether the first occurrence of one of these events indicates that coronary heart disease was before or after a certain age. If the participant cannot answer this question confidently, the response should be coded as “Don’t Know.”

Has your spouse ever had heart surgery or a cardiac (heart) procedure, such as coronary bypass surgery, balloon angioplasty, or stent placement because arteries of the heart were blocked?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*at what age did your spouse have this procedure?”*

Enter the age when the first procedure was done into the boxes then go to the next column.

Definition: *Coronary bypass* can be defined as surgery on the heart to improve blood supply/flow to the heart muscle. This surgery is most often performed when narrowed coronary arteries reduce the flow of oxygen-containing blood to the heart. *Balloon angioplasty* can be defined as a procedure to dilate (widen) narrowed coronary arteries. A catheter with a deflated balloon on its tip is passed into the narrowed artery segment, the balloon is inflated, and the narrowed segment is widened. Acceptable symptoms for heart attack, coronary bypass surgery, or balloon dilation are listed in the Definition and Synonyms for Medical Terminology at the end of these instructions.

The intent is to record whether the first occurrence of one of these procedures indicating coronary heart disease was before or after a certain age. If the participant cannot answer this question confidently, the response should be coded as "Don't Know."

Has your spouse ever had a stroke?

If "no" or "don't know", fill in the appropriate circle then skip to the next column.

If "yes", ask, "at what age did your spouse have the first stroke".

Enter the age at first stroke into the boxes then proceed to the next column.

Definition: To aid the participant, a *stroke* can be defined as the sudden loss of muscle function, vision, sensation, or speech resulting from brain cell damage caused by insufficient supply of blood to a part of the brain. A stroke may result from blockage of the arteries by blood clots, atherosclerotic plaques, or rupture of an artery. *Cerebral hemorrhage* can be defined as bleeding into or around the brain caused by a rupture of an artery or aneurysm in the brain. Cerebral hemorrhage usually causes sudden onset of very severe headache and is associated with other symptoms described above for stroke or with loss of consciousness. **DO NOT include TIAs or 'mini-strokes'.** Acceptable synonyms for stroke and cerebral hemorrhage are listed in the Definition and Synonyms for Medical Terminology at the end of this instruction.

The intent is to determine whether the first time one of these events occurred prior to 60 years of age. If the participant cannot answer this question confidently, the response should be coded as "Don't Know."

Does/Did your spouse have high blood pressure or hypertension diagnosed by a physician?

If "no" or "don't know", fill in the appropriate circle then skip to the next column.

If "yes", ask, "at what age was your spouse first diagnosed with high blood pressure?"

Enter the age at first diagnosis of high blood pressure into the boxes then proceed to the next column.

A "Yes" response means that, to the best of the participant's knowledge, a physician specifically told his/her spouse of the diagnosis of high blood pressure or hypertension. Other conditions, such as being anxious, tense, or "hyper" should not be confused with high blood pressure.

Definition: To aid the participant, the interviewer can explain that blood pressure is measured using a device similar to the one used in this study. Based on those measurements, a physician may have diagnosed the person as having *high blood pressure or hypertension*. A spouse who may have made a self-diagnosis based on readings obtained using an automatic device in a mall or other location should not be

considered as having high blood pressure, unless a doctor also verified the condition.
In most cases, unless a doctor has prescribed anti-hypertensive medication or arranged a plan for follow-up blood pressure readings, a diagnosis of high blood pressure is in doubt.

Does/did your spouse have Diabetes or high blood sugar diagnosed by a physician?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*at what age was your spouse first diagnosed with Diabetes?*”

Enter the age at first diagnosed of diabetes into the boxes then proceed to the next column.

A “Yes” response to the question means that a physician has specifically told the participant’s spouse that he/she has diabetes or high blood sugar. Other blood sugar conditions, such as “hypoglycemia”, should not be considered a “Yes” response to this question. If a physician had told the participant’s spouse that he/she has “borderline” or “early” diabetes (or high blood sugar), code the response as “yes”.

Does/did your spouse have Cancer diagnosed by a physician?

If “no” or “don’t know”, fill in the appropriated circle then skip to the next column.

If “yes”, ask, “*was this a cancer other than skin cancer such as basal cell or squamous cell skin cancer?*”

A “Yes” response to the question means that a physician has specifically told the participant’s spouse that he/she had cancer. An attempt should be made to encourage the participant to specify the type of cancer. If this is not possible, leave the fill in box blank.

If this cancer was a type other then the two mentioned then continue below; otherwise skip to the next column of disease/condition:

“Do you know the specific type of the cancer your spouse had?”

“At what age was your spouse diagnosed with this cancer (or the very first cancer if more the participant gives more than one cancer)?”

Enter the age when first diagnosed and the specific type of cancer into the appropriate boxes then proceed to the next column of disease of interest.

Does/did your spouse have any other heart diseases/problems diagnosed by a physician i.e. heart valve problems, heart defects, heart enlargements, heart conduction disorders or irregular rhythm disorders?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*At what age was your spouse first diagnosed with this heart disease/problem?*” Enter the age at first diagnosed into the boxes then proceed to the next column.

A “Yes” response to the question means that a physician has specifically told the participant’s spouse that he/she had some other heart disease/problems other than those specifically asked previously. An attempt should be made to encourage the participant to specify type of heart disease/problem. This will ensure that indeed some other heart disease exists.

Does/did your spouse have Asthma diagnosed by a physician?

If “no” or “don’t know”, fill in the appropriated circle then skip to the next column.

If “yes”, ask, “*At what age was your spouse first diagnosed with Asthma?*”

Enter the age at first diagnosed with asthma into the boxes.

A “Yes” response to the question means that a physician has specifically told the participant’s spouse that he/she had Asthma. It can be explained to the participant that a person with asthma usually has an inhaler that is used to improve breathing.

The interviewer has now completed all questions in the first row regarding “Spouse”. The interviewer should now proceed to the second row.

Questions regarding the Biological/Birth Mother:

The following questions are regarding your Biological or Birth Mother, do you have any comments before we begin?

If the participant was adopted or for some reason does not have information about his/her biological/birth mother, the interviewer should confirm whether the participant responded “no,” or “don’t know” and fill in the appropriate circle.

If “no”, skip to the next row with question about the biological father.

If “yes” or “don’t know”, the interviewer will continue with the questions across the column i.e.

What was your mother’s age at the last birthday (if deceased, age at death)?

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column.

Has/Did your mother ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*at what age did your mother have the first heart attack?*”

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next “Relation” column.

Questions regarding the Biological/Blood Father.

The interviewer should begin with:

The following questions are regarding your Biological Father; do you have any comments before we begin?

If the participant was adopted or for some reason does not have information about his/her biological/birth father, the interviewer should confirm whether the participant responded “no,” or “don’t know” and fill in the appropriate circle.

If “no”, skip to the next row with question about the biological siblings.

If “yes” or “don’t know”, the interviewer will continue with the questions across the column i.e.

What was your Father’s age at the last birthday (if deceased, age at death).

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column of disease of interest.

Has/Did your Father ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “at what age did your Father have the first heart attack?”

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next “Relation” column.

Questions regarding the Biological/Blood Siblings

The interviewer should begin with:

The following questions are regarding your Biological Siblings; we will begin with the oldest sibling and work our way to the youngest. Do you have any comments before we begin?

If the participant has *no siblings*, skip this and proceed to the next “Relation” category – Child.

If the participant has *more siblings than available on the form*, use a second form to obtain complete information on all siblings.

If the participant for some reason does not have information about his/her biological/blood siblings, the interviewer should confirm whether the participant said “no,” or “don’t know” and fill in the appropriate circle.

If “no”, skip to the next row with question about the biological Children.

If “yes” or “don’t know”, the interviewer will continue with the questions across the column i.e.

What was your Sibling’s age at the last birthday (if deceased, age at death).

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column.

Has/Did your Sibling ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*at what age did your Sibling have the first heart attack?*”

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next sibling or “Relation” column.

Questions regarding the Biological/Blood Children

The interviewer should begin with:

The following questions are regarding your Biological Children. We will begin with the oldest child and work our way to the youngest. Do you have any comments before we begin?

If the participant has *no children*, skip this and the interview is now completed.

If the participant has *more children than available on the form*, use a second form to obtain complete information on all children.

If the participant for some reason does not have information about his/her biological/birth children, the interviewer should confirm whether the participant said “no,” or “don’t know” and fill in the appropriate circle.

If “no”, skip this section and the interview is now complete. Thank the participant.

If “yes” or “don’t know”, the interviewer will continue with the questions across the column i.e.

What was your child’s age at the last birthday (if deceased, age at death).

Round off to the nearest whole number and enter the digits into the boxes then proceed to the next column.

Has/Did your child ever had a heart attack or Myocardial Infarction?

If “no” or “don’t know”, fill in the appropriate circle then skip to the next column.

If “yes”, ask, “*at what age did your child have the first heart attack?*”

Enter the age at first heart attack into the boxes then proceed to the next column.

The interviewer will continue in this fashion as done for the first set of questions until the last question regarding Asthma. Then, the interviewer will begin with the next child.

Once all the information has been obtained for the last child, the interview is 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," select whether the form was self-administered or interviewer-administered. Record reviewer (if form self-administered) or interviewer (if form interviewer-administered) ID number.

Table of Medical Terms

MEDICAL CONDITION OR PROCEDURES	DEFINITIONS & SYNONYMS
Hypertension or high blood pressure	A chronic increase in blood pressure above its normal range that does not include being anxious, tense or "hyper".
Diabetes or high blood sugar	A chronic disease where the body doesn't produce or properly use insulin that occurs at times other than during pregnancy. Also called diabetes mellitus.
Heart attack	Death of, or damage to, part of the heart muscle due to an insufficient blood supply. Also called myocardial infarction or MI.
Coronary bypass surgery	Surgery to improve blood supply to the heart muscle. This surgery is most often performed when narrowed coronary arteries reduce the flow of oxygen-containing blood to the heart itself. Also called CABG, "cabbage operation"; by-pass graft or operation; vein by-pass operation; open heart surgery.
Balloon Angioplasty	A procedure used to dilate (widen) narrowed arteries. A catheter with a deflated balloon on its tip is passed into the narrowed artery segment, the balloon inflated, and the narrow segment widened. To keep arteries from collapsing, stents (stainless steel supports) can be inserted into the artery during angioplasty. Also called percutaneous angioplasty, balloon dilation, PTCA, stents, etc.
Stroke	Loss of muscle function, vision, sensation or speech resulting from brain cell damage caused by an insufficient supply of blood to part of the brain. Also called apoplexy, cerebrovascular accident, or cerebral vascular accident.
Transient ischemic attack, or TIA	A temporary stroke-like event that lasts for only a short time and is caused by temporarily blocked blood vessels to part of the brain.

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Section 7: Laboratory

7.1 Background & Rational

The general goal of the MESA Family Study is to apply modern genetic analysis and genotyping methodologies to delineate the genetic determinants of early atherosclerosis. This will be accomplished by utilizing all the current organizational structures of the Multi-Ethnic Study of Atherosclerosis (MESA). MESA itself is a study of the characteristics of sub-clinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and risk factors that predict progression to clinically overt cardiovascular disease, and that predict progression of sub-clinical disease itself, in a diverse, population-based sample of 6,814 men aged 40 and older and women aged 50 and older. The Central Blood Analysis Laboratory (CBAL) will have responsibilities for special blood collection and handling protocols as well as training and QC monitoring at the Clinical Centers. The laboratory will also be responsible for performing assays and reporting results.

The blood samples collected and processed by Clinical Center technicians are the foundation for all of these tests. The most important step (and potentially the most variable) is the collection and processing of the blood samples. If the blood sample itself is not correctly drawn and processed, the laboratory results may not be precise or may not be valid.

MESA Family involves the collection of 66.5 mls of blood from Sibling participants and 27 mls of blood from Probands and parents at the MESA Family Exam.

7.2 Equipment & Supplies

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

- 5 ml SCAT-I tubes. Must be stored refrigerated until used.
- Cryovials – 0.5 ml and 1.5 ml with color-coded caps
- 4 mL clear-capped cryovials (#41)
- 10 mL white-capped cryovials (#42 and #16)

The following supplies will be provided in bulk by the CBAL for Parent and Proband collection:

- 10 mL white-capped cryovials (#1)

The blood collection area should have the following supplies:

- Lab coats and gloves
- Phlebotomy chair
- Basin (just in case)
- Washcloths/Towels
- Smelling salts
- Lab mats and wipes
- 10% bleach solution or approved biohazard disinfectant

Plastic cart with wheels for phlebotomy supplies (or plastic tray with compartments)
Butterfly needles (21 G) with luer adapter (B-D # 7251)
Vacutainer barrels
Tourniquets
Alcohol prep pads
Gauze (2x2)
Surgical tape - paper tape (easier on participants)
Band-Aids
Blood collection tubes (keep extras on hand):
 2 – 8.5mL ACD'A' tubes (B-D 364606)
 2 – 10 ml Serum tubes (B-D 366430)
 2 – 10 ml EDTA tubes (B-D 366457)
 1 – 4.5 ml Citrate tubes (B-D 366415)
 1 – 5 ml SCAT-I*
For Blind Duplicate Samples: 5 ml Serum (B-D 366534) & 5 ml EDTA (B-D 366452)
Blood tube rocker
Blood tube racks
Ice bucket and crushed ice - filled 10 min before draw
Stopwatches or timers (ex. Fisher Scientific # 06-662-9)
Scissors
Pens
Labels
Phlebotomy / Processing Form
Blood Spill Kit
Biohazardous waste container
Needle/sharps container
Material for urine collection

* provided by CBAL

7.3 Safety Issues / Precautions for Handling Blood Specimens

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

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

Use of aerosol containers in all centrifuges.

Follow 'Universal Precautions' when handling any blood products.

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

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

7.4 Participant ID Labels

The Coordinating Center will supply each field center with sheets of sample ID barcode labels to use for labeling draw tubes, working tubes, cryovials, and freezer boxes.

Sibling Participant Labels:

There will be a total of *55 labels*: 8 labels for the draw tubes plus 3 extra labels, 2 labels for two pooling tubes, and *42 cryovial* labels. Each participant set of barcode labels has the same 7-digit sample ID number (the first digit identifies the clinic – Wake Forest =3, Columbia = 4, John's Hopkins = 5, UMinn = 6, Northwestern = 7, UCLA = 8). The cryovial labels have a 10-digit number. The first 7-digit s are the participant ID number , the 8th digit will be a "1" - identifies participant is a Mesa Family Sibling, and the final 2-digit extension (01 to 42) serves as a unique identifier for each cryovial within a sample ID – this helps in tracking the repository. See Appendix for proper orientation of the barcode label on the cryovial. The extra labels are used for the urine collection cups.

Parent Participant Labels:

There will be a total of *5 labels*: 3 labels for the draw tubes, 1 label for the 10ml packed cells tubes, and 1 extra label. Each participant set of barcode labels has the same 7-digit sample ID number (the first digit identifies the clinic – Wake Forest =3, Columbia = 4, John's Hopkins = 5, UMinn = 6, Northwestern = 7, UCLA = 8).). The cryovial labels have a 10-digit number. The first 7-digit s are the participant ID number, the 8th digit will be a "2" - identifies participant is a Mesa Family Parent, and the final 2-digit extension (01) serves as a unique cryo identifier – this helps in tracking the repository. See Appendix for proper orientation of the barcode label on the tube.

Proband Participant Labels:

There will be a total of *5 labels*: 3 labels for the draw tubes, 1 label for the 10ml packed cells tubes, and 1 extra label. Each participant set of barcode labels has the same 7-digit sample ID number (the first digit identifies the clinic – Wake Forest =3, Columbia = 4, John's Hopkins = 5, UMinn = 6, Northwestern = 7, UCLA = 8).). The Proband participants will use their MESA Exam3 ID number. The cryovial labels have a 10-digit number. The first 7-digit s are the participant ID number, the 8th digit will be a "0" - identifies participant is a Mesa Family Proband, and the final 2-digit extension (01) serves as a unique cryo identifier – this helps in tracking the repository. See Appendix for proper orientation of the barcode label on the tube.

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

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

7.5 Phlebotomy/Processing Forms

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

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

Separate and uniquely identifiable Phlebotomy and Processing Forms will be required for MESA Family Probands, Parents and Sibling participants.

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

7.6 Participant Refusal to Provide Urine Sample or Refusal of Phlebotomy

Rarely, a participant will refuse to provide a urine sample or will refuse phlebotomy. Please keep a list of participant ID #s of any of these participants and identify which test they refused. MESA Family siblings must provide blood to be a contributing participant in the study.

7.7 Description of Participant Tubes

Sibling Participants:

Tubes #1 and #4 are 8.5mL ACD'A' tubes (B-D#364606). These tubes will be shipped overnight to Cedars Sinai Hospital for genetic testing.

Tubes #2 and #6 are 10ml EDTA tubes (B-D#366457). After centrifugation, plasma from these tubes are pooled and will be aliquoted into 15 purple-coded aliquots. This plasma will be used for lipid levels and banked for future testing. Once the plasma is removed, the packed red cells from both tubes are removed into one 10.0 ml white-capped tube. The packed red cells will be used for possible additional DNA extraction. For selected Blind Duplicate participants; an additional 5 ml EDTA tube (BD# 366452) will be used.

Tubes #3 and #7 are 10ml red-topped Serum tubes (B-D# 366430). After filling, let these tubes stand at room temperature for a minimum of 40 minutes to allow the blood to clot. They are then centrifuged and the sera are pooled and aliquoted into 16 red-coded aliquots. The serum will be tested for glucose and stored in repository for later analysis.

For selected Blind Duplicate participants, an additional 5 ml Serum tube (BD# 366534) will be used.

Tube #5 is a 4.5ml blue topped citrate tube, a silicon-coated glass tube containing 0.5ml of 3.2% sodium citrate (B-D# 366415). After centrifugation, plasma is aliquoted into 4 blue-coded aliquots. The plasma will be banked for future testing.

Tube #8 is a 5ml red-topped "Special Coagulation" tube (SCAT-I) containing a white, powdered anticoagulant provided by CBAL. This tube contains a special combination of anticoagulants that ensure long-term stability of the plasma sample. Specifically, this tube, when filled, will contain 4.5 mm EDTA, 150 KIU/ml aprotinin and 20 uM D-Phe-Pro-Arg-chloroketone. The SCAT-1 tube must be stored refrigerated. It must be drawn after at least one other tube has been drawn. Important to note, this tube is 'non-sterile', therefore it must be drawn using a butterfly apparatus with 12 inches of tubing; alternatively, a syringe may be used for the venipuncture, and expressed through the cap (with great care to limit turbulence) into the SCAT tube. It is critical that the SCAT-1 tube is mixed well (>30 sec of gentle inversion) before being placed on ice to await further processing/centrifugation. The four aliquots for this tube are yellow-coded, and the plasma will be stored in repository for future testing.

Parent and Proband Participants:

Tubes #1 and #2 are 8.5mL ACD'A' tubes (B-D#364606). These tubes will be shipped overnight to Cedars Sinai Hospital for genetic testing.

Tube #3 is a 10ml EDTA tubes (B-D#366457). After centrifugation, plasma from this tube will be discarded into biohazard waste. Once the plasma is removed, the packed red cells from the tube are removed into one 10.0mL white-capped tube. The packed red cells will be used for possible additional DNA extraction.

7.8 URINE COLLECTION & PROCESSING (For Sibling Participants Only)

7.8.1 Preparation for Urine Collection

Urine should be collected before venipuncture; preferably as early in the visit as possible. Urine will be collected on all participants. Collection will be random and therefore considered a 'spot' urine collection. Samples should not be collected after exertion or an acute fluid load (>24 oz), however participants should be encouraged to stay hydrated (water only) even while fasting for the visit. Participants having difficulty producing a urine specimen may be offered a glass of water, and a second (and third) urine specimen may be collected later in the visit to bring the volume up to the required amount.

It is suggested that female participants may prefer to use the Sage Commode Specimen & Measuring System #2500 for urine collection. If so, follow the instructions provided with this collection kit. Male participants, and female participants if they would prefer to, may urinate directly into a specimen collection container.

Specimen containers for routine random urine collection should be chemically clean, and have a tight fitting lid to prevent leakage during transportation. (Suggestion for standard sterile specimen container: Lab Safety Cat# OR-29091). The urine container must be clearly labeled with participant ID#.

7.8.2 Urine Collection:

Instructions for Participants:

The participant's privacy should be assured.

The following steps should be followed:

1. Wash hands before and after voiding.
2. Preferably cleanse area with antiseptic wipes prior to voiding (optional).
3. Remove the cap from the collection container and void into the container.
4. Carefully seal the cap of the container so that it is tight and leak proof.
5. Bring filled container to MESA staff for processing.

7.8.2.1 Urine Processing:

For urine storage, you will prepare one 4.0 mL Sarstedt clear-capped tube (#41) and one 10.0 mL white-capped tube (#42). Process using the following instructions:

Do not overfill the tubes. There must be room for the urine to expand when frozen.

1. Pipette a minimum of 3.0 mL urine (no more than 4.0mL) into the clear-topped tube #41. Place in freezer box to Vermont in appropriate space. Place on dry ice or put into freezer. Do not OVERFILL.
2. Pipette 9.0mL urine into the white-capped 10.0mL tube #42. Place in freezer box to Vermont in appropriate space. Place on dry ice or put into freezer. Do Not OVERFILL.
3. Double-check that cryovials have correct participant ID label.
4. Check off on the MESA Family Sibling Processing form the number of urine aliquots made (#s 41 and 42).
5. Discard any extra urine.
6. Freeze cryovials in an upright position at -70°C .
7. Blind Duplicate Urine Sample. Process a blind duplicate urine sample from all participants who are selected to provide a blind duplicate blood sample. Aliquot 3 mL urine into a tube similar to #41. Freeze *separately* cryovials in an upright position at -70°C . Ship with blind duplicate blood samples, one week after shipping tubes 41 and 42 (see 6.77 in the following chapter of this manual, "Collection, Processing, and Shipping of Blood Samples").

7.9 VENIPUNCTURE

7.9.1 Preparation

Preparation of Participants for Phlebotomy - Informed consent must have been obtained before blood is drawn. It should be stressed that this study requires the voluntary cooperation of the participants. These people are donating their time – (and blood and urine) on a purely voluntary basis, with no reward other than the knowledge that they are contributing to progress in medicine. Thus, the whole experience must be made as pleasant as possible.

Blood drawing is standardized for the sitting position. The 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. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. The 12 inches of tubing also allows the collection of non-sterile SCAT-1 tubes by eliminating the possibility of blood back-washing from tube to participant. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with the technician who draws the blood and their general attitude and competence. Do NOT under any circumstances force the participant to have blood drawn.

Phlebotomy Room - The blood drawing takes place in an isolated room or participants are separated by room dividers. The room is equipped with all of the necessary blood drawing supplies. A separate counter or work table is equipped with all of the materials and vials that are used in the blood handling and processing. The centrifuge, refrigerator, and freezer should be nearby.

Setup of Draw Tube and Aliquot Racks

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

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

Preparation for Specimen Collection

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

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

Phlebotomy Form Questions

There are five questions to ask the participant before the start of venipuncture. The first three questions deal with the participant's experience with venipuncture. If they answer yes to any of these three questions, the phlebotomist can take extra care with the procedure. Question 4 deals with diabetes status. Check yes only if the participant is taking medication for diabetes. *Question 5 deals with fasting status. The participant should be fasting (nothing to eat or drink*

except water) for 12 hours with a minimum acceptance of 8 hours. If they have not fasted for at least 8 hours, the blood collection needs to be rescheduled, if possible.

Specific Preparation for Siblings:

Eight tubes of blood of various sizes are collected, each containing about 1-2 teaspoons (5-10ml) of blood. On 20%, an extra 5 ml will be collected as a blind duplicate sample for QC purposes. Participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is less than 5 tablespoons, although it may look like more. The phlebotomist may also assure participants that they donate more than 6.5 times as much blood (450ml) when they donate a unit of blood.

Priority of Collection Tubes - A total of approximately 66.5 ml of blood will be drawn from each Sibling participant in 8 tubes. (20% will have 9 tubes collected for a total of 71.5 ml of blood)

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

- | | |
|------------------|------------|
| 1. 8.5mL ACD'A' | yellow top |
| 2. 10 ml EDTA | purple top |
| 3. 10 ml Serum | red top |
| 4. 8.5mL ACD'A' | yellow top |
| 5. 4.5ml Citrate | blue top |
| 6. 10 ml EDTA | purple top |
| 7. 10 ml Serum | red top |
| 8. 5 ml SCAT-I | red top |

Summary of Blood Mixing During Venipuncture of Siblings

Each tube should be treated as follows:

- | | |
|----------------|---|
| Tube #2 and 6 | EDTA – place on mixer for ~30 seconds, then place in ice bath. |
| Tube #5 | Citrate – place on mixer for ~30 seconds, then place in ice bath |
| Tube #8 | SCAT-1 – place on mixer for AT LEAST 30 seconds, then place in ice bath. |
| Tube #3 and 7 | Serum – do NOT mix; place in rack at room temperature for AT LEAST 40 minutes |
| Tubes #1 and 4 | ACD'A' - gently rotate up and down 5-6 times; place in rack at room temperature until shipping. |

Specific Preparation for Proband and Parents:

Three tubes of blood are collected, each containing about 1-2 teaspoons (5-10 ml) of blood. Participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is less than 2 tablespoons, although it may look like more. The

phlebotomist may also assure participants that they donate more than 15 times as much blood (450 ml) when they donate a unit of blood.

Priority of Collection Tubes – A total of approximately 27 ml of blood will be drawn from each Proband and Parent participant in 3 tubes.

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

1. 8.5mL ACD'A' yellow top
2. 8.5mL ACD'A' yellow top
3. 10 ml EDTA purple top

Summary of Blood Mixing During Venipuncture of Parents/Probands

Each tube should be treated as follows:

- Tubes #1 and 2 ACD'A' – gently rotate up and down 5-6 times; place in rack at room temperature until shipping.
- Tube #3 EDTA – place on mixer for ~30 seconds, then place in ice bath.

7.9.2 Venipuncture Procedure:

ALWAYS WEAR LATEX GLOVES AND LAB COAT

1. Arrange draw tubes in order of draw on the table top or in the tube rack within easy reach. Assemble butterfly apparatus and Vacutainer holders, gauze, and alcohol prep prior to tourniquet application.
2. Apply tourniquet.
3. Examine participant's arms for the best site for venipuncture. Release tourniquet.
4. Cleanse venipuncture site by wiping with alcohol prep pad in a circular motion from center to periphery. Allow area to dry.
5. Reapply tourniquet and start timer and document start time. **NOTE:** If possible, it is best to release the tourniquet as soon as possible after flow has been established. Tightened tourniquet should be on no longer than 2 minutes recommended or loosen tourniquet, then reapply if necessary. In our experience, however, especially with sick and/or elderly subjects, this may result in flow stopping, and the trauma of a second venipuncture. Therefore, this is a “judgment call” based upon the phlebotomist’s experience and skill.
6. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches below the venipuncture site.
7. With the needle bevel upward, enter the vein in a smooth continuous motion.
8. Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.
9. Grasp the flange of the Vacutainer holder and gently push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.

NOTE: Attention should be paid to minimizing turbulence whenever possible. Small steps, such as slanting the needle in the Vacutainer to have the blood run down the side of the tube instead of shooting all the way to the bottom, may result in significant improvement.

10. Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly. Try moving the needle slightly without causing discomfort to the participant.
11. Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.
12. Fill each Vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a Vacutainer tube fills only partially, remove the tube and attach another of the same type without removing the needle from vein. Tubes are not acceptable if $< \frac{1}{2}$ full.
13. When the blood flow ceases, remove the tube from the Vacutainer holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted (if necessary). Place all tubes, except serum, on tube mixer for a minimum of 30 seconds.
14. Release tourniquet, if still applied. The ideal tourniquet time is two minutes.
15. To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma. Discard needle into puncture-proof sharps container. Record on Phlebotomy form duration tourniquet was applied and end venipuncture time.
16. The Citrate, EDTA, and SCAT-I, tubes are placed on wet ice. The serum and ACD'A' tubes are held at room temperature.
17. Clean up the venipuncture area (if necessary). Dispose of needle and tubing in the appropriate biohazard needle sharps containers. Complete the Phlebotomy Form.
18. Bring the filled blood collection tubes to the processing area, keeping the EDTA, citrate and SCAT-I tubes on ice and the ACD'A' and serum tubes at room temperature.

7.9.3 Guidelines for Difficulties

HANDLING PARTICIPANTS WHO ARE EXTREMELY APPREHENSIVE ABOUT HAVING BLOOD DRAWN. Do not under any circumstances force the participant to have blood drawn. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. If the participant has "good veins" the phlebotomist can reassuringly say, "Oh, you have good veins; there should be no problem."

1. Bandaging the Arm. If the patient continues to bleed apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops. A gauze bandage can be tightly wrapped around the arm over the pad, and left on for at least 15 minutes.

2. Procedures for Difficult Draw. If a blood sample is not forthcoming, the following manipulations may be helpful.
 - a. If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel edge away from the wall of the vein.
 - b. If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.
 - c. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.
 - d. The phlebotomist should not attempt a venipuncture more than twice.
 - e. Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on their part.
 - f. If venipuncture is unsuccessful, this should be noted on the Phlebotomy Form.

3. WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD COLLECTION.
 - a. Have the person remain in the chair, if necessary have him/her sit with head between knees.
 - b. Provide the person with a basin if he/she feels nauseous.
 - c. Have the person remain seated until he/she feels better.
 - d. Place a cold washcloth on the back of the person's neck.
 - e. If the person faints, use smelling salts to revive by crushing the ampoule and waving it under the person's nose for a few seconds.
 - f. If the person continues to feel sick, contact a medical staff member who will advise you on further action.

4. Other Possible Problems:

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

5. Collection tube does not fill.

First, try another tube of the same type. Partially filled plasma tubes are not acceptable if less than ½ full. If a tube is less than ½ filled, it should be discarded. Partial tubes for serum and ACD'A' are acceptable, but will result in a reduced number of aliquots for serum. If a tube is not completely filled (> ½), clearly note on the Processing Form as this can affect future assays.

7.10 PROCESSING:

7.10.1 Overview

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

7.10.2 Aliquoting: Citrate, EDTA, SCAT-1 and Serum tubes (Siblings Only)

Collection Tube	# Aliquot cryovials/ tubes	Color Code	Volume per Aliquot Tube
#1: 8.5mL ACD'A'	0	n/a	Collection tubes #1 and 4 are mailed overnight at room temperature to Cedars
#4: 8.5mL ACD'A'			
#2: 10mL EDTA	16	Purple	(Combine plasma from tubes #2 & 6 before aliquoting) 1x 1.0mL in 1.5mL cryo #01 14 x 0.5mL in 1.0mL cryo #s 02 to 15
#6: 10mL EDTA		White (packed red cells)	1x 9.0mL packed cells in 10 mL cryo #16 (from both collection tubes #2 and #6)
#3: 10ml Serum	16	Red	(Combine sera from tubes #3 & 7 before aliquoting) 16x 0.5mL in 0.5mL cryo #s 25 to 40
#7: 10mL Serum			
#5: 4.5mL Citrate	4 cryovials	Blue	4 @ 0.5mL cryo #s 17-20
#8: 5mL SCAT-I	4 cryovials	Yellow	4 @ 0.5mL cryo #s 21-24
#9: Urine Cup	2 tubes	Clear	1 @ 3.0mL in 4.0mL tube cryo #41
		White	1 @ 9.0mL in 10.0mL tube cryo #42

Aliquot Assignments:

1. Aliquoting consists of removing the serum or plasma in small amounts (e.g.: 0.5ml) by pipette and placing it into the appropriate color-coded cryovials (provided). Color-coding is predetermined and used to identify sample type such as citrated plasma vs. SCAT-I plasma, etc. If color-coding is not available, the sample type must be clearly identified on the label.
2. Packed cells from EDTA tubes 2& 6 are transferred by pouring into Cryovial # 16. Do not OVERFILL.
3. This process must be done while the tubes and cryovials are on ice (unless otherwise noted).
4. When aliquoting serum and plasma, be careful not to disturb the top of the cell layer with the pipette tip, as this will result in platelet, white cell and red cell contamination.
5. Use a new pipette tip for each draw tube.

- Once the sample is aliquoted, cryovials should be immediately (< 10 minutes) frozen in an upright position at -70°C or promptly placed on dry ice for quick freezing.

After centrifugation, pool plasma or serum of like tubes from the same participant, (e.g.: EDTA plasma from tubes 2 & 6; Serum tubes 3 & 7). A disposable transfer pipette may be useful in transferring the plasma or serum from the centrifuged blood collection tube into a 15 ml or similar 'pooling tube'. Make sure the pooling tubes are clearly labeled with ID#s. From the pooled plasma or serum, now in the 15 mL tube, pipette the appropriate volume into each cryovial for that draw tube type (i.e. EDTA plasma = 15 purple- capped cryovials).

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

Upon completion of the processing steps, aliquots must be frozen at -70°C or below within 10 minutes, or place immediately on dry ice. Make sure all cryovials and tubes are frozen in the upright position. However, remember that ACD'A' tubes are NEVER to be refrigerated or placed on ice.

7.10.3 Preparing the Packed Cells Tube (Parents/Probands Only)

Aliquot Assignments:

Collection Tube	# Aliquot Tubes	Color Code	Volume per Aliquot Tube
#1: 8.5 mL ACD'A'	0	n/a	Mailed overnight at room temp to Cedars Sinai
#2: 8.5 mL ACD'A'	0	n/a	Mailed overnight at room temp to Cedars Sinai
#3 10mL EDTA	1-9.0mL tube	White	5.0mL packed red cells in 10.0mL cryo # 01

- Once the EDTA tube has been centrifuged the plasma layer can be removed.
- When discarding the EDTA plasma, a disposable transfer pipette may be useful in transferring the plasma into a biohazard waste container.
- Packed cells from EDTA tube are transferred by pouring into Cryovial #1 (10.0mL clear-capped tube).
- Processing the EDTA tube must be done while the tube and cryovial are on ice (unless otherwise noted).
- Use a new pipette tip for each participant.
- Once the sample is aliquoted, tube #01 containing the packed red cells should be immediately (< 10 minutes) frozen in an upright position at -70°C or promptly placed on dry ice for quick freezing.

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

Upon completion of the processing steps, aliquots must be frozen at -70°C or below within

10 minutes, or place immediately on dry ice. Make sure all cryovials and tubes are frozen in the upright position. However, remember that ACD'A' tubes are NEVER to be refrigerated or placed on ice.

7.10.4 Summary of Timing Issues

After blood drawing, time before centrifugation:

EDTA, SCAT-1 - store on ice; preferably < 15 minutes (maximum < 30 minutes) before and Citrate centrifuging.
 Serum - store at room temp for at least 40 minutes, but < 90 minutes prior to centrifuging.
 ACD'A' - store in a rack at room temp until shipping to Cedars Sinai

After aliquoting, ALL aliquoted samples other than ACD'A' must be frozen within 10 minutes or placed immediately on dry ice.

7.10.5 Daily Preparation

The following items should be on hand before beginning processing:

Lab coats and gloves, splash shields, other Personal Protective Equipment as needed.

Refrigerated Centrifuge: 2,000 g-force minimum, 4 °C, Swinging bucket.

10% bleach solution (or approved biohazard disinfectant)

test tube holders (adapters) for centrifuges

Harvard Trip Balance / Pan balance

water bottles for balance

freezer (-70°C or colder)

emergency eye wash station

biohazard trash can, with large and small biohazard bags (*biohazardous waste puncture proof containers*)

test tube racks / cryovial racks

Fixed volume pipettes with tips (MLA) and adjustable pipettes (Rainin, Finn, etc) with tips. Volumes needed to pipette: 0.5 ml, 1.0 ml (200 to 1000 µl)

cryogenic vials* (0.5 ml and 1.5 ml)

color-coded cryovial caps (purple, red, yellow, and blue)*

Tubes (Sarstedt 4.0mL and 10.0mL for urine)*

cryovial labels (from the CC)

Revco Boxes and dividers (9 x 9 and 7x7)

Styrofoam/insulated shipping coolers

Refrigerator- for storage of special blood tubes

- Can be a household fridge.

- Cannot be the same as food fridge.

Labels/lab tape for reagents

Sharpie pens

ID labels for cryovials and freezer boxes

* = provided by CBAL

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

Low sample volume

If there is insufficient sample of a tube type to make the full set of aliquots, start filling the lowest numbered cryovial for that tube type. Any partially filled cryovial (less than the specified volume) should be marked with a dot on the cap and a "P" in the comment field on the Processing Form next to that cryovial number.

7.10.6 Centrifugation – EDTA, SCAT-1, & Citrate Tubes

If centrifugation is not immediate, these tubes are stored upright on wet ice. Tubes are centrifuged at 4°C at least at 2,000g x 15 minutes or 3,000g x 10 minutes for a total of 30,000 g-minutes. Maximum time elapsed before centrifugation is 30 minutes from time of collection. For the Sibling Processing, please note all start times on the Processing Forms. For Probands and Parents, EDTA centrifuge start times do not need to be recorded. Once centrifugation is complete, tubes are carefully placed on ice and are ready to aliquot.

1. EDTA

Place the EDTA tube in the centrifuge.

For the Siblings: After centrifugation, the EDTA plasma is pooled and aliquoted, by specified volume into purple-capped cryovials #01 – 15. After plasma has been pooled, the packed red cell layer is removed from collection tubes # 2 and 6. Transfer the packed red cell layer by pouring from both tubes into white-capped 10.0mL tube #16.

For Probands and Parents, after centrifugation the EDTA plasma is removed and discarded. The packed red cells are transferred into white-capped 10.0mL tube #01.

2. SCAT-1

For Siblings: After centrifugation, carefully pipette 0.5ml of this plasma into each of the yellow-capped cryovials #21 – 24.

3. CITRATE

For Siblings: After centrifugation, carefully pipette 0.5ml of this plasma into each of the blue-capped cryovials # 17 – 20. .

7.10.7 Serum - Centrifugation

Allow serum tubes to clot for at least 40 minutes at room temperature (maximum time before centrifugation is 90 minutes). These tubes are centrifuged at 4°C at 2,000g x 15 minutes or 3,000g x 10 minutes for a total of 30,000 g-minutes.

After centrifugation is complete, pool the serum before aliquoting and place on ice.

Carefully pipette 0.5ml of pooled serum into each of the red-capped cryovials # 25-40.

The remaining red cells in the serum draw tube (and the tube itself) can be discarded in the biohazardous waste.

7.10.8 Special Circumstances

1. Blood specimens (EDTA, Citrate, SCAT-I) cannot be processed within 30 minutes of collection.

If centrifugation cannot be performed within 30 minutes of collection, try to process specimens as soon as possible after that time. **Note time of collection and centrifugation** on the P/P form. Maintain the EDTA, citrate, and SCAT-I tubes on wet ice until centrifugation.

2. Serum and plasma cannot be frozen within 10 minutes of aliquoting.

Every effort should be made to freeze serum and plasma cryovials at -70°C or below as soon as possible after aliquoting. If specimens cannot be placed immediately at -70°C or below, they may be temporarily (< 2 hours) stored at -20°C or placed on dry ice until transfer to -70°C or below. Dry ice is the preferred solution.

3. ACD'A' tubes are not centrifuged.

ACD'A' tubes collected for all participants in MESA Family are kept at room temperature and sent uncentrifuged to California. After these tubes have been filled, it is very important that they be gently rotated up and down 5-6 times to thoroughly mix the blood with the preservative.

7.10.9 Processing Completion

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

Filled and correctly labeled, *frozen* cryovials from each participant are packed in 9x9 freezer boxes. One freezer box can hold two participant cryovial sample sets (cryos#01-15, 17-40). (The packed cells (#16) and the urine tubes (#41 and #42) go in 7x7 freezer boxes. The labels for the corresponding participants are attached to the front cover of the 9x9 freezer box.

Frozen 4 mL and 10 mL urine tubes #s 41 and 42 from Sibling participants are packed in 2 separate 7x7 boxes and shipped frozen to Vermont in the same shipment as the corresponding participant blood samples.

Filled and correctly labeled frozen packed red cell tubes #16 from Sibling participants are packed together in 7x7 freezer boxes and shipped to Vermont in the same shipment as the corresponding participant's blood samples. **If there are 12 or less Sibling samples, the packed cell tube # 16, urine tube #41 and urine tube #42 from 12 or less participants can be packed in the same freezer box to conserve space. See "Alternative Sibling Freezer Box Diagram" for recommended packing design.**

Filled and correctly labeled frozen packed red cell tubes # 01 from Parent and Proband participants are packed in 7x7 freezer boxes and shipped to Vermont. If there are only a few Parent and Proband samples, they can be packed in the same freezer box to conserve space, but be sure to clearly identify and separate the two Family groups. See “Alternative Parent/Proband Freezer Box Diagram” for recommended packing design.

After each participant visit, room temperature ACD’A’ tubes will be shipped overnight to California immediately following the visit.

Be sure the correct Sibling, Parent, or Proband Phlebotomy and Processing Forms are completely filled out.

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

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

7.11 SHIPPING BLOOD SAMPLES

7.11.1 General

Blood samples are shipped only on Mondays or Tuesdays to the CBAL by an overnight carrier (Federal Express is preferred).

ACD’A’ tubes are shipped only on Monday through Thursday to the Cedars Sinai Genetics Lab by an overnight carrier (Federal Express is preferred). If blood is drawn late in the day or after the FedEx pickup, it can and should be kept at room temperature until the next day. Blood drawn Friday, Saturday or Sunday can be kept at room temperature and shipped on Monday.

Samples will be shipped on a pre-arranged schedule.

7.11.2 Packaging Samples

Sample Shipping Checklist (Frozen samples to Vermont):

- Coolers
- Rubber bands for freezer boxes
- Ziplock plastic bags for freezer boxes
- Absorbent material (i.e. paper towels, newspaper)
- Packaging tape
- Dry ice (~10kgs per mailing container)
- Labels: Fedex address labels, air eligible label (airplane)
- Dry Ice Labels (class 9, UN1845)
- Labeled freezer boxes with participant samples
- Completed Phlebotomy and Processing Forms
- Completed Shipping Forms (to be faxed)

Sample Shipping Checklist (Room Temperature samples to Cedars Sinai)

STP 600: bubble wrap, which will hold up to 5-10 mL vacutainer tubes
STP 150: 3" absorbent strips with a 50 mL capacity
STP 700: inner leakproof poly bag and Tyvek outer envelope
STP 306 or STP 300: outer diagnostic shipper
STP 305 or STP 300: insulated polystyrene cooler
STP 317: 15-30°C PCM (Phase Change Material) bricks
STP 805: Inner Packages Comply labels
STP 814: Air Eligible labels
STP 815: diagnostic specimen labels (UN3373)
Completed Processing Forms
Completed Shipping Forms
Completed Cedar lab form
A copy of the pedigree

7.11.3 Procedure

For frozen shipment to the University of Vermont:

1. Line cooler with absorbent material (i.e. paper towels).
2. Place approximately 1/2 the dry ice (per mailer) on the bottom of the cooler.
3. Place another layer of absorbent material (i.e.: paper towels) on top of the dry ice – so it will be between the dry ice and the freezer boxes containing the samples.
4. Collect the freezer boxes containing samples to be shipped, and check the sample ID numbers against the Processing Forms for that shipment. Each cryovials box contains the samples from 2 participants. Urine tubes and packed red cell tubes are stored in taller boxes with a 7 x 7 grid.
5. Place a rubber band around each cardboard freezer box containing samples before enclosing each box with absorbent material in a Ziploc plastic bag. Then carefully place these bagged boxes containing samples in the mailer. The rubber band is important for aiding in the prevention of a cryovial spill; the Ziploc bag & absorbent material are for compliance with commercial carrier specifications.
6. Another layer of absorbent material is placed on top of the sample freezer boxes.
7. The remaining dry ice is placed on top of this last layer of absorbent material.
8. The top of the cooler is sealed on with tape.
9. The Shipping Form and Phlebotomy/Processing Forms for all samples included in that particular shipment are placed in a plastic Ziploc bag on the top of the absorbent material before the top of the cooler is securely taped 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).

Fill out the Shipping Log including the Fedex airbill #s and fax to the University of Vermont at (802) 656-8965.

For room temperature shipment to Cedars Sinai Hospital

1. Insert tubes in bubble wrap tube holder (STP 600).
2. Be sure absorbent paper (STP 150) is in the inner leak proof poly bag (STP 700 or a Ziploc bag).

3. Wrap tube individually in bubble wrap or place in bubble wrap holder (STP 600) with tubes in the inner bag. Seal the bag completely
4. Place the plastic bag into the outer envelope.
5. Place the plastic bag into an insulated polystyrene cooler (STP305 or STP 300).
6. Enclose 2 phase change material pacs (STP 317).
7. Place cooler into a cardboard outer box (STP 306 or STP 300). Seal the carton.
8. Affix the shipping labels. **If pickup is not immediate, DO NOT REFRIGERATE, no matter how long the time delay. Keep tubes at room temperature at all times.**
9. Do NOT put the name “Cedars-Sinai” anywhere on the shipping label or the blood will be delivered to the hospital loading dock and not the lab.
10. An “Air Eligible” (STP 814) label must be applied next to the Diagnostic Specimen label-UN 3373 (STP 815) on the outer package. Each outer package must be labeled to indicate that it meets all these requirements with the Inner Packages Comply labels (STP 805). All samples being shipped as diagnostic samples, must have the exact words “**Diagnostic specimen packed in compliance with IATA packing instruction 650**” clearly marked on the outside of the shipping box.

Complete infectious (STP 310) and diagnostic (STP 320) shipping kits with all supplies included are also available through SafTPak. The previously mentioned catalog numbers are suggestions only. Please feel free to purchase similar products from another company that is more convenient for your institution. **Just be sure that the products comply with all the terms of PI 650.**

Email the completed Cedars Sinai laboratory form each day that a package ships to mesafamily@cshs.org.

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

7.11.4 Mailing Addresses:

Frozen shipments:

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

Room temperature ACD’A’ tubes:

S, Pressman/C. Hernandez/K. Roll
8723 Alden Drive, Room SSB378
Los Angeles, CA 90048

7.12 QUALITY ASSURANCE

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

7.12.2 Field Center Technician Training & Certification

Standardization of venipuncture and blood processing procedures is of utmost importance for the quality of the blood samples and subsequent data analysis. There will be a one time training session on blood collection and processing of the MESA Family samples. The training session will present information relating to the collection of the blood sample (i.e.: infection control, safety precautions including OSHA regulations, handling equipment, venipuncture procedure and possible venipuncture problems), and proper processing procedures for the varied array of draw tubes, including centrifugation and temperature requirements, and aliquoting the multitude of corresponding color-coded cryovials.

Field Center Technician Requirements.

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

Field Center Technicians should have read the MESA Family Manual of Operations before attending the CBAL training session. Certification in MESA Family blood collection and processing is required before working with actual participants and blood samples.

Field Center Technician Certification

Field Center technicians who attend the CBAL training session and successfully complete both the written and practical examinations will be certified in MESA Family blood collection & processing. Once fully certified, this technician is qualified to certify other technicians at their site in the complete or partial process with final approval from the CBAL.

The steps for certification are:

1. Read the Lab Manual of Operations.
2. Observe the process performed by a certified technician.

3. Successful completion of practical exam (using the Certification Form/Supervisor Checklist), which involves observation by a certified personnel of complete phlebotomy/processing procedure on a volunteer.
4. Successful completion of written exam (prepared by CBAL).

Completed written exams will be corrected and kept on file at the CBAL.

7.12.3 Field Center Equipment Records

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

7.12.4 Field Center Supervisor Checklist

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

7.12.5 Maintaining Certification

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

7.12.6 Field Center Acknowledgement Forms

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

7.12.7 Alert Values

The University of Minnesota will be analyzing these samples for Lipid Panel, Glucose, and Creatinine. The University of Vermont will run microalbumin on the urine samples.

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

7.12.8 Sibling Blood Blind Duplicates

Blind duplicates will be collected on Sibling participants, but not on Probands or Parents. Blind duplicate samples will be collected on 5% of the participants on four different tube types. This results in 20% of all participants having a blind duplicate sample collected for QC.

Participants will be selected to have a QC sample drawn based on the last two digits of their ID numbers. All participants whose MESA Family ID numbers end in **6** or **7** will have a seventh tube of blood drawn, and those whose MESA Family ID numbers end in **6** and having a sixth digit of **0 – 4** (the same participants who have a QC EDTA tube drawn) will also have a QC cryovial of urine.

Each participant will have a pre-assigned QC ID number in case he/she is selected for any type of quality control activity. This number will be printed on the Clinic Reception form. You will have blood labels with QC ID numbers for those participants whose MESA Family ID numbers end with 6 or 7.

Below is the procedure for collecting these blind duplicate samples.

The QC tube and cryovials should be labeled with the QC ID number that is printed on the Clinic Reception form, not a MESA Family ID number. The urine cryovial can be filled out of the 25 mL minimum urine cup and should be the same type as the regular required cryovial (#23 on the processing form). This should also be labeled with the QC ID number labels. The aliquoting scheme is as follows:

Sixth & last digit of MESA Family ID# (<i>example</i>)	Tube type	Cryovial color	# of cryovials	Cryovial volume	BD Cryovial #
Last digit is 6 and sixth digit is 0 – 4 (e.g. 3 0 2 2 7 <u>1 6</u>)	EDTA	5 mL EDTA purple	2	1.0 ml	01,02
	Urine	Urine cup white	1	3.0 ml	41
Last digit is 6 and sixth digit is 5 – 9 (e.g. 7 0 1 8 2 <u>7 6</u>)	5 mL Serum	Red	4	0.5 ml	25, 26, 27, 28
Last digit is 7 and sixth digit is 0 – 4 (e.g. 8 0 1 7 9 <u>0 7</u>)	4.5 mL Citrate	blue	4	0.5 ml	17, 18, 19, 20
Last digit is 7 and sixth digit is 5 – 9 (e.g. 6 0 1 4 8 <u>9 7</u>)	5 mL SCAT-I	Yellow	4	0.5 ml	21, 22, 23, 24

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

- 5 ml EDTA (B-D# 366452)
- 5 ml Serum (B-D# 366534)
- 4.5 ml Citrate (B-D# 366415)
- 5 ml SCAT-I*

*Provided by CBAL

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

Aliquoting Scheme:

Tube Type	Cryovial color	# x sample volume
EDTA	purple	2 x 1.0 ml Cryo #s 01 - 02
Serum	red	4 x 0.5 ml Cryo #s 25 - 28
Citrate	blue	4 x 0.5 ml Cryo #s 17 - 20
SCAT-I	yellow	4 x 0.5 ml Cryo #s 21 - 24

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

After aliquoting, cryovials are frozen immediately at -70°C in an upright position. Blind Duplicate cryovials are placed in their own freezer box with a 9 x 9 grid. More than one participant's samples are included in one box. These samples are shipped a week or so after the original samples are sent out to the CBAL, so that the laboratory cannot match them with the original participant. Include the completed Blind Duplicate Shipping Log when these frozen samples are shipped.

7.12.9 Sibling Urine Blind Duplicates

Blind duplicates will also be processed for urine. Whenever an EDTA blind duplicate is being collected on a participant, a urine blind duplicate should also be processed. On each participant selected for EDTA QC, the QC ID# will be the same for both the EDTA and the urine blind duplicate cryovials.

After aliquoting the participant's urine into tube #41 and 42, aliquot 3 ml of the remaining urine into a 4 ml tube. Carefully label this tube with the QC ID# and freeze 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.

It is important to complete the Blind Duplicate Shipping Log and include a copy in the shipping box with the frozen samples. Ship frozen urine blind duplicates at least one week after the original participant's samples are shipped to CBAL.

<u>Sample</u>	<u>Cryovial</u>	<u>Cryovial Color</u>	<u># x sample volume</u>
Urine	Cryovial# 41 = 4.0 mL tube	Clear	1 x 3.0mL

FIELD CENTER FORMS:

MESA Family Sibling Phlebotomy and Processing Forms
MESA Family Sibling Shipping Log

MESA Family Proband Phlebotomy/Processing Forms
MESA Family Parent Phlebotomy and Processing Forms
MESA Family Proband/Parent Shipping Form
MESA Family Cedars Sinai Shipping Log
MESA Family Sibling Blind Duplicate Shipping Log
MESA Family Sibling and Parent/Proband Field Center Supervisor Checklists
MESA Family Sibling and Parent/Proband Field Center Technician Certification
Examinations
MESA Family Equipment Temperature Logs (Same as MESA Exam 3)

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Section 8: COMPUTED TOMOGRAPHY

8.1 Background and Goals

The MESA Family study is studying genetic phenotypes regarding sub clinical atherosclerosis including computed tomographic coronary calcium measurements.

8.2 Qualifications of Personnel

Each Field Center has a designated physician to oversee the general performance of CT examination. A reading center investigator will provide the necessary on-site training.

Field Center technologists should have appropriate knowledge of cross-sectional anatomy, physiology, and pathology related to the heart. Technologists must be certified as RTs in their state. It is recommended that technologists also have at least two years of experience in chest computed tomography. The technologist should also have a basic knowledge of cardiac CT, knowledge of computer software applications, data formatting, and experience with the workstations and data formatting / transmission procedures used.

Each technologist involved in the study should also have a complete understanding of this protocol, be experienced at providing breath-holding instruction, ECG gating, operation of the EBCT or HCT equipment. To ensure quality control, each Field Center should have designated CT technicians who will perform the MFAM examinations, once appropriate training, including the March 2000 training course, has been provided.

8.3 Scanning Equipment and Centers

The scanners used will be:

Imatron C-150:	Saint Francis Hospital; Roslyn, New York
Imatron C-150	Northwestern University, Chicago, Illinois
Imatron C-150	UCLA School of Medicine, Los Angeles, CA
General Electric LightSpeed:	Wake Forest Bowman Grey School of Medicine
Siemens S4+ Volume Zoom:	Johns Hopkins Univ. Medical Center, Baltimore
Siemens S4+ Volume Zoom:	Univ. of Minnesota Medical Center, Minneapolis
As of Spring 2004:	
Siemens Sensation 16:	Johns Hopkins Univ. Medical Center, Baltimore
Siemens Sensation 16:	Univ. of Minnesota Medical Center, Minneapolis

Each center will be responsible for scanning approximately one sixth of the cohort of 6500 individuals. For purposes of increased reliability and quality control, each subject will be scanned twice during each session.

8.4 Training for Field Center CT Technologists

Training visits will not be required since technologists are already performing scans according to the MESA protocol which will also be used for the MESA Family Study.

Reader Training and Certification

Readers likewise have already been trained and will not require further training.

8.5 Participant Scheduling

The MESA Family operations committee will arrange details of scheduling. Each scanning site will provide their local Field Center with the days/times when MESA participants may be scheduled and the Field Center will contact participants to arranging their appointments for their CT scan. Whether certain days/times will be held for MESA participants only, until 2 weeks or a certain amount of time prior to the day of the appointment, will be agreed upon between the scanning center and Field Center.

A Field Center interviewer/scheduler will be responsible for explaining and obtaining consent for the CT examination. Participants will be scheduled for a certain date and time, and transportation arrangements, if necessary, will be arranged by the Field Center interviewer/scheduler. A confirmation appointment letter will be sent, providing the time, date, and directions to the scanning center, and describing the procedure.

8.6 Scanning Table and Calibration Phantom.

A special gel stuffed mat will be provided with the calibration pad phantom to prevent artifacts and for subject comfort. This pad is placed between the phantom and the patient. The calibration phantom will be placed inside the blue catcher bag with its long axis parallel to the long axis of the scanning table. The phantom is of tissue equivalent plastic with rods of hydroxyapatite of known radiographic density. The technologist will position the subject so the length of the phantom covers the expected length of the heart phantom will be the one pictured on the right.



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8.7 Scanning Procedure

The scanning procedure described below, **consisting of two scans done in succession on each subject**, will require approximately 20 minutes of the subject's time. Minimal respiratory motion and maximum accuracy and reproducibility require this duration. In unusual cases, this may require as many as 30 minutes. In many cases, the procedure will be completed in 15 minutes or less.

Entering the MESA Family ID and ACROSTIC

This very important step must be done correctly to prevent any irretrievable loss of CT data. All necessary information needs to be entered into the system, including your technician ID number, Field Center identification. The MFAM seven digit ID number must be placed in the ID Field, and an acrostic defined below will be placed in the name field. The acrostic will consist of the first four letters of the last name followed by the first two letters of the first name and one letter for the gender. Each will be preceded by the letters "MFAM".

Example:

ID: MFAM1212345 NAME: MFAMDETRROM

The time stamp on each CT record will determine whether the scan is the first or second in the sequence of two scans.

Preparation of the Subject (3 minutes). Subjects weighing more than 300 pounds will not be scanned due to technical difficulties. The technologist will ask women if they might be pregnant and will not scan them if they answer affirmatively. The technologist will attach three electrocardiographic electrodes under the left clavicle and on either side of the thorax near the axillae (to maximize ECG signal). The technologist will ask each subject for their self reported weight in pounds and will use this value to decide if the subject satisfies the “Large Patient” criterion.

Breath Holding Instruction (3 minutes). The technologist will instruct the subject on the importance of breath holding and immobility during scanning. An interpreter will assist in the instruction of subjects, not fluent in English. All centers will have access to interpreters fluent in languages spoken by at least 95 percent of potential volunteers.

Checking the Scout Image (1 minute). The technologist will instruct the subject to take three deep breaths, and then to hold his/her breath (at end-inspiration), while acquiring an 11 cm scout image, beginning 180 mm below the sternal notch. This will provide views of the chest on the image monitor at the operator console. From this, the technologist will check patient centering and choose the position for the highest scan (at the lower margin of the bifurcation of the main pulmonary artery). The couch will be moved to the start position. The technologist will check subject and phantom positioning in the scout image.

Default Scanner Settings. At least 10.5 cm of data in the z direction will be acquired with each scan and the scan Field of View will be 35 cm for all scanners (to incorporate the phantom in the image). For each scanner, the default settings will be as follows:

- **GE Light Speed (800msec rotation time) - 120 kVp, 200 mA, .8 sec scan, 4 x 2.5mm collimation, sequential axial scans, segmented reconstruction, standard filter.**
- **GE Light Speed (500msec rotation time) - 120 kVp, 320 mA, .5 sec scan, 4 x 2.5mm collimation, sequential axial scans, segmented reconstruction, standard filter.**
- **Siemens Volume Zoom –140 kVp, 50 mAs, (139 mA, .361 sec scan), 4 x 2.5 mm collimation, sequential axial scans with prospective cardiac gating, standard filter reconstruction (as designated on the VZ).**
- **Siemens Sensation 16 –140 kVp, 50 mAs, .5 second/rotation scan time(139 mA, .361 sec exposure time), 3 mm slice thickness using 12 x 1.5 collimation, sequential axial scans with prospective cardiac gating 35 cm FOV and standard filter reconstruction (B35F HeartView medium), ECG triggering would be at 50% of RR.**
- **Imatron EBT scanners: 130 kVp, 630 mA, scan time 100 msec, 3mm collimation, sharp reconstruction filter. For EBT scans, prospective cardiac gating will be used with scanner triggering at 80% of the electrocardiographic RR interval. The EBT scanner table will pause after each table increment of 3 mm (sequential axial scans) . The technologist will acquire 35 to 40 image slices as needed to ensure that the entire heart is scanned**

Large Patient Scanner Settings

For the MDCT scanners *only*, the mA will be adjusted upwards for larger patients. For patients > 220 lbs, the mA will be increased by 25%. Specifically:

- **For General Electric LightSpeed scanner (800msec rotation time): increase to 250 mA. All other parameters are to remain the same.**
- **For General Electric LightSpeed scanner (500msec rotation time): increase to 400 mA. All other parameters are to remain the same.**
- **For Siemens Volume Zoom scanners: increase to 174 mA. All other parameters are to remain the same. Note, increase mAs to 63.**
- **For Siemens 16: 140 kVp, 63 effective mAs, .5 second/rotation scan time, (174 mA, .361 sec exposure time)**

Reconstruction The technologist will use the 35 cm field of view and the sharp reconstruction kernel for all EBT scans and 35 cm field of view and the standard kernel for all other scans.

Imaging (6 minutes). All scanning will be done with a single breath hold. The Siemens Volume Zoom helical scan acquisition requires a breath hold for the participant ranging from 15 to 25 seconds depending upon the combination of heart rate and heart length. EBCT and General Electric scan acquisition requires a breath hold of between 25 and 40 seconds depending on the same factors. The technologist will always ask the subject to take two deep breaths and then a third before imaging commences.

Though total imaging time will be approximately 30 to 40 seconds, double scanning will require about 5 to 7 minutes to complete. The technologist will first acquire one entire series of image slices. The technologist will instruct the subject to relax on the table while he/she reconstructs and assesses the adequacy of positioning, ECG gating and lack of respiratory motion. If these are adequate, the technologist will immediately acquire another series of image slices while the subject remains immobile and in an identical position. Once again, he will assess the adequacy of the images.

Identification, Storage and Transport of Image Data. The following will ensure proper scan identification and subject confidentiality. The technologist will record each volunteer's study id number in the medical record number field of each scan. A five character acrostic consisting of the subject's first, middle and last initials and a digit for the field site will be put in the last name field of the header. The date and times of the two scans and the identity of the scanning site and technologist will be identified in the appropriate fields. The technologist will store images in retrievable format on optical disk (MODs). Images will be transferred to a local work station which has an INTERNET interface to be used for transfer to the reading center.

8.8 Data Transmission of Studies

The technologist will transmit the studies to the local work stations if this has not been done during scanning. He/she will archive each study locally and leave a copy on the hard disk of the work station until it has been successfully transferred to the reading center. He/she will transmit the studies electronically using DICOM transfer on the local workstation to the reading center on a given afternoon of each week. He will simultaneously FAX the list of MFAM ID numbers and acrostics transmitted at that time to the FAX number 310-533-1616.

The CT Data Manager will check each study to ascertain if it has been completely received. If it has been so received, the study will be put on the queue to be read by the reader. If it has not been received, the data manager will immediately communicate the identity of the study to the

field site technologist and request that another attempt be made to send that study. The CT data manager will enter the identity, scan date, scan time and date and time of transfer of each study into a running log, a copy of which will be sent weekly to the coordinating center.

The CT reader will backup studies onto CDs as they are read. The reader will label each disc with a volume name and the date the CD was created. The reader will print a directory to be stored with each CD. Each CD-R that is archived will thus have a printed list of its contents inserted into its sleeve. The Data Manager will back check the CDs to be sure that they contain complete studies.

8.9 Data Flow

A complex data flow diagram is contained in the Appendix. In brief, the following illustrates the principles of data flow:

8.9.1 Images

1. The coordinating center will keep a list of all id numbers of subjects that are scanned. This list will be the master list.
2. The scanning tech will FAX to the reading center (310-533-1616) a separate list of all subjects for whom an attempt is being made at INTERNET electronic transmission of images.
3. Simultaneous with this FAX in 2, the scanning tech will attempt transmission of images for all subjects whose ID numbers are on the FAXed list.
4. The reading center data manager will check the work station for the arrival and completeness of the images corresponding to the id numbers on the FAXed list.
5. The data manager will log in study id numbers on an electronic spread sheet in EXCEL for all studies completely received.
6. The data manager will print this spreadsheet as soon as it is complete and deliver it to the reading center director to begin the process of reading.
7. Scans will be reviewed by a certified radiologist for non-coronary pathology. This radiologist will be working from a remote work station. He/she will enter the readings on an approved results form which will be electronically transferred to the coordinating center and the field site.
8. The reader will then read the scan studies.
9. The data manager will e mail this spread sheet labeled with the date sent to the coordinating center as soon as it is complete.
10. The coordinating center will check the spread sheet from the reading center against the master list. On a weekly basis, the coordinating center will inform the Reading center data manager of missing studies.
11. The reading center data manager will track down the studies that have not been received by communicating with the scanning sites on a weekly basis.
12. The same procedure will be applied to studies which have not arrived.

8.9.2 Reading Results

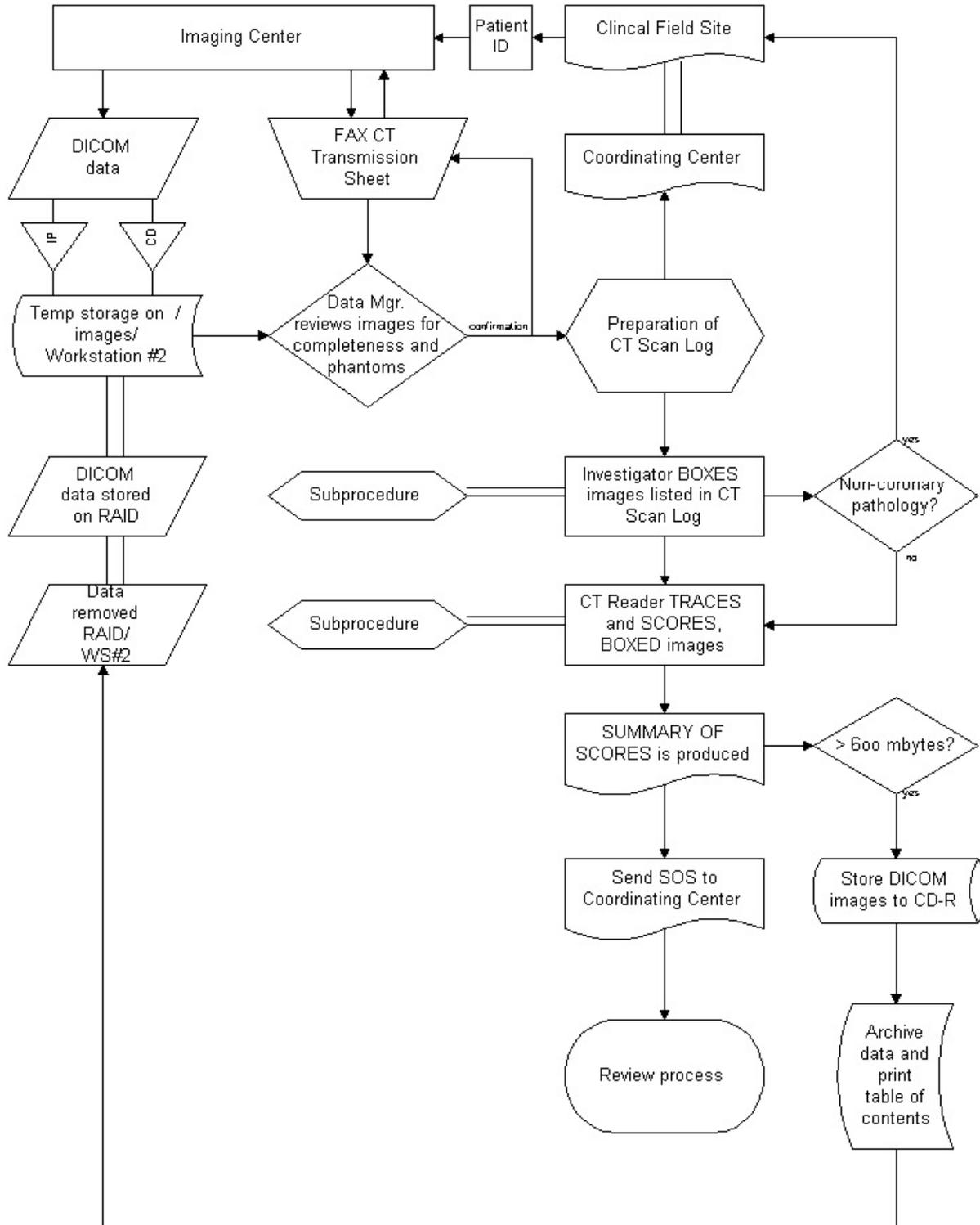
1. The readers will automatically accumulate results as they read in their results files called summary of scores.
2. The data manager will FTP these results to the coordinating center every Friday afternoon. The results file will be labeled with the readers initials and the date and time that it was electronically sent to the coordinating center.
3. The coordinating center will communicate to the data manager whenever expected results are incomplete or not received.

8.9.3 Completing the CT Completion Form

1. The CT Completion form must be filled out by either the CT technologist or the attending Field Center staff member. If the Field Center staff member completes the form, he/she will need to get information from the CT technologist before leaving the scan area. The form will be taken back to the clinic and scanned into the computer along with the other forms.
 - 1.1 Question 1: Results of CT scan.
 - Complete: Select this response if the scan was complete or partially complete and a file containing the scan data will be sent to the CT Reading Center.
 - Incomplete: Select this response if the scan was only partially completed due to a technical or participant problem.
 - If Incomplete is selected, indicate whether a file containing this exam information will be sent to the Reading Center. If the scan was Complete or Not Done, leave this question blank.
 - Not Done: Select this response if the scan was not even started, due to a technical or participant problem.
 - If Incomplete or Not Done is selected, indicate the reason the scan was not completed. If the scan was complete, leave this question blank.
 - 1.2 Question 2 is a comments section.

APPENDIX

MESA CT DATA FLOW SHEET



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Section 9: Vision

9.1 INTRODUCTION

MESA Family-Eye is an ancillary study to MESA Family which also includes Proband participants who were also studied under MESA Eye. It will be completed on all siblings and probands in participating in the MESA Family study. MESA Family-Eye has one questionnaire, and two procedures, MESA Family-Retina, i.e. Retinal Photography, and MESA Family Vision, i.e. Vision Refraction. The specific guidelines for completing the procedures are described below.

9.2 MESA Family – Retina, Retinal Photography

9.2.1 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 2 beginning September 3, 2002.

9.2.2 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.

9.2.3 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 infarct⁵ 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.

9.3 Equipment and Material

9.3.1 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.

9.3.2 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) | Lens cotton
(long-grain Red Cross sterile cotton batting) | One 4 oz. box |
| (b) | Lens cleaning alcohol | One 8oz. bottle (100% alcohol) |
| (c) | Facial tissues | Six boxes (200 tissues/box) |
| (d) | Spare view lamp (#BH3-3277) | One |
| (e) | Canon camera fuses (125V, 4 amp) | Two |

The Ocular Epidemiology Reading Center (OERC) will provide each FC with the following: 100 CDs and jewel cases, CD labels, shipping envelopes, and preprinted shipping labels.

9.4 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.

9.4.1 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.

9.4.2 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.

9.4.3 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.

9.4.4 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.

9.4.5 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, Michael Neider and Hugh Wabers and Katie Nigl can be reached at 608-263-9858.

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).

9.5 Subject Photography Overview

Digital images will be captured into a table mounted laptop computer, archived to CDs, and sent weekly to 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 as 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, the images are archived to CD, making one copy for the reading center and a backup copy, which will be kept at the clinical site.

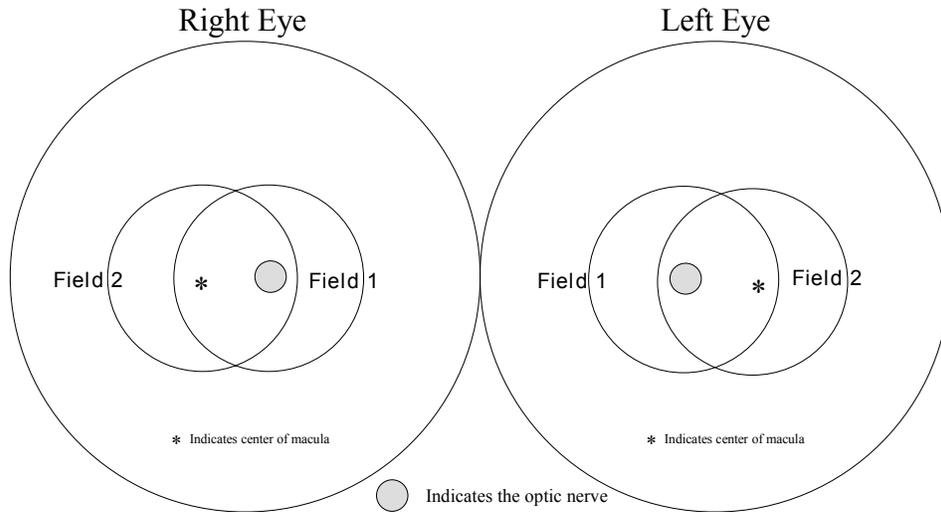


Figure 1

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 Photography Completion 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.

9.5.1 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.

9.5.2 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.

9.5.2.1 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.

9.5.2.2 Completing the MESA Eye History Form

At some sites, before photographing the subject, the photographer will complete the MESA Eye Vision History Form, 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.

9.5.2.3 The MESA Photography Completion Form

The MESA Photography Completion Form 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. Photocopies of this form will be mailed along with the images to the OERC in Madison.

9.5.2.4 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.

Comment: Is this true?

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

9.6 Examination Procedures

9.6.1 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 Digital Healthcare Client icon and selecting the EyeQSL program. A “Login” screen will appear in which the name and password that has been setup for that computer are entered. 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 appropriate boxes. 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.

9.6.2 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 Photography Completion form.

9.6.3 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.

9.6.4 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.

9.7 Photographic Challenges

9.7.1 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 ≤ 2 mm 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 Photography Completion form.

9.7.2 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.

9.7.3 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.

9.8 Shipping Manifest

At the end of each week, all MESA Eye Study Photography Completion forms will be given to the person who will archive the images. Whoever creates the CDs should compare the photography logs 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 Photography Completion Forms and MESA Eye Study Non-Myd Digital Photograph Shipping Manifest, or other deviations from protocol should be recorded in the comments box on the shipping manifest. See Attachments 3 and 4 for editing participants information and deleting visits.

9.9 Digital File Handling

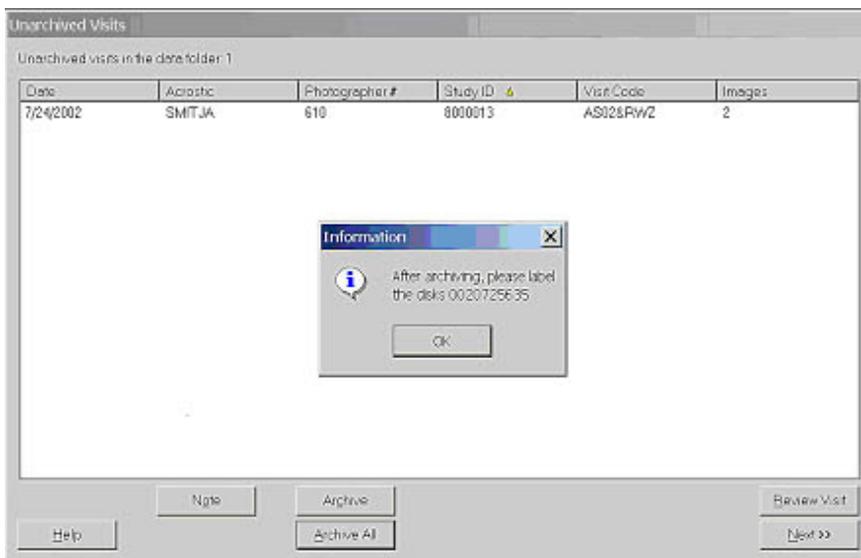
9.9.1 Archive Procedure

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 first step in the archiving process involves formatting the CD. A blank CD is placed into the CD drive on the laptop computer. After a few seconds the "Roxio EZ CD Creator 5.0", a CD writing program, should appear on the screen. If it does not, the CD writing program can be selected from the Start Menu/Programs.

When prompted, select "data" and then "Direct CD". Next, select "format CD". When prompted to type a label for the disc, leave the space blank and click "start format". The program will begin formatting. When finished, select "OK" and close the CD writing program. You will need to format both CDs separately as you begin each archive process. You will be unable to preformat them, as the CDs will need to be "closed" before ejecting them (see next paragraph).

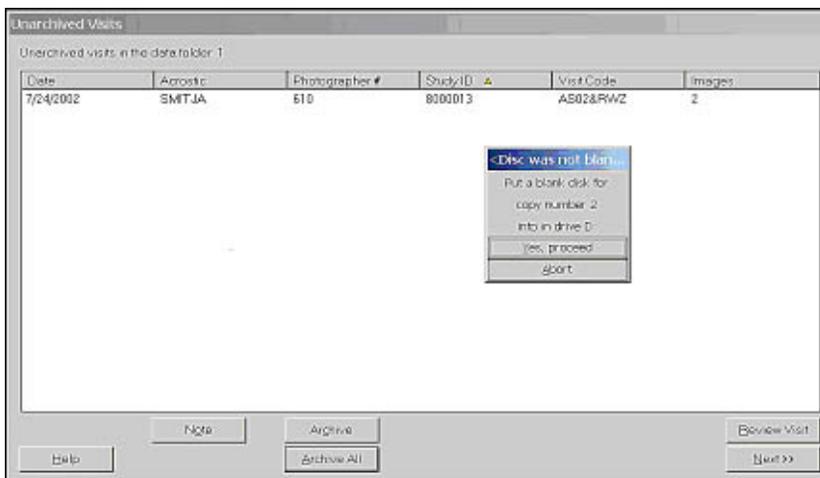
Once the CD has been formatted, the photographer may then proceed to the "Identify a Patient" screen and select the "Archive" button. All of the visits for the week that have not already been archived will appear on the "Unarchived Visits" screen.

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 saved onto a CD. If more than fifteen visits are unarchived, select the first fifteen and hit "Archive". Record the information for these visits on the shipping manifest. An information box will appear before the archiving process begins, which prompts the photographer to label the disk with a specific code number. **This number must be written on the CD label and on the shipping manifest.**



Once this information is written down, press “OK” and the computer will begin archiving. This may take a while depending on how many images are being archived.

When the archiving process is completed on the first CD, a prompt box will open indicating that a “blank CD must be inserted” to creating the second CD.



Eject the first CD by pressing on the eject button located on the front of the CD drive. There will be options on how to save the information. Select “close and read on any computer,” also check the box for protecting the CD from being written to again.



The first CD may now be ejected and the second CD (the clinic copy), placed in the drive. The steps for formatting the second CD will need to be repeated. After completion of formatting, select the “yes, proceed “ option on the prompt box.

9.9.2 CD labeling

The archived CDs, and their jewel cases (one copy for the reading center and a second clinic copy) will be labeled (Figure 2) using preprinted CD and jewel case labels.

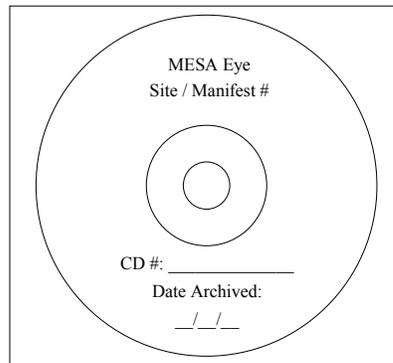


Figure 2

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 that will remain at the site should be placed in an Imation or Fellowes Slim Line jewel case; the CD that will be shipped to the OERC should be placed in a Verbatim Trim Pak case.

9.9.3 Photo Shipping

The package containing the CD, MESA Photography Completion forms and an Eye Study Non-Myd Digital Photograph Shipping Manifest will be sent weekly to Jennifer Reinke at the Ocular Epidemiology Reading Center. 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.

9.9.4 Shipping Materials

CDs to be shipped to Madison should be placed in Verbatim Trim Pak CD cases. These CD cases are designed for shipping CDs; no cushioned mailer is necessary. The CD will then be placed, along with the appropriate paperwork, into a FedEx Priority mailer. Study sites will be supplied with preprinted Federal Express labels. Sites should keep the sender's copy for each shipment until the shipping manifest had been faxed back to them, confirming the CD's arrival in Madison. The person preparing the shipment must fill in the sender's address, phone number and shipping date.

9.10 Photographer Certification

9.10.1 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 at the University of Wisconsin. The Reading Center Photographic Consultant, Michael Neider, will conduct this training, which will involve training on equipment set up. 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 2). This form is submitted along with 10 eyes (5 right eyes and 5 left eyes) photographed following the study protocol. A photographer is fully certified after submitting satisfactory quality photographs of 10 eyes taken on non-study volunteers. 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, ect.)

Acrostic: CERTIFY

Photographer: 109

The photographs must be archived to CD, completely labeled and shipped according to protocol. A separate CD should be submitted for each technician. Once certification is complete, the photographer's information and ID number will be entered into the database and notification of certification will be forwarded to the photographer.

9.10.2 Certification of New Photographers

As additional personnel need training to become certified, a certified photographer at the Field 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.

9.10.3 Certification Maintenance Requirements

In order to maintain certification, photographers must complete photograph 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.

9.11 Quality Control

9.11.1 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.

9.11.2 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 the study eye, if possible. Repeat photographs will be taken after the study photograph session has been completed. The photographer will then start a new patient session, entering the participant's QC ID information as usual. All repeat photographs will undergo an A/V grading by the same grader who initially graded the original photograph at the reading center.

9.12 Communications Channel

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 53726-2397

Jen Reinke Study Coordinator	(608) 263-0285 reinke@epi.ophth.wisc.edu
Stacy Meuer Senior Grader	(608) 263-8835 meuers@epi.ophth.wisc.edu
Michael Neider Photography Consultant	(608) 253-9858 neider@rc.ophth.wisc.edu
Hugh Wabers Photography Consultant	(608) 253-9858 wabers@rc.ophth.wisc.edu
Katie Nigl Photography Consultant	(608) 264-9858 nigl@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

Attachment 1

MESA Family Eye Study
 Non-Myd Digital Photograph Shipping Manifest

This manifest is to be completed whenever a CD is to be sent to the Reading Center.

Prepare **two (2)** copies of this form. Retain one copy for your office and send the original, with the CD to:

Jennifer Reinke Ocular Epidemiology Reading Center 610 N. Walnut Street, 405 WARF Madison, WI 53726-2397 (608) 263-0285 reinke@epi.ophth.wisc.edu (608)263-0279 fax															
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.). Each CD sent should be accompanied by shipping manifests listing all images on the CD															
Patient ID#						Acrostic (Name Code)				Photo Date		Eye (Circle)		Fields Present	
												OD	1	2	
												OS	1	2	
												OD	1	2	
												OS	1	2	
												OD	1	2	
												OS	1	2	
												OD	1	2	
												OS	1	2	
												OD	1	2	
												OS	1	2	
												OD	1	2	
												OS	1	2	
Received By:						Date Archived:									
Date Received:						CD Number:									
Comments:						FAX No:				ATTN: Notification of receipt of CD will be faxed to you.					

Attachment 2

**Photographer Certification Request Form
MESA Family 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: Multi-Ethnic Study of Atherosclerosis – Eye Study

*Fundus Photography Protocol: Multi-Ethnic Study of Atherosclerosis – Eye Study (MESA Eye) – 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 MESA Eye Study

Patient Identifier	Photo Date	Eye	Photograph	Field Type <i>(circle fields present)</i>
_____	____/____/____ Day Month Year	RE LE	Digital Image: Digital Image:	1 2 1 2
_____	____/____/____ Day Month Year	RE LE	Digital Image: Digital Image:	1 2 1 2
_____	____/____/____ Day Month Year	RE LE	Digital Image: Digital Image:	1 2 1 2
_____	____/____/____ Day Month Year	RE LE	Digital Image: Digital Image:	1 2 1 2
_____	____/____/____ Day Month Year	RE LE	Digital Image: Digital Image:	1 2 1 2

Send completed form and photographs to: Questions may be directed to Michael Neider, phone 608-263-9858 Michael Neider
Park West One
406 Science Drive Suite 400
Madison, WI 53711-1068
or Hugh Wabers, phone 608-263-9858
e-mail: wabers@rc.opth.wisc.edu

For Reading Center use only	Certification Approved: ____/____/____ Month Day Year
Reviewer's Signature _____	

Attachment 3

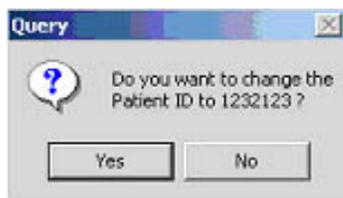
Procedure to edit participant information

If when reviewing unarchived visits it is discovered that incorrect participant information has been entered, hit “Next” to open the “Identify a Patient” screen. Select “Next” again and the “Patient Found in Database” screen will appear. Select the visit that contains the incorrect participant information and hit “Next”; the “Select Visit/Study” screen will appear.

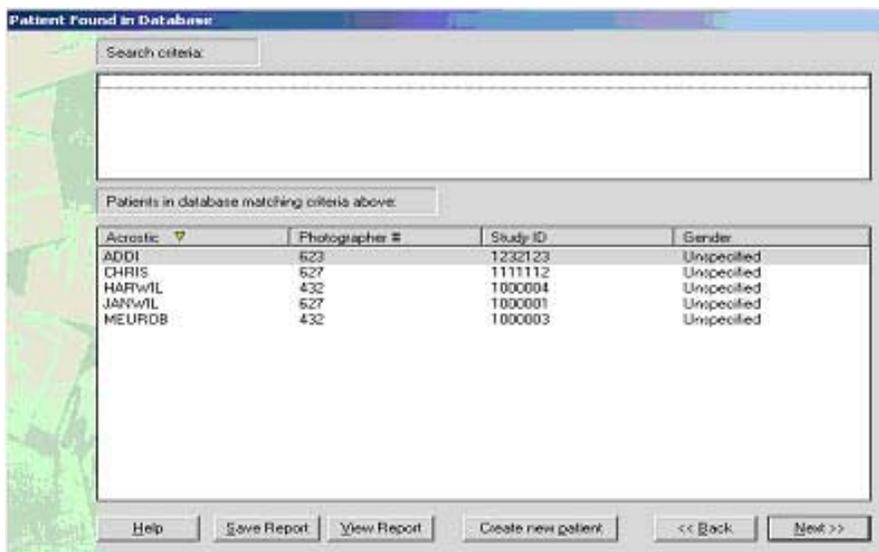
Select “Edit Details”; this will bring up the Patient Details screen.

To correct the participant's acrostic, simply edit the text in the acrostic field and hit "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 acrostic should appear in the listed visits. Hit "Back" again to return to the Identify a Patient screen.

To correct a participant's ID number, follow the same steps as above, editing the Study number in the Patient Details screen. After correcting the number, hit "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.



Attachment 4

Draft Protocol for Database Deletion

Occasionally it is necessary to delete a visit or patient record from the EyeQ capture station databases; on rare occasions it may be necessary to delete (re-initialize) the entire database. It may be necessary to delete a visit, which was entered for test or quality control purposes, for example.

This is done from the Microsoft Windows interface/desktop. To do this, delete all files in the C:\PROGRAM FILES\DIGITAL HEALTHCARE\CLIENT\DB\ folder and all files in the C:\PROGRAM FILES\DIGITAL HEALTHCARE\CLIENT\IMS\ folder. (Do not run the Digital Healthcare software (EyeQ Super Lite) while doing this.)

This can be accomplished by the following step-by-step procedure:

Start laptop if not on

Click on My Computer

Click on Drive C: in the next Window

Click on the Program Files folder in the next window

Click on Digital Healthcare folder in the next window

Click on the Client folder in the next window

Click on the DB folder in the next window

Enter Control – A (selects all files)

Enter Delete

Enter yes to confirm deletion

Enter Backspace (to move to Client folder again)

Click on IMS folder

Enter Control – A (selects all files)

Enter Delete

Enter Yes to confirm deletion.

(This completes the actual deletion.)

Start EyeQSL.

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

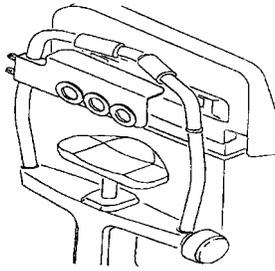
Tolerance range for Axis is + or - 3 degrees

3.3.2 Quarterly Calibration Procedures

Autorefractor

Approximately every quarter year, you will receive artificial test eyes. 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 chin 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 Autorefractor

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 box at left]

SAMPLE →

VD = 13.75mm -10.27D -0.16D +10.08D
--

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.

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 and
- those with a severe eye infection in either eye.

The Technician will make these determinations by observation and enters 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 subconjunctival 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.

The screenshot shows a software window titled "Eye Data Capture" with a subtitle "MESA-Eye Data". The date is "8/13/02". The participant information is "IDNO: 9900001" and "Name: Barbara Anderson". The question is "Do you normally wear glasses or contact lenses for distance vision, such as when you watch television, read street signs or drive?". The answer options are: "Yes, glasses", "Yes, contacts", "Yes, either glasses or contacts", "Yes, both at the same time", "No", "Don't know", and "Refused".

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.

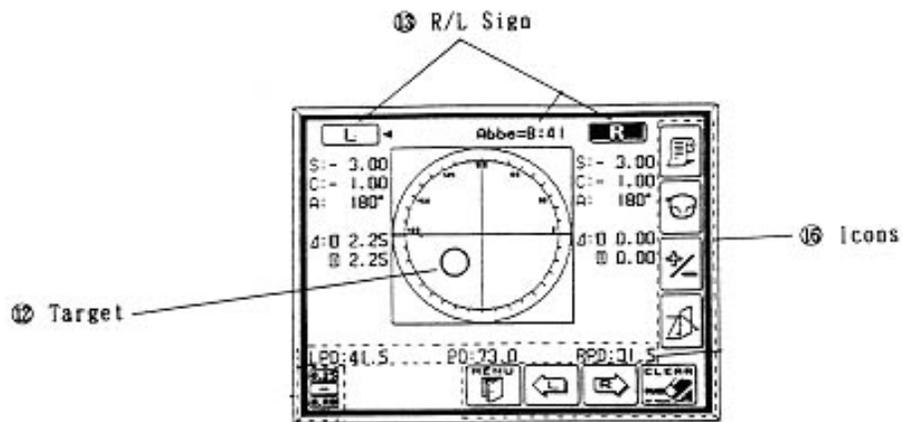
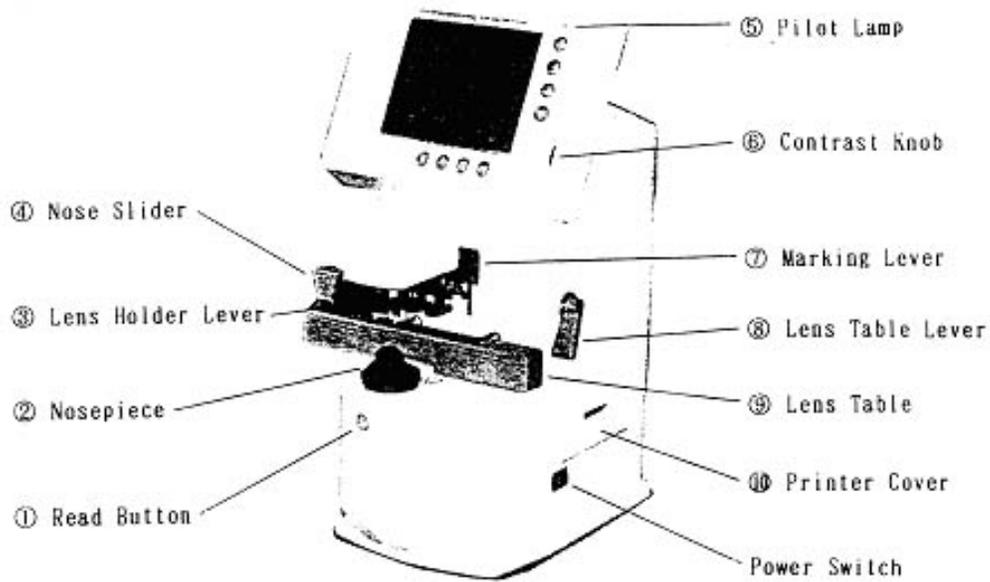
NOTE: REMEMBER TO CLEAR THE LENSMETER AND AUTOREFRACTOR!!!!
The autorefractor should be set so the [CYL +] appears on the LED screen.

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.

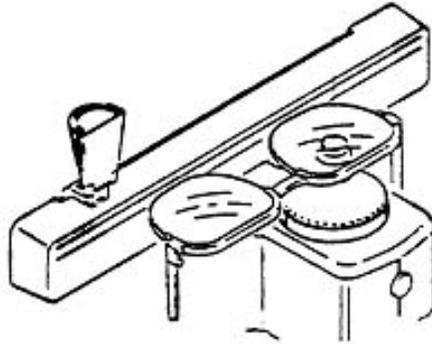


Nidek Lensmeter LM 990-A

- Ask the study participant to hand you the eyeglasses he/she wears to see distant objects.
- Inspect the lenses. If they are exceptionally dirty, 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:

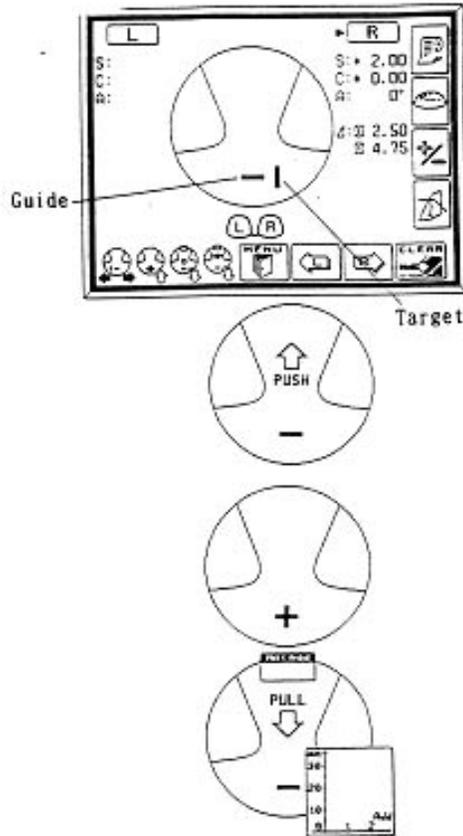
- Keep the eyeglass frames horizontally flush against the platform. (Otherwise, the axis measurements may be skewed.)
- Use the lens holder to avoid tilting the lenses. (Otherwise, the cylinder measurements may be skewed.)

4.3 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:

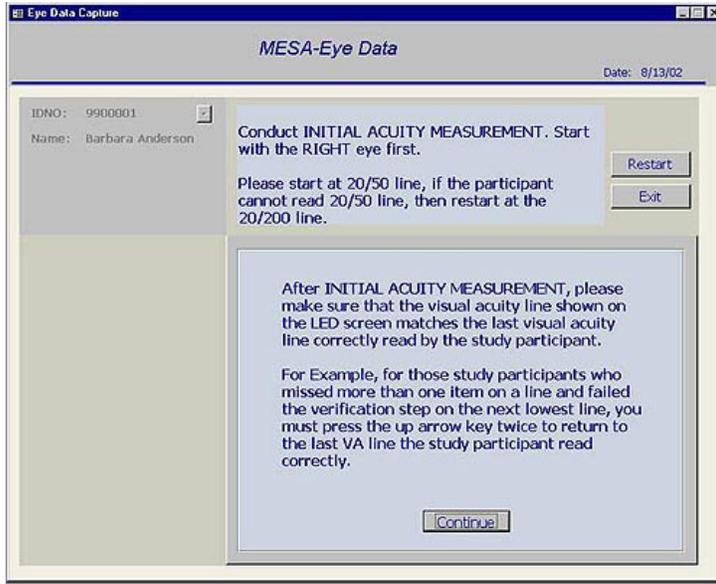
- If you forget the order of the steps, follow the lens graphics on the bottom left hand side of the screen.
- Always remember to start in the top distance portion of the lens, especially when switching to the left lens.
- If the plus sign never appears in Step 6 and the PUSH sign appears all the way 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.

IMPORTANT NOTE FOR MEASURING ANY LENS

If the sphere is equal to or greater than $\pm 5D$ 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.

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

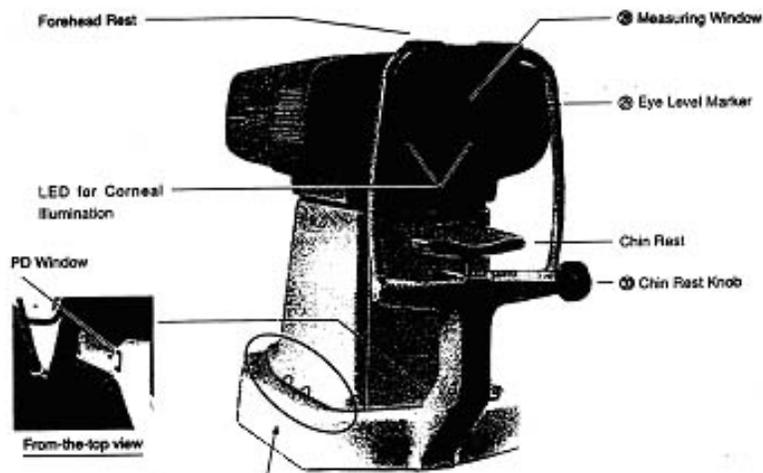
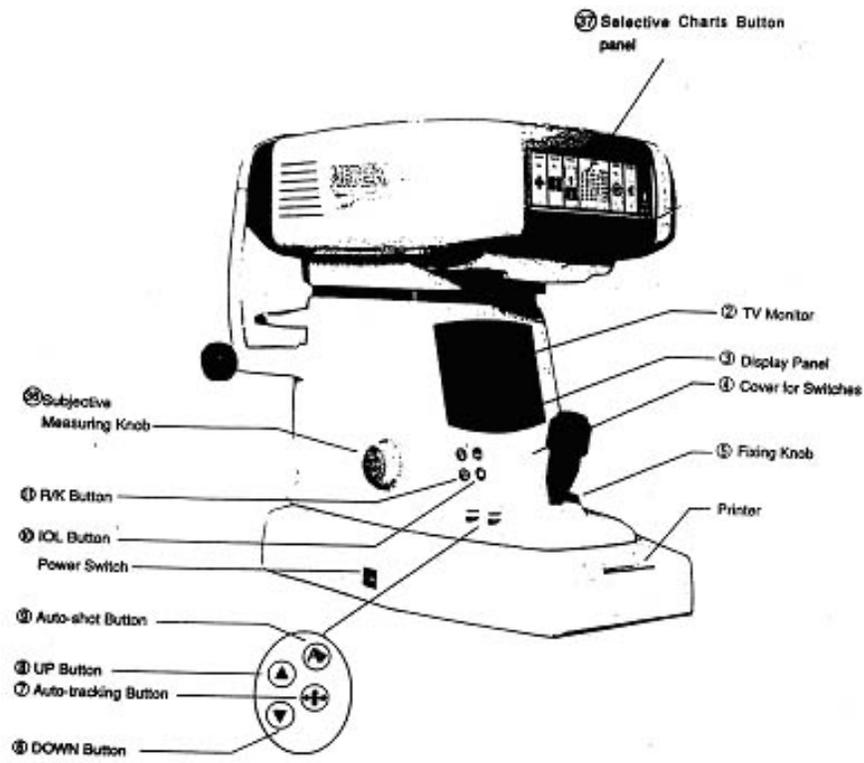
Perform the visual acuity and enter data into the computer screen as prompted as you move along the visual acuity protocol.

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.

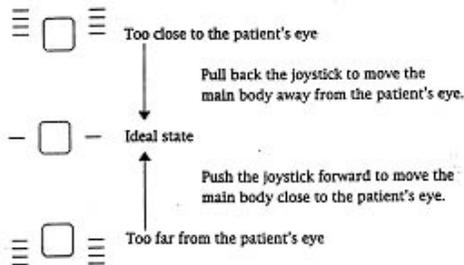


Autorefractor ARK760

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 see 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.”)

VA Chart Button

6		F			20/200
2	5	O	P	H	20/80
8	2	V	D	F	20/60
7	6	K	R	H	20/50
5	8	P	O	E	20/40
6	2	C	N	K	20/30
8	7	R	P	O	20/25
2	5	D	H	V	20/20

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 all 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.
- If you are unable to get 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 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.

1.6 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 indicated 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.



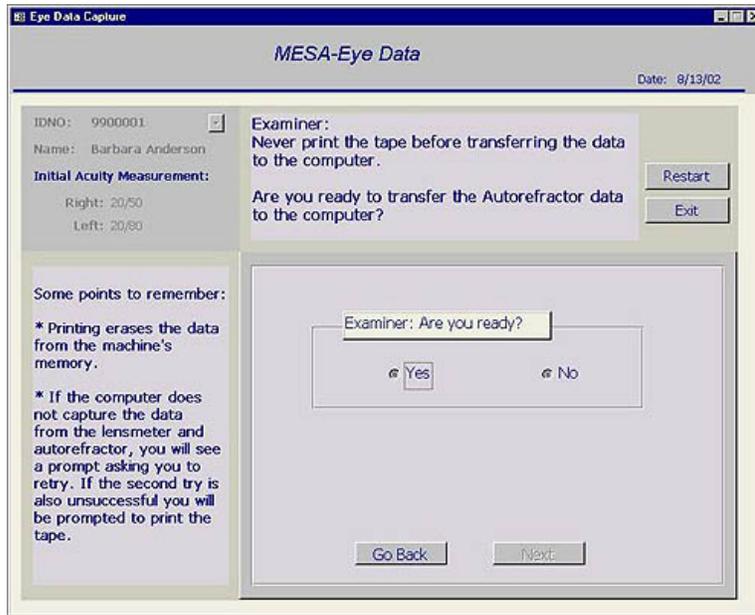
5. Using the up arrow keys, change the visual acuity chart to the 20/50 line.
6. Ask the study participant to read the line: ***Now please read this line slowly and from left to right for me. Remember that you may see both numbers and letters on the screen.***
 - If the study participant can read the 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 capture and transfer the information from the equipment to the MESA –Vision database (see below).



If the computer does not capture 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

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.
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



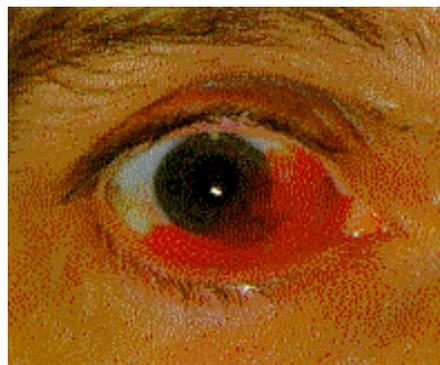
(2) Acute anterior cellulitis.
Excluded from vision assessment



(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.(Oph)
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.

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Section 10: Carotid Ultrasound

10.1 General Guidelines

Standardization and Probe Frequency

MESA Family ultrasound scans will all be performed with GE Logiq 700 ultrasound machines using ML probes. The probe operates at two different frequencies: 9 and 13 MHz. Images of the brachial artery and common carotid are captured at 9 MHz, and the internal carotid images are captured at 13 MHz. Pulse Wave Doppler measures are not affected by the probe frequency setting. When making Pulse Wave Doppler measurements, the probe frequency can remain set at the frequency that makes the execution of the protocol most efficient.

Data Transmission

Ultrasound scans are recorded to Super VHS videotape and sent to the Ultrasound Reading Center for analysis. Field Centers send tapes to the Reading Center every Thursday. Two separate tapes are used, one for both Brachial Artery Endothelial Function and Carotid Distensibility scans and the other for Carotid IMT scans. The separate tapes are used, because Endothelial Function and Carotid Distensibility analysis involves the capture of video loops, whereas Carotid IMT analysis involves static images. Since the some scanning is done at separate clinical sites at Columbia University (MESA Field Center 4) this field center may use up to three separate videotapes.

Forms

There are two different forms that the MESA Family sonographers will be using. One is the IMT Videotape Log Sheet and the other is the Ultrasound IMT Case Report Form (titled *Carotid Ultrasound*).

A videotape log sheet, which can consist of more than one page, is the reader's guide to the scans that are recorded on the videotape. It is sent to the Ultrasound Reading Center with the videotape. Photocopy the log sheets before sending them.

MESA Family ultrasound case report forms are completed for each participant. These forms are scanned into the MESA Family database at the field center. Do not send these forms to the Ultrasound Reading Center.

Ultrasound Videotape Log Sheets and Labels

A videotape log sheet is to a MESA Family ultrasound videotape what a table of contents is to a book. Just as it is impossible to find out what is in a book without a table of contents, it is also impossible to determine what is on the tape and to analyze it without a log sheet. The process is simply much more efficient with the log sheet.

Matching numbered labels for both the log sheets and videotapes have been provided to facilitate the long-term organization of MESA Family ultrasound data. A new log sheet is started for each new videotape. Label each new videotape and log sheet with matching labels before scanning. If more than 10 participants will be recorded on videotape, attach a second page and continue.

Though most of the entries to the Videotape Log Sheet are self-explanatory, take the time to review the details below. It is important to check subject IDs and to write legibly. Transposition errors can be difficult to catch after the participant has left the scanning area.

VCR Start Time

The one item on the log sheet that can cause confusion is the "VCR Start Time." The "VCR Start Time" recorded on the log sheet is analogous to the page numbers in a table of contents. Without the "VCR Start Time," finding a study on a videotape can be very difficult and time consuming.

General Guidelines for "VCR Start Time"

1. Start each new tape with the counter set at 0:00
2. At the end of each study mark down the VCR counter time of the study just completed as the "VCR Start Time" for the next study on the log sheet.
3. If the next scan is immediately consecutive and on the same videotape, great. Start the next scan and when it is complete, mark down the VCR counter time as the "VCR Start Time" for the next study.
4. If the scans are not consecutive, there are two options:

Option A (more efficient)

1. Don't rewind the videotape, or, if you have reviewed, have it queued up (ready) at the end of the last recorded scan.
2. Re-set the VCR counter to 0:00.
3. Videotape the new study.
4. When the scan is completed, jot down the time displayed on the VCR counter. Add it to the "VCR Start Time" of the scan just completed.
5. Write the result in the "VCR Start Time" space of the next scan.

Option B

1. Rewind the tape
2. Reset the VCR Counter to 0:00.
3. Fast-forward to the end of the last study.
4. The VCR counter should now display at time within seconds of the time written in as the "VCR Start Time" on the log sheet.
5. Videotape the new study.
6. When the scan is completed, record the VCR counter time at the end of the study just completed as the "VCR Start Time" of the next study.

Videotape Log Sheet Details

Item	Description
Scan Date	<u>Date scan is performed</u>
Subject ID	Participant's MESA ID
Plaque Location <i>Carotid IMT log sheet only</i>	<i>If there is (are) plaque(s), indicate the location(s) by circling the appropriate acronym. RB = right bulb RICA = right internal carotid LB = left bulb LICA = left internal carotid</i>
VCR Start Time	VCR Counter time, not the time of day. Start new videotapes at 0:00. Re-set the VCR counter if necessary.
Sonog ID	Sonographer's MESA Technician ID
Comments	<i>This space has been provided for general comments regarding each scan. Note any significant findings, including plaque, wall thickening, or difficulties encountered in trying to obtain good images. This section of the log sheet is invaluable to the ultrasound Reader, who must rely on the sonographer to communicate what is seen.</i>

Sample Carotid IMT Videotape Log Sheet

Only Carotid IMT scans are recorded on the Carotid IMT videotape.

	Scan Date	Subject ID	Plaque *	VCR Start Time	Sonog. ID	Comments
1.	6/13/2000	3551212	RB RICA LB LICA	0:00	317	<i>Nice clean and easy scan</i>
2.	6/13/2000	3899223	RB RICA LB LICA	3:10	319	<i>Plaque in right ICA</i>
3.	6/14/2000	3221555	RB RICA LB LICA	5:25	317	<i>Use second LCC image, thick neck difficult to scan</i>

10.2 Right Common Carotid Dynamic Acquisition

MESA Family Case Report Forms

All MESA Family case report forms require the entry of the MESA Family participant ID, acrostic and date at the top right, and the sonographer, reviewer and data entry IDs along the bottom.

Protocol: Carotid Progression and IMT

Form Name: Carotid Ultrasound

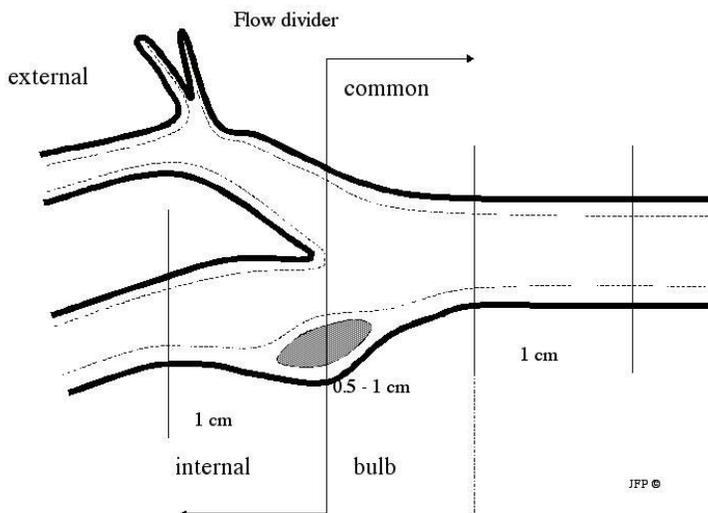
Item	Response Options	Explanation
ID #	primary MESA Family Subject Identifier	
Acrostic	secondary MESA Family Subject Identifier	
Date	scan date – today’s date	
1. Results of Carotid IMT Progression Examination (20 seconds of right common carotid dynamic acquisition):	<input type="radio"/> Done <input type="radio"/> Incomplete <input type="radio"/> Not Done	Indicate whether or not the scan was successfully conducted and recorded. Skip to #3 if it was “Done.”
2. Reason Carotid IMT Progression Examination incomplete or not done:	<input type="radio"/> Equipment Malfunction <input type="radio"/> Time/Staff/Room Constraints <input type="radio"/> Examinee refused/uncooperative <input type="radio"/> Examinee physically unable <input type="radio"/> Other (specify)	If scan is “Incomplete” or “Not Done,” indicate why.
3. Tape #	3 digit number	The number on the videotape and log sheet. No letters.
4. VCR Start Time	1 digit for hour 2 digits for min 2 digits for sec	VCR Counter time, not the time of day.
5. Were Right CCA Doppler blood flow signals detectable?	<input type="radio"/> Yes <input type="radio"/> No	Indicate whether or not PW Dopplers could be heard.
6. Right CCA Pulse Wave Doppler measurement (cm/s)	3 digits cm/sec	Enter PW Doppler measurement from image screen in cm/sec.
7. Exam 1 image evaluation	<input type="radio"/> None available <input type="radio"/> Device malfunction – not available <input type="radio"/> Landmarks identifiable? If Yes for “Landmarks,” select one: <input type="radio"/> All <input type="radio"/> In part <input type="radio"/> None	Skip to #10 if “None available” or “Device malfunction—not available” is marked. If “Landmarks” is marked, then also fill in “All,” “In part,” or “None.”

8. Quality of Exam 1 image	<input type="radio"/> Excellent <input type="radio"/> Very Good <input type="radio"/> Satisfactory <input type="radio"/> Poor	
9. Quality of current examination	<input type="radio"/> Excellent <input type="radio"/> Very Good <input type="radio"/> Satisfactory <input type="radio"/> Poor	
10. Post-imaging Blood Pressure and Pulse	3 digits for systolic 3 digits for diastolic 3 digits for pulse	Left arm blood pressure (use right arm if there is a gradient ≥ 15 mmHg; check box if right arm used)
11. Results of Carotid IMT scan	<input type="radio"/> Done <input type="radio"/> Incomplete <input type="radio"/> Not Done	Indicate whether or not the scan was successfully conducted and recorded. Skip to #13 if it was "Done." If "Not Done," then complete Question 12 and skip to end of form.
12. Reason Carotid IMT scan incomplete or not done	<input type="radio"/> Equipment malfunction <input type="radio"/> Time/Staff/Room constraints <input type="radio"/> Refused/Uncooperative <input type="radio"/> Examinee physically unable <input type="radio"/> Other (specify)	
13. Were ICA Doppler blood flow signals detectable? (Right and Left)	<input type="radio"/> Yes <input type="radio"/> No	Indicate separately for the right and the left sides whether or not PW Dopplers could be heard.
14. Pulse Wave Doppler measurements (cm/s) (Right and Left ICA/Bulb)	3 digits cm/sec	Enter PW Doppler measurement from image screen in cm/sec.
15. Quality of scan (Carotid IMT scan)	<input type="radio"/> Good <input type="radio"/> Fair <input type="radio"/> Poor	Based on your assessment, is the quality of this Carotid IMT scan good, fair, or poor?

Carotid Imaging Protocol

The protocol includes review of previous common carotid artery images taken during Exam 1 on the participant. This Exam 1 image is distributed on a CD-ROM with the subject ID as an identifier. A reading program included on the CD permits image display on any standard Window PC. This image is used to confirm orientation and location of the previous study and serves as an aid for the sonographer in localizing the bulb and other landmarks.

The common carotid artery (CCA) dilates to form the carotid bulb proximal to the bifurcation into the internal and external carotid arteries.



The origin of the bulb can be recognized in most, though not all, subjects. The common carotid artery bulb is defined as the site where the artery begins to dilate slightly and the vessel walls curve out, i.e.: they are no longer parallel to each other. The bulb is elliptically shaped and geometrically complex in the longitudinal view. The tip of the flow divider defines the bulb's upper limit. The tip of the flow divider also marks the origin of the internal and external carotid arteries.

Evaluation of the common carotid artery IMT is made on a segment at least 1 cm below the carotid bulb. The common

carotid artery is, at this level, straight. Blood pressure measurements will be made to verify that there are no significant interval changes in blood pressure since the previous carotid IMT examination. Variations in blood pressure may affect IMT estimates. For example, an increase in blood pressure will increase the diameter of the common carotid artery. The short-term effect of a diameter increase of the common carotid artery is to slightly decrease the IMT at the level where the IMT measurement is being made.

Videotape Identification – Labels and Log sheets

The Ultrasound Reading Center will provide videotape labels and log sheets to the field centers. Matching numbered labels for the videotapes enable the long-term organization of MESA Family data. A new log sheet is started for each new videotape. Before beginning the first scan, attach the matching labels to the videotape and log sheet. Labels are put on the spine and the face of the videotape and the matching label is put in the specified area of the log sheet.

MESA Family Carotid Ultrasound procedure form

A procedure form will be completed for every participant by the sonographer. The forms are scanned into the MESA Family database. In addition, to facilitate the analysis process, photocopies of the form are to be made and sent to the Ultrasound Reading Center together with the videotape and videotape log sheet.

Participant Position and Blood Pressure Measurement

The subject is supine during the carotid artery examination and is made comfortable in a position that allows head rotation to the left side. The sonographer stands or is seated at the end of the examination table near the participant's head. The top of the head is about three inches from the end of the examination table and the head is rotated 45 degrees to the left. Participants may wish to turn their heads to look at the screen. This should be discouraged. The sonographer may gently place his/her hands on the subject's head as an aid to rotate the head in the correct position. The sonographer should emphasize the importance of not moving. A blood pressure cuff is connected to an automated sphyngomanometer (Dinamap) placed over the left upper arm. Blood pressures are recorded from the left arm. The left arm is held slightly abducted from the body. Record the blood pressures and pulse to the MESA Family Ultrasound IMT procedure form.

Image review

Exam 1 common carotid images will be distributed to the field centers. They are in a standard file format that can be recognized by most digital imaging software including "Imaging" a program installed as part of the Windows operating system. The image file names include the MESA Family participant's subject ID numbers. For example, the digital image for the Subject ID# 5021399 is 5021399.DIB.

The images are displayed by double clicking on the file name to open it or by starting the program "Imaging" and selecting File → Open and navigating to the appropriate file. Sonographers should verify that the MESA Family Subject ID imprinted upon the displayed Exam 1 CCA image is that of the participant being scanned. If the image file cannot be found or the imprinted Subject ID is does not match, please notify the Ultrasound Reading Center immediately.

Machine Set-up

All images are taken only from the right common carotid artery with the Common Carotid Presets entered in the GE Logiq 700 device.

Common Carotid Presets

Frequency: 13 MHz

66 Dyn Range

Res

Edge 3

Map G

Ave. 2

Number focal zones: 2 (*position of focal zones: mid artery*)

Videotape Demographic Information and Label Images with Study Identifiers

Participant information is entered on the ultrasound machine's demographic information screen to identify the scan as a MESA Family scan for a particular subject. This page is videotaped before scanning begins to ensure the identification of the scan data with the proper participant. Videotape the demographic information page for five seconds before beginning the imaging protocols. The following information should be included on the demographic screen and on the image throughout the scan:

- The study name and the exam: **MESA Family**
- Subject ID
- Subject Acrostic
- Date of ultrasound scan
- Sonographer ID
- Protocol: **IMT Progression**

Enter subject information to subject information screen

The participant ID number is entered

Press "New patient"

Hit "return" until you reach the new patient question (Y/N); type "Y" and then return

Keep hitting return until the Last Name field: Type "right cca"

Keep hitting return until the ID# field: enter the ID number

Hit return for gender (M or F)

Hit return and enter your Sonographer ID

Videotape screen for 5 seconds

Hit the "Exit" key to exit to begin scanning

Initial Scan (Videotaped for 10 seconds at a 4 cm field-of-view)

The purpose of the initial scan is to orient the sonographer to the subject's carotid anatomy:

1. To identify the relative location of the jugular vein to the common carotid artery,
2. To locate the bifurcation and;
3. To locate the common carotid artery bulb.
4. To confirm proper orientation of the transducer

Videotape ON

This initial scan is done in the plane transverse to the artery and vein. The transducer is slowly swept from the low common carotid artery (just above the clavicle) to just above the origins of the internal and external carotid arteries: the transducer is kept over the internal jugular vein so that the vein is stacked on top of the artery. Once the location of the bifurcation is identified, the transducer is slowly rotated to a plane parallel to the axis of the artery and vein. The jugular vein then serves as an acoustic window for imaging of the common carotid artery. Apply only a minimal amount of pressure to the transducer in order to prevent collapse of the vein and loss of the acoustic window.

Videotape OFF

Summary of imaging sequences:

1. **Video stream 1:** A pulse-wave Doppler tracing is recorded over at least 5 cardiac cycles, approximately 5 seconds of *real-time* imaging.
2. **Video stream 2:** A recording is made with the optimal "zoomed" image of the common carotid artery. Both near and far wall interfaces defining the intima-media thickness should be clearly imaged over at least a 1 cm length. This recording is made for 20 seconds (approximately 20 cardiac cycles)

Imaging sequences:

Video Stream 1 - Pulse-wave Doppler

A 4 cm field-of-view is used as the default image size.

The critical information is the peak-velocity in the common carotid artery at peak systole at the point of maximum flow acceleration. This confirms location and patency of the carotid artery.



Procedure:

- Hit the "cursor" key.
- Move the cursor to the middle of the artery with the trackball
- Press the toggle switches ("angle steer" and "angle correct") until there is an angle of 60 degrees or less between artery axis and the ultrasound beam.
- Press the PW button. Adjust the velocity scale so that the Doppler waveform fits the display (Y-axis).
- Turn the videotape on for 5 -10

seconds until a satisfactory Doppler tracing is obtained

- Freeze the image and let the videotape record the frozen image for 5 seconds
- N.B. The velocity scale is set so the Doppler waveform fills the display.
- Record peak Pulse Wave Doppler measurement to MESA Family Ultrasound IMT procedure form.

Video Stream 2 - Right Common Carotid real-time gray scale imaging

Verification of image location:

If it is available, review the appearance of the Exam 1 CCA image on laptop computer screen. Note the location and orientation of the bulb, artery wall and if visible the tip of the flow divider. Document the visibility of the landmarks and assess the quality of the Exam 1 image on the procedure form.

Image acquisition

Imaging is done in the lateral projection with the jugular vein lying immediately above the common carotid artery (or at 45 degrees if the internal jugular vein is not present). The image is centered on a 10 mm segment of the right common carotid artery at least 10-mm below (caudad to) the right common carotid artery bulb. The carotid bulb may be displayed on the left side of the monitor (when facing the screen). The bulb occupies 1/4 up to 1/3 of the image along the X-axis. If the bulb cannot be identified, but the tip of the flow divider can, this may substitute as the internal landmark on this view.

After locating the tip of the flow divider on the transverse image, the transducer is slowly moved down the neck by 2 to 3 cm. Rotate the transducer into the lateral plane keeping the jugular vein in the same imaging plane. The probe is then centered on the upper 2 cm of the common carotid.

The sonographer magnifies the longitudinal image (jugular vein above common carotid artery).



- Press the "ZOOM" button
- Use the trackball to center the distal common carotid artery (edge of bulb occupying up to 1/3 of the image to the left hand side of the screen)
- Press the "SET" button
- A videotape is taken for a minimum of 20 seconds so that the images in the video sequence are from at least 20 cardiac cycles. The common carotid artery wall interfaces (near and far wall IMT interfaces) are clearly depicted during this recording.

Procedure Form Details

A MESA Family Carotid Ultrasound procedure form will be completed by the sonographer for every MESA Family subject scanned. The sonographer is responsible for the numbered items and to verify the Subject ID, Acrostic and the Date. **NEW PROCEDURE: the procedure forms are to be photocopied and the copies sent to the Ultrasound Reading Center together with the videotape and the log sheet.** The originals are to be returned to the clinic coordinator who will oversee their being scanned into the MESA Family database system.

Summary of MESA Family Procedures

Preparation	
Videotape	Labeled and queued up
Log sheet	Labeled and completed
Exam 1 Image Review	Locate and display Exam 1 image on laptop
Ultrasound IMT procedure form	Verify Subject identifiers, date, Sonographer ID
Pre-sets – Common Carotid	Verified
Protocol	
Blood pressure	Measure with Dinamap and record on form
Demographics	Enter Subject and scan information Videotape for 5 seconds
Transverse sweep	Videotape 10 second transverse sweep from the low common to above origins of ICA and ECA with the jugular vein stacked on top of artery
Video stream 1 (PW Doppler - CCA)	4 cm field of view Videotape 5-10 seconds of live Doppler tracing Videotape 5 seconds of measured peak velocity Record peak PW velocity on form
Video stream 2 (Longitudinal gray scale of CCA)	ZOOMED field of view Match the Exam 1 image Videotape 20 seconds of right CCA: clear near and far walls

Data Transmission

Videotapes are sent weekly to Dr. Polak through a package delivery service with tracking capabilities at the following address. The package includes the appropriate videotape and log sheet and copies of the procedure forms.

Joseph F. Polak, M.D., M.P.H.
MESA Ultrasound Reading Center
New England Medical Center, Box 380
750 Washington Street
Boston, MA 02111

Carotid Acquisition/Analysis quality assurance

All new MESA sonographers will travel to Boston for a one-day training session. This training session is similar in content to the MESA training session and consists of a review of basic carotid physiology and instrumentation. Following didactic lessons, the sonographers will practice imaging according to the protocol on volunteers, using the GE Logiq-700 ultrasound device available at the Field centers. Dr. Polak will be in charge of this session. Following this training session, the sonographers will conduct 10 examinations in order to be certified. The certification scans will be reviewed for protocol adherence and quality and a written critique provided. Once certified, they will start imaging the MESA Family cohort.

Sonographers who have been certified by the Ultrasound Reading Center during Exam 1 or Exam 2 are eligible to scan for Exam 3 after participating in a telephone conference call with Dr. Polak and the URC staff. The sonographers will have hardcopies of the manual, procedure form and videotape log sheet, which will be distributed via the Internet, with them for reference during the call. The protocol will be discussed, administrative details will be reviewed and questions will be answered. The first tapes will be carefully reviewed for protocol adherence and image quality and a written critique provided to each sonographer.

All readers will need to be certified. This will be done after training (one month of performing IMT measurements). Dr. Polak will review at least 30 common carotid IMT measurements in order to certify the readers.

Quality assurance will be conducted in order to determine inter-sonographer (if applicable), intra-sonographer variability, intra-reader variability and inter-reader variability.

Since new sonographers may be working at some of the Field centers, we need to make allowances for differences in sonographer performance. This will be done by reader evaluation of the relative location/presence of the carotid bulb on the current study image as compared to the Exam 1 image. The reader will score concordance of location for the current study image, as compared to the Exam 1 image, on a 3 level Likert scale (exact concordance, partial concordance, no concordance). The reader will also score concordance of image gain for the current study image, as compared to the Exam 1 image, on a 3 level Likert scale (exact concordance, partial concordance, no concordance). The reader will also judge the quality of the Exam 1 study image and the current study image according to a five level Likert scale: (perfect alignment, almost perfect, interpretable, poorly interpretable, uninterpretable).

We will perform intra-sonographer variability evaluations at the beginning of the examination, mid-way into the examination and at the end of the examination. Sonographer will repeat 30 ultrasound image acquisitions at each time point. At those centers where there are more than one sonographer, we will ask the sonographers to perform, in the first month of the study, separate image acquisitions on all these same participants (estimated 30 studies). This will serve as a baseline for inter-sonographer variability. This will also be repeated at 9 months into the examination and during the last month of the examination. These replicate scans will be measured by two different reader in order to determine (using a nested design) the relative contributions of inter-sonographer, intra-sonographer and, if applicable, inter-reader variability.

The consistency in subjectively evaluating the quality of the images and concordance between image pairs will be evaluated by weighed kappas (expected level of 0.6 or better). Errors in paired measurements will be quantitated using paired absolute differences in IMT values (inter-reader, intra-reader, intra-sonographer and inter-sonographer, if applicable) in addition to ANOVA.

Since baseline IMT may turn out to be an important co-variate (see data analysis section below), appropriate corrections will be needed to account for bias between different readers. Variability of human drawn interfaces and edge detector interfaces will be determined for the whole cohort. Both types of IMT measurements will be available for estimating the extent of atherosclerosis progression.

10.3 Carotid IMT

I. OVERVIEW OF THE CAROTID ULTRASOUND SCANNING PROTOCOL

1. Introduction to the Carotid Ultrasound Scanning Protocol
2. Participant Position
3. Anatomical Sites
4. Initial Scan
5. Standard Carotid Ultrasound Images
6. Criteria for Satisfactory Images
7. Imaging Priorities
8. Sonographer Response to a Significant Stenosis – ALERT
9. A *Good* Carotid Ultrasound Image

II. OVERVIEW OF CAROTID ULTRASOUND ANALYSIS PROTOCOL

1. Preview and Digitization of a Carotid Ultrasound Study
2. Calibration
3. The Interfaces
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5. Quality Scores
6. Subjective Assessment
 - 6.1 Surface
 - 6.2 Morphology
 - 6.3 Percent Stenosis
 - 6.4 Location
 - 6.5 Density

III. DATA TRANSMISSION

1. Videotape
2. Labels and Log Sheets
3. Shipping Videotapes and Log Sheets to the Ultrasound Reading Center
4. Data Transmission to the Coordinating Center

IV. SONOGRAPHER QUALITY CONTROL

1. Clinical Center Sonographers
2. Ultrasound Reading Center – Sonographer Communication
3. Ultrasound Reading Center Report on Sonographer Performance
4. Performance Standards during Examination Period
5. Replacement or Retraining of a Sonographer
6. With-in Sonographer Variability Assessment
7. Achieving and Maintaining Quality Ultrasound Data

I. Overview of the Carotid Artery IMT Ultrasound Scanning Protocol

1. Introduction to the Carotid IMT Ultrasound Scanning Protocol

The extracranial carotid arteries are the largest arteries in the neck. The right common carotid artery (CCA) originates from the innominate artery on the right, and the left CCA originates directly from the aortic arch. Each common carotid artery ascends in the neck, lateral to and posterior to the trachea. At the approximate level of thyroid cartilage, slightly below the angle of the mandible, the common carotid artery bifurcates into the external and internal carotid arteries. Proximal to the bifurcation, the common carotid artery dilates to form the carotid bulb. The origin of the bulb can be recognized in most, though not all, subjects. It is defined to be where the common carotid artery begins to dilate slightly and where the vessel walls curve out and are no longer parallel to the skin surface. The bulb is elliptically shaped and geometrically complex in the longitudinal view. Its upper limit is defined by the tip of the flow divider. The tip of the flow divider also marks the origin of the internal and external carotid arteries. The external carotid artery lies anterior and slightly medially to the internal carotid artery in 90% of individuals. In the remaining 10%, the orientation is reversed. The external carotid artery is usually smaller than the internal and it has branches that supply the neck and face. The internal carotid artery has no branches in the neck and ascends into the calvarium to supply the brain.

2. Participant Position

The subject is supine during the carotid artery examination and is made comfortable in a position that allows head rotation to either side. The sonographer stands or is seated at the end of the exam table near the participant's head. The top of the head is about three inches from the end of the exam table and the head is rotated 35° away from the side examined. Participants may wish to turn their heads to look at the screen. This should be discouraged. This is best achieved by the sonographer using his hands at the start of the study to rotate the head while stressing to the participant the importance of not moving.

3. Anatomical Sites of Interest

3.1 The extracranial carotid arteries are divided into four anatomically defined segments:

- Distal common carotid artery
- Carotid bulb
- Internal carotid artery
- External carotid artery

3.2 The lateral extent of each segment is defined relative to the tip of the flow divider, which is typically the most clearly defined anatomical reference in the carotid system. The three segments of interest are the distal common carotid artery, the carotid bulb, and the internal carotid artery. No external carotid images will be recorded.

3.3 Anatomical Definitions

- Distal common carotid is the segment of the common carotid artery immediately proximal to the origin of the carotid bulb, where the near and far walls of the artery are parallel to one another. The end of the distal common carotid artery is marked by the dilatation of the vessel walls, which is the carotid bulb.
- Carotid bulb: the inferior extent of the bulb is the beginning of dilatation, or 8 mm below the tip of the flow divider. The superior extent of the bulb is defined by the very

tip of the flow divider.

- Internal carotid artery: the caudal, or inferior, extent is defined by the tip of the flow divider. The vessel then ascends in the neck and enters the base of the skull. For the purposes of this protocol, the ultrasound study will be limited to the initial 10 mm of the internal carotid artery.

4. Initial Scan

The purpose of the initial scan is to orient the sonographer to the subject’s carotid anatomy. The sonographer should locate the bifurcation and distinguish which vessel is the internal and which is the external carotid artery. The site of maximal wall thickening in the near or far wall, in the bulb or internal carotid artery should also be identified during the initial scan. Color and pulse-wave Doppler can be used as identification aids. The initial scan is not recorded.

5. Standard Carotid Ultrasound Images

5.1 Summary overview: one videotaped transverse (short-axis) scanning sequence and five videotaped longitudinal images are taken from both the right and the left carotid arteries for each subject. The transverse (short-axis) scanning sequence is a sweep of the carotid from the base of the common up through the bulb into the internal and back down to the base of the common. The purpose of the transverse scanning sequence is to help the reader distinguish between real echoes that represent plaque and spurious echoes that represent artifact. Occasionally when viewing the transverse images the reader is uncertain whether echoes represent real plaque or not. The transverse view serves as the tiebreaker. The first of the five longitudinal images is a pulse-wave Doppler measurement of the peak systolic velocity in the internal carotid. Next is a standard view of the common carotid. The other three longitudinal images are of the internal carotid artery or bulb. The frozen images of the common carotid and the internal or the bulb are videotaped as both frozen images and as part of a cine loop.

5.2 The magnification function is used when obtaining the lateral the common carotid and the internal carotid images. It is turned “ON” immediately after the pulse-wave Doppler measurement and before imaging the common carotid. The magnification must be kept constant throughout the remainder of the scanning on each side. This is because the reader calibrates on the first images and assumes that the magnification remains constant. An unexpected magnification change can result in erroneous measurements.

5.3 In summary, the sonographer first videotapes a transverse (short-axis) scanning sweep of the common through the bulb. He/she then finds the site of peak systolic velocity as measured by pulse-wave Doppler. The common carotid is then imaged. And finally the site of maximal thickening in the internal artery or in the bulb is then imaged from three different angles, anterior, lateral and posterior, to obtain as much circumferential information at this site as possible.

5.3 Collect and videotape the carotid ultrasound images in this order:

IMAGE DESCRIPTION	TO BE VIDEOTAPED
RIGHT SIDE	
<i>Standard zoom-depth = 4 cm</i>	

1. Transverse (Short-axis) sweep	15 second scanning sweep
2. Pulse Wave Doppler ICA	5 seconds of frozen, measured image
3. Common Carotid	5 seconds of frozen image followed by the cine loop
4. Anterior ICA or Bulb	5 seconds of frozen image followed by the cine loop
5. Lateral ICA or Bulb	5 seconds of frozen image followed by the cine loop
6. Posterior ICA or Bulb	5 seconds of frozen image followed by the cine loop
<i>Magnification "OFF"</i>	
LEFT SIDE	
<i>Standard zoom-depth = 4 cm</i>	
1. Transverse (Short-axis) sweep	15 second <i>real time</i> scanning sweep
2. Pulse Wave Doppler ICA	5 seconds of frozen, measured image
<i>Magnification "ON"</i>	
3. Common Carotid Artery	5 seconds of frozen image followed by the cine loop
4. Anterior ICA or Bulb	5 seconds of frozen image followed by the cine loop
5. Lateral ICA or Bulb	5 seconds of frozen image followed by the cine loop
6. Posterior ICA or Bulb	5 seconds of frozen image followed by the cine loop

5.3.1 Image 1: Transverse (Short-Axis) Sweep

The transverse sweep begins at the base of the common carotid and smoothly travels up through the bulb, into the internal and back down to the common. Videotape in the full sweep, up from the common, into the internal and back, for 15 seconds. The transverse scan is done with the ultrasound machine in standard zoom mode at a depth of 4 cm.

5.3.2 Image 2: Pulse-wave Doppler

The critical information is the peak velocity in the internal carotid artery at peak systole at the point of maximum flow acceleration. To locate it a 2-mm Doppler sample gate should first be placed in the center of the distal common carotid artery. The sample gate is then moved from the common through the bulb to the proximal internal. If there is no site of disturbed or turbulent flow, the Doppler measurement should be taken from the first centimeter of the internal carotid artery. The audible signal can be used to facilitate placement of the Doppler sample gate. Angle correction must not exceed 60°. The frozen image of the measurement is videotaped for approximately five seconds. The pulse-wave Doppler measurement is done with the ultrasound machine in standard zoom mode at a depth of 4 cm.

5.3.3 Image 3: Common Carotid Artery

Image 3 is a view of the distal 10-mm of the common carotid artery. Magnification is turned "ON". The carotid bulb is displayed on the left side of the monitor (when facing the screen). If the bulb cannot be identified, but the tip of the flow divider can, this may substitute as the internal landmark on this view. After locating the tip of the flow divider and centering it on the screen, the probe is rotated into the lateral plane and moved downward 2 cm. The probe is then centered on the upper 1 cm of the common carotid. The probe should be angled to obtain the best view of the common carotid artery intima-media thickness (CCA IMT). In general the best angle to achieve the optimal CCA image is 45 degrees.

5.3.4 Images 4, 5 and 6: Internal or Bulb at the site of maximum wall thickness

Images 4, 5 and 6 are images of the internal artery or the bulb. Magnification is left "ON". Images are centered on the site of maximum wall thickness in the internal carotid artery. The objective is to image the segment of the internal that contains the single largest wall abnormality, on either near or far wall, in any one view. THE IMAGES SHOULD NEVER BE CENTERED ON THE COMMON CAROTID EVEN IF IT IS THE SITE OF MAXIMUM DISEASE. The location of the site of maximum wall thickness is determined during the initial scan. It is not the site of average maximum wall thickness, but rather it is the single site of maximum wall thickness in any single view. The sonographer can use any available technique to make this decision including long axis or short axis views, color or pulse-wave Doppler.

5.3.5 The three internal carotid images are taken from three different scanning angles, the anterior-oblique, the lateral and the posterior-oblique. The point of maximal wall thickness should be centered in the middle of the screen for each image. The sonographer should adjust the probe to maximize the lesion and wall interfaces in each projection to not exaggerate the size of the focal plaque by scanning across the vessel on an oblique axis. The Ultrasound Reading Center readers are completely dependent upon the sonographer to provide a legitimate display of a plaque. The philosophy of the URC is that image drives angle rather than angle drives image.

5.3.6 The anterior-oblique, lateral, and posterior-oblique scanning angles are defined as follows:

- Anterior-oblique: the arch on the surface of the neck from the midline (trachea) to 55° to a line drawn from the mid-trachea to the center of the back of the neck.
- Lateral: the arch along the lateral surface of the neck, from 55° to the perpendicular to 100° (hence 45°). The sternomastoid muscle can be palpated beneath this portion of the skin's surface.
- Posterior-oblique: the arch from 100° to the perpendicular to 145°. The probe almost always lies just behind the posterior margin of the sternomastoid muscle.

(Probe angle ranges are defined above to help guide the sonographer. The URC emphasizes optimal images over specific probe angle, however, we don't want three identical views of the ICA.)

5.3.7 If the artery is normal, the study will be centered on the initial 10 mm of the internal carotid artery. The tip of the flow divider is used as the point that identifies the most caudal portion or inferior boundary of the internal carotid artery. It will not always be possible to identify the tip of the flow divider in the frozen image. The priority is to display the wall interfaces of the proximal internal artery, not the tip of the flow divider.

5.3.8 A cine loop of each of the four images described above, one of the common carotid and three of the internal carotid arteries, is captured by selecting the freeze button. The sonographer then cycles through the cine loop images to select the one that best displays the intimal walls. It is videotaped for five seconds. The cine loop is

then set in motion and it is videotaped in its entirety.

6. Criteria for Satisfactory Images

- 6.1 The criteria for optimal B-mode ultrasound image of the carotid arteries is defined as the clear visualization on long axis views of arterial interfaces, internal arterial landmarks, and lesions.
- 6.2 Near wall - arterial wall nearest the probe
Adventitial - medial boundary
Intima - lumen boundary

Far wall - arterial wall furthest from probe
Lumen - intimal boundary
Medial - adventitial boundary
- 6.3 The area of interest will be centered in the middle of the image. Align the probe to show as much of the vessel cephalad and caudad as possible. The sonographer should optimize the visualization of the interfaces by adjusting the gain settings, beam steering, and probe placement.
- 6.4 It is expected that the lumen of a good carotid ultrasound study image will contain a significant amount of artifact. To clearly visualize the intimal linings, the gain setting will need to be set considerably higher than it is for a typical clinical study.
- 6.5 Satisfactory images are properly magnified and the magnification level is the same for all longitudinal views. If a sonographer mistakenly uses an incorrect magnification level or changes it mid-scan, he/she should make a note of the problem in the "Comments" section of the videotape log sheet. Without a note from the sonographer the reader will assume the magnification is the same for all of the images. Thus, the image will be calibrated incorrectly, resulting in erroneous measurements.

7. Imaging Priorities

Some participants will not have vascular lesions or easily visible intimal linings. For these cases the sonographer will prioritize the far wall intimal linings over the near wall intimal linings. For the more difficult cases, where there is some disease, the top priority is to provide a quality image of disease. Disease located in the carotid bulb presents a special case. When a lesion is detected in the bulb and the internal carotid artery is normal, capture images to characterize the bulb disease. Instead of capturing an anterior, a lateral and a posterior view of the internal carotid artery, image the carotid bulb disease from these three perspectives.

- 7.1. Lesion: If there is a focal lesion in either the distal 10 mm of the common or in the proximal 10 mm of the internal, focus on the lesion.
- 7.2. Bulb lesion: If there is a lesion in the bulb and the internal is normal, focus on the bulb lesion.
- 7.3. Far wall interfaces: If there is no disease, the clear visualization of the far wall is more important than that of the near wall

7.4. Near wall interfaces

8. Sonographer Response to a Significant Stenosis – ALERT

- 8.1 Some participants will have significant carotid stenoses, which are discovered, perhaps for the first time, during this examination. An ALERT is defined as an 80% or greater stenosis in the common carotid, the bulb or internal carotid artery. The only criteria used to estimate stenosis is the peak systolic pulse-wave Doppler. An 80% or greater stenosis is indicated by a pulse-wave Doppler measurement of 250 cm/s. Imaging data should **not** be used in arriving at this conclusion; its role is limited to determining the site of the abnormality.
- 8.2 If a sonographer believes a significant vascular abnormality is present, he should double-check this finding by repeating the Doppler measurement. Under no circumstances should this impression be conveyed either directly or indirectly to the participant by the sonographer. *The clinic coordinator should be told immediately after the participant has left the scanning area.* An inquiry is thereby triggered at the field center regarding the presence of relevant symptoms in the participant. It will be determined whether he is under care for the vascular abnormality and if necessary appropriate referrals will be provided
- 8.3 The responsibility of the participant's health care is completely with the field center. Whenever a participant presents with what the sonographer suspects is a problem he is to communicate it immediately to the field center medical personnel. *Do not wait for confirmation from the Ultrasound Reading Center.* The scan will not be reviewed until at least a week later. The readers and the project manager are not qualified to provide any sort of diagnostic report.

9. A Good Carotid Ultrasound Image

- 9.1 Wall boundaries can be demonstrated with high-resolution ultrasound imaging. They appear as two parallel echogenic lines separated by a hypoechoic space in longitudinal views of the carotid arteries. The artery walls are usually best observed in the common carotid artery where the vessel courses parallel to the skin surface and thus presents a target which is at a right angle to the ultrasound beam.
- 9.2 The first echo along the far wall is derived from the lumen-intima interface and the second, normally brighter, echo along the far wall originates from the media-adventitia interface. Between these interfaces lies the media, which appears as an echolucent zone. The distance between the first two lines corresponds to the combined thickness of the intima and media. Because of its collagen content the adventitia is quite echogenic and appears as a bright zone highlighted along its inner margin by the media. However the periadventitia, depending on location, is composed of loose areolar tissue and in most instances is echolucent.

Common Carotid Image**Internal Carotid Image**

- 9.3 It is more difficult to image the interfaces when the near and far walls of the vessels are curvi-linear and not at right angles to the ultrasound beam. In the carotid bulb, where the walls flare, only short wall segments may be seen on any single frame. This same phenomenon is observed in the proximal portion of the internal carotid artery when the walls are not parallel and hence sub-optimally visualized. Other causes for loss of wall interfaces that are not related to scanning technique are the presence of plaques and the presence of fat in the arterial wall. The interfaces can also be seen along the near wall but the lines may be disrupted and the echoes weaker because the ultrasound beam is passing from tissue to fluid. At times, it is impossible to maximize both the near wall and far wall interfaces on the same image. On such occasions the priorities are to first optimize lesion, second the far wall, and third the near wall. *Lesion* refers to the site of maximum single wall thickness, near or far wall.
- 9.4 The very earliest changes that can be seen in the arterial wall indicating the onset of

atherosclerosis are fatty streaks. It is not clear that this finding can be detected by ultrasound. Fat is weakly echogenic and would not be expected to generate a signal. Picano et al. pointed out that the gaps seen in the lumen-intima interface, the "first interface" of the double line echo pattern along the far wall in a study of 75 fresh human aorta specimens might be a reflection of the presence of fatty plaques. However, such breaks may be present for multiple other reasons, including less than optimal scanning technique.

- 9.5 The first definite abnormality that can be detected by B-mode is intimal-medial thickening. As progressive development of atherosclerotic lesions occur, there is an increase in the error of estimating anatomic abnormalities because acoustical shadowing is often present with large lesions. Doppler, on the other hand, becomes progressively more accurate with increasing levels of disease. The Ultrasound Reading Center uses both grayscale imaging information and Doppler to compile a complete lesion profile for each carotid artery. It is important that both portions of the examination be done well.

II. Overview of Carotid Ultrasound Analysis Protocol

1. Preview and Digitization of a Carotid Ultrasound Study

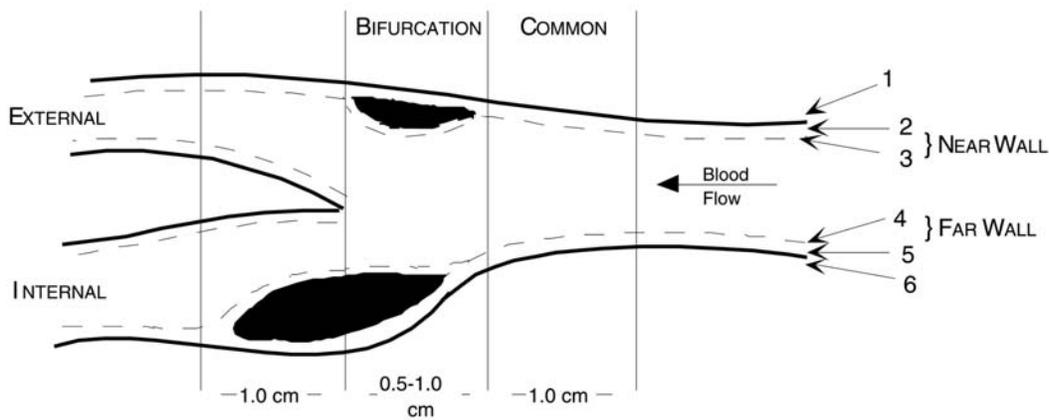
- 1.1 The first thing the reader does when analyzing a carotid ultrasound scan is to read the sonographer's comments on the log sheet and preview the videotape of the entire study. Through the preview process the reader learns of any unusual situations specific to the study, such as images videotaped out of the standard order. Carotid system structures are identified: the common carotid artery, the carotid bulb, the internal carotid artery, and if visible the external carotid artery. The site where the interfaces are most clearly imaged is identified in both the common carotid and non-diseased internal/bulb images. If there is disease in the internal/bulb, the site of the largest lesion is determined. In addition, the pulse wave Doppler measurements are assessed.
- 1.2 After previewing the videotape, the images are digitized. The computer-based ultrasound image analysis system combines the digitization and analysis functions. The program is designed to permit the user to immediately proceed with the analysis after digitizing a study.

2. Calibration

The first step in carotid image analysis is calibration. It is necessary to calibrate each ultrasound image in order to convert the computer unit, pixels, to "real world" units, centimeters. To calibrate an image the reader positions the calibration tool such that it demarcates the 1-cm distance between two calibration marks. Readers assume that the magnification level does not change within a Carotid IMT scan.

3. The Interfaces

- 3.1 The reader's goal is to draw all six of the lines as shown on the each carotid artery image. The figure below demonstrates the anatomy of the carotid artery in the longitudinal perspective and the correspondence of the vessel layers to the line numbers used by the ultrasound image analysis program.



3.2 The correspondence between the line numbers and the interfaces are defined:

Wall	Line Number	Interface
Near Wall	Line 1	Adventitia – Periadventitia
	Line 2	Media – Adventitia
	Line 3	Lumen – Intima
Far Wall	Line 4	Lumen - Intima
	Line 5	Media – Adventitia
	Line 6	Adventitia – Periadventitia

3.3 Measurements from carotid study images are made from the six lines drawn on the image. The lines are drawn with the stylus pen and are approximately 1-cm long. In the common carotid view, the measurements are taken in the 1-cm segment proximal to the bulb. The common carotid view is the only view from which measurements are made in the common carotid artery. It is a relatively simple task for a trained reader to draw the lines on a high quality image of a healthy artery. It is more difficult to analyze diseased arteries.

3.4 The measurements that are made are of the near wall, far wall, vessel width and lumen. The measurement algorithm calculates the distance between pairs of lines and reports the minimum, maximum and average (and standard deviation) values. The vessel structures are defined by the lines pairs as follows:

Vessel Structure	Line Pair
Near Wall	2 – 3
Far Wall	4 – 5
Vessel Width	1 – 6
Lumen	3 – 4

4. Priorities

4.1 The goal is to draw six lines on each carotid image. Due to varying degrees of image quality, which is highly dependent upon sonographer skill and subject anatomy, it will not always be possible to draw all six lines. It is, therefore, necessary to set priorities. The physics of ultrasound make the far wall is easier to image. The far wall interfaces will

typically be more distinct than those of the near wall. Thus the far wall data set will be more complete and therefore be considered most important. Wall thickness is of greater importance than vessel thickness because this is a study of atherosclerosis.

4.2 The carotid ultrasound analysis priorities in order of importance are:

Measurement	Line Pair
Far Wall	4 – 5
Near Wall	2 – 3
Vessel Width	1 – 6

5. Image Quality Scores

5.1 An image quality score is assigned to every analyzed image. This score is based on the quality of the image and the reader's confidence that the lines drawn represent the true interfaces. Although the score is subjective, the following is a loose guideline for scoring the common and non-diseased internal carotid arteries. Note that the criterion used to assign a quality score to a diseased vessel differs slightly from that of a healthy vessel

5.2 Common & Healthy Internal Arteries

- Unacceptable: only one or no lines drawn.
- Poor: only two lines, one pair, drawn, giving a lumen measurement (i.e.. 3&4, or 2&5) or vessel width (i.e.. 1&6)
- Acceptable: only two or three lines on either near or far wall with an opposing line (lumen measurement).
- Very Good: all six lines drawn, all measurements made.
- Excellent: all six lines clearly visualized and easily drawn, with good gain and positioning.

5.3 Atherosclerotic Carotid Arteries

- Unacceptable: only one or no lines drawn.
- Poor: only two lines, one pair, drawn, yielding a lumen measurement (i.e.. 3&4, or 2&5) or vessel width (i.e.. 1&6) or can draw lines on wall opposing the lesion but no lesion measurement can be made.
- Acceptable: four to five lines drawn, lesion traceable but not clearly defined, with at least one posing line.
- Very good: all six lines drawn, lesion clearly defined, with opposing line.
- Excellent: lesion clearly defined, with opposing wall measurement, lines are easily drawn and the image has good gain and positioning.

6. Subjective Assessment

In addition to obtaining measurements of wall thickness, vessel width and lumen the reader characterizes the internal/bulb disease. Lesions in the common carotid artery are not characterized. Assessments are made of the surface, morphology, percent stenosis, location and density. Each characteristic and the categories the Ultrasound Reading Center uses to qualify them are briefly described.

6.1 Surface

The surface of the artery or lesion is categorized as one of the following:

- Smooth - no lesion is present in the artery and the lumen-intima surfaces (lines 3 & 4) are free of irregularities. If there is a lesion present then this category is applicable when the lumen-intima surface (lines 3 & or 4) of the lesion is smooth and free of irregularities.
- Mildly irregular - the lesion has minute surface irregularities.
- Markedly irregular - the lesion has noticeable surface irregularities. This can be best described by saying that the surface is “bumpy” but without a prominent pit or crater.
- Ulcerated - the lesion surface has prominent ulceration, pit(s) or crater(s) that is at least 1mm in depth and is at least 1mm in width. The “back wall” of the lesion, defined as the media-adventitia interface, can be clearly seen at the point of ulceration.
- Can’t tell - the surface characteristics of the artery/plaque cannot be determined due to insufficient information (images are unclear or missing).

6.2 Morphology

The morphology, the form and structural characteristics, of the lesion are categorized as:

- No lesion - no lesion present in either the internal or bulb
- Homogeneous - a lesion that demonstrates an echogenicity that does not vary across its width and thickness.
- Heterogeneous - a lesion with have a mixture of lucent and echogenic zones.
- Can’t tell - the morphology of the plaque cannot be determined due to insufficient information (images are unclear or missing).

6.3 Percent Stenosis

- 6.3.1 All available information is used to determine the percent stenosis. Information sources include digitized images, cine loops and Doppler values. The degree of stenosis is crudely estimated from the images and cine loop as a ratio of artery width. The Doppler value (velocity) which is known to be a more reliable measure is incorporated to refine the estimate.
- 6.3.2 It may not be evident from the images the images that there is a 100% occlusion. In some cases, there may not be a Doppler value because the sonographer was unable to produce a Doppler signal. When Doppler values are nonexistent and the images are unclear, the reader must rely on comments from the sonographer to determine the degree of stenosis.
- 6.3.3 In all instances where the PW Doppler is greater than 1.5 m/s, it is used to categorize the percent stenosis according to the following table.

% Stenosis	Doppler Values
0-49%	<1.5 m/sec

50-74%	> 1.5 m/sec but < 2.5 m/sec
75% -99%	> 2.5 m/sec
100% (occlusion)	0.0 m/sec

6.3.4 The categories from which the percent stenosis must be chosen are more specific:

- Normal - no focal lesion present in either the internal or bulb.
- 1–24% - Doppler values that are <1.5 m/sec and reader's estimate based on the three images of the internal showing that the lesion occupies 1-24% of the vessel width.
- 24–49% - Doppler values that are <1.5 m/sec and reader's estimate based on the three images of the internal showing that the lesion occupies 25-49% of the vessel width.
- 50–74% - Doppler values that are \geq 1.5 m/sec but <2.5 m/sec.
- 75–99% - Doppler values that are \geq 2.5 m/sec.
- 100% - Doppler values that are 0.0 m/sec.
- Bad image/NI (normal) Doppler - the images are so unclear that % stenosis can't be estimated by looking at them but Doppler values fall within normal range, < 1.5 m/sec.
- Can't tell – the percent stenosis cannot be determined due to insufficient information (images are unclear or missing).

6.4 Location

The plaque location describes the site of the lesion. The categories are:

- No lesion - no lesion is present in either the internal or bulb
- Can't tell - cannot determine the location of the plaque cannot be determined due to insufficient information (images are unclear or missing).
- Internal - the lesion is located in the internal carotid
- Bulb - the lesion is located in the bulb
- Can't tell - cannot determine the location of the plaque cannot be determined due to insufficient information (images are unclear or missing).

6.5 Density

The lesion density qualifies the compactness of the plaque. The categories are:

- No lesion - no lesion is present in either the internal or bulb
- Hypodense - a plaque that appears darker than the surrounding tissue.
- Isodense - a plaque that has same level of brightness as the surrounding tissue.
- Hyperdense - the plaque appears brighter than the surrounding tissue and some of the tissue beneath the plaque is shadowed.
- Calcified - the plaque appears much brighter than the surrounding tissue and all of the tissue beneath the plaque is shadowed.
- Can't tell - cannot determine the density of the plaque cannot be determined due to insufficient information (images are unclear or missing).

III. Data Transmission

1. Videotape

- 1.1 Carotid ultrasound images are transmitted to the Ultrasound Reading Center on videotape. The ultrasound machines are equipped with Super VHS videocassette recorders. **Super VHS tape must be used.** It can be purchased at Radio Shack or through the field center's vascular lab or radiology department. Standard, non-Super VHS, tape is not acceptable.
- 1.2 When the image displayed on the ultrasound machine monitor is optimal, "Freeze It". Page through the cine loop of saved images and select the image that best demonstrates the intimal linings. Videotape five seconds of the frozen image, start the cine loop on *loop preview* and videotape it in its entirety. It is important to wait for the mechanical delay of the VCR mechanism to engage when timing recordings. A few seconds of over-recording is infinitely more desirable than a non-recording. If at any time during an examination a better image than that previously recorded is obtained, **do not** erase or tape over the previous recording. Add the new images to the videotape and make a note in the *Comments* section of the log sheet to inform the reader.
- 1.3 At the Ultrasound Reading Center the readers digitize (computerize) and analyze the images from the carotid ultrasound videotapes. The computer-based system to accomplish these tasks was developed by the Ultrasound Reading Center.

2. Labels and Log Sheets

- 2.1 At the training session the sonographers will be given sets of labels and master copies of a log sheet. These items make it possible to track and organize the numerous videotapes that arrive at the Ultrasound Reading Center. It is of utmost importance that the sonographers carefully label the videotapes and the corresponding log sheets. The labels contain the study name, field center name, exam number and tape number. See the label illustrated in the upper right corner of the sample log sheet below. The log sheet enables the reader to quickly locate each study on the individual videotapes and to obtain pertinent information regarding particular cases.
- 2.2 The sonographer completes the appropriate sections of the log sheet for each subject he scans. The necessary items are relatively obvious.
 - **Scan Date** – the date of the carotid scan
 - **Subject ID** – the ID number assigned to the participant (no names please)
 - **Plaque** – if there is a plaque(s) circle the location(s). *RB = right bulb, RICA = right internal carotid, LB = left bulb, LICA = left internal carotid*
 - **VCR Start** – the VCR counter/timer that indicates where the particular study is located on the tape
 - **Sonographer ID** – the MESA Technician ID assigned to the sonographer

- **Comments** – the place for the sonographer to tell the reader about the case
- **Sample of Typical Videotape Log Sheet Entries**

	Scan Date	Subject ID	Plaque*	VCR Start Time	Sonog. ID	Comments
1.	8/2/2000	4125993	RB RICA LB LICA	0:00	456	Good study, easy subject
2.	8/2/2000	4328713	RB RICA LB LICA	5:34	413	Lesion in left bulb

2.3 The value of sonographer - reader communication cannot be overemphasized. **Use the Comment section of the log sheet to communicate** any abnormalities, problems or difficulties encountered. For example, if a scan is canceled or if there are images missing or out of order, **write it down**. The readers base all of their decisions on the information the sonographer provides. It is not easy for a reader who is not medically trained, to distinguish plaque from artifact. Efforts by a sonographer to assist the reader in the analysis process are greatly appreciated.

3. Shipping Videotapes and Log Sheets

3.1 The videotapes are to be sent monthly to the Ultrasound Reading Center. The package should include the videotape, log sheet and a hard copy packing slip. A copy of the log sheet should be made prior to packaging it with the videotape. This will enable the sonographer to maintain a record of his scanned subjects. Mailing labels will be provided at the training session, the delivery address is:

Daniel H O'Leary, MD
 Ultrasound Reading Center
 25 Stuart Street, 2nd Floor
 Boston, MA 02116

3.2 The Ultrasound Reading Center insists that a delivery service with tracking capabilities be used. This will reduce the possibility of lost data. United Parcel Service ground delivery has proven to be both satisfactory and economical.

4. Data Transmission to the Coordinating Center

The carotid ultrasound analysis program output is an MS Access database that contains the calculated measurements and subjective scores for each analyzed participant study. The

calculated measurements include values for the far wall, near wall, vessel width and lumen. The subjective scores that characterize the surface of the artery or lesion include assessments of the morphology, the percent stenosis, the location of the lesion, and the morphology. Also included with each participant data record are items such as sonographer ID, scan date, reader ID, read date and pulse-wave Doppler measurements. The data files will be appropriately formatted and sent on a regular basis to the Coordinating Center on either floppy disk or via e-mail.

IV. Sonographer Quality Control

Following initial certification sonographers will be evaluated continuously to monitor and assure quality control. Since reliable data is the essential element in studies that seek to monitor arterial change over time, great effort must be expended upon all aspects of quality control. The Ultrasound Reading Center has found it best to minimize the number of sonographers and to maintain the same reader thereby limiting problems prospectively rather than retrospectively adjusting data.

1. Field Center Sonographers

- 1.1 If possible, two sonographers should perform all examinations at each field center on the same ultrasound machine. Ideally, these individuals would be an experienced vascular sonographer, preferably RVT certified or equivalent. If not the sonographers should have experience in an ICAVL accredited laboratory. The sonographers do not necessarily have to have experience performing research protocols. The Ultrasound Reading Center Carotid IMT protocol is such that it can be quickly taught to someone who routinely performs high quality clinical ultrasound examinations.
- 1.2 The Ultrasound Reading Center assumes that at most of the six field centers there will be two sonographers performing all of the carotid studies. Together with the Coordinating Center the Ultrasound Reading Center will assess inter-sonographer variation. Intra-sonographer variability measurements, which will involve the replicate scanning of approximately twenty subjects by the same sonographer on two different occasions, are highly recommended.

2. Ultrasound Reading Center – Sonographer Communication

- 2.1 Experience with prior studies demonstrated the need for rapid and possibly frequent communication between the Ultrasound Reading Center and the sonographers. The project manager and readers will periodically call each of the field centers. Problems encountered by the sonographers and sonographer performance will be discussed and the importance of sonographer compliance with the protocol will be stressed. Examples of the kind of problems that required immediate attention in the past include the apparent non-recording of images on videotape and discordance between Doppler and imaging data. Sonographers are asked to keep notes when problems are recognized so that they may provide direction to the Ultrasound Reading Center when necessary. Sonographers are encouraged to call the project manager to discuss problems or questions as they arise. If necessary, a member of the Ultrasound Reading Center staff will visit field centers. The execution of the carotid protocol will be observed and equipment function evaluated. The purpose of the visits is to be a direct assistance to the sonographers and the field centers.

- 2.2 If a sonographer submits unacceptable or un-analyzable studies, the reader will alert the project manager. An unacceptable study is one from which very little or no data can be obtained. Unusable studies can be the result of poor images or failure to follow the protocol (incorrect images). Poor performance by a sonographer will result in a telephone call to the sonographer and the clinic coordinator from the project manager. Severe cases will warrant a memo to the field center and Ultrasound Reading Center Director documenting the persistent poor quality.
- 2.3 Throughout the exam period, the sonographer will receive sets of *Reader Comments*. The *Reader Comments* are written by the reader at the time of study analysis. Typically, the comments contain constructive criticisms and suggestions for improving the study images, i.e., gain adjustments, check the Doppler gate angle or position. More often than not, the *Reader Comments* include such positive feedback as “Great images, thanks”.

3. Ultrasound Reading Center Report on Sonographer Performance

- 3.1 In addition to the *Reader Comments*, sonographers will receive feedback from the Ultrasound Reading Center in the form of a sonographer performance quality control report. The maintenance of high performance requires immediate and direct feedback. The reports, which will be published bi-monthly, quantitatively track each sonographer’s performance. Copies of the reports will be distributed to the field centers, the Ultrasound Reading Center PI and the coordinating center. Cumulative summaries will be published periodically to serve as an ongoing benchmark against which to judge individual results.
- 3.2 The report will include the number of studies, average image quality scores and percentages of possible lines drawn and Doppler velocity measurements tabulated for all of the field centers, and then by each individual field center, by sonographer and reader. A one page summary report that highlights the overall study results as well as the best and the worst field center and sonographers will accompany each report. Consistently poor performance by a sonographer will initiate a call of encouragement and investigation by the project manager. Continued poor performance will result in the sonographer being re-trained or replaced.

4. Performance Standards During Examination Period

A baseline quality control score, derived from the score assigned to each image and the number of lines drawn for each image, will be established after analysis of pilot and pre-pilot data, below which sonographer performance will be considered unacceptable if maintained for three consecutive weeks. The readers will be monitored to ensure uniform scoring throughout the exam period. Additional training may be required of sonographers and/or readers in the event of any significant temporal drift in accuracy or precision of sonography study performance and/or reading. In terms of the analysis of the actual measurements produced, we will subscribe to those suggested by echocardiography - namely, a goal of mean variability of 10%, with a cut-off point of 15% acceptable variability.

5. Replacement or Retraining of a Sonographer

If a sonographer resigns or is replaced due to poor performance, a replacement must be trained and certified prior to performing scans on research subjects. The Ultrasound Reading Center must conduct all sonographer training. A new sonographer can be introduced to the protocol by a certified sonographer, but the new sonographer will not be certified to conduct participant

exams until he attends an Ultrasound Reading Center training session. Contact the Ultrasound Reading Center Project Manager immediately when the need to train a new sonographer becomes apparent. Due to the Ultrasound Reading Center's involvement in numerous national multi-center research studies, it may be possible to identify a local sonographer who is certified to execute the carotid ultrasound protocol to assist with the training.

6. Within Sonographer Variability Assessment

Intra-sonographer variability during the examination period will be assessed from the data obtained by repeating complete carotid ultrasound studies on a number of subjects. Each sonographer will complete two scans on a subset of approximately twenty participants distributed throughout the exam time period. The videotapes will be sent to the Ultrasound Reading Center for analysis coded with random number assigned by the Coordinating Center. The reader will be blinded – he will not know that a study he is analyzing is a standard or a sonographer intra-variability quality control study. Subjects for the replicate studies will be recruited during the exit interview. They will not be recruited by the sonographer. The repeat scan should be scheduled to occur no sooner than one week and no later than one month after the initial scan. On the return visits, each volunteer will be scanned once by each sonographer.

7. Achieving and Maintaining Quality Ultrasound Data

7.1 Quality carotid ultrasound data begins with the sonographer. The sonographer produces the images from which the readers extract the data. A poor set of images results in a poor and sometimes incomplete set of data. The readers are entirely dependent upon the sonographers to provide clear images and to explain any conditions, anatomical or procedural that vary from the norm.

7.2 To maintain high quality ultrasound data:

7.2.1 Acquire nothing but clean images that clearly display the intimal linings and the lesions. The intimal linings and the lesions are the top priority, they are what is measured by the reader. It is expected that in order to clearly display the intimal linings the lumen will contain more artifact than the typical clinical study. Review Section 2.4, "Priorities."

7.2.2 Videotape the images in the order stated by the protocol. A study that is videotaped out of order is not as easy for the readers to analyze.

- Right transverse sweep of common and internal
- Right pulse wave Doppler measurement
- Right common carotid
- Right internal/bulb anterior oblique
- Right internal/bulb lateral oblique
- Right posterior/bulb oblique
- Left transverse sweep of common and internal
- Left pulse wave Doppler measurement
- Left common carotid
- Left internal/bulb anterior oblique
- Left internal/bulb lateral oblique
- Left internal/bulb posterior oblique

- 7.2.3 Annotate the images with the following information:
- MESA
 - Subject ID
 - Subject Acrostic
 - Sonographer ID
 - Image acronyms: RPW, RCC, RAO, RLO, RPO, LPW, LCC, LAO, LLO, LPO
- 7.2.4 Magnify the views of the CCA and ICA. Keep the magnification level constant throughout the scan.
- 7.2.5 Annotate the images and use arrows to distinguish the vessels when the images are atypical. Don't force the reader to guess which vessel is the internal and which is the external carotid.
- 7.2.6 **Use the Comment section of the log sheet to communicate** any abnormalities, problems or difficulties encountered. For example, if a scan is canceled or if there are images missing or out of order, **write it down**. The importance of this communication cannot be stressed enough. The readers are not medically trained and must base their decisions on the information the sonographer provides.
- 7.2.7 Videotape the frozen images for 5-10 seconds. Any less than 5 seconds makes the image difficult to capture and any longer than 10 seconds reduces the reader's efficiency.
- 7.2.8 Videotape the complete cine loops. Cine loops enable the reader to discriminate between true anatomy and artifact.
- 7.2.9 Take the time to verify subject ID's. Check the ID typed into the ultrasound machine and written on the log sheet. If an incorrect ID is videotaped because the wrong ID was typed into the ultrasound machine, tell the Ultrasound Reading Center about it with a note in the Comments section of the log sheet. After the videotape leaves the field center sorting out erroneous ID's can be a very difficult, if not impossible task. Watch for transpositions.