Manual 7

Blood Collection

The National Heart, Lung, and Blood Institute
of the National Institutes of Health

THE UNIVERSITY OF NORTH CAROLINA
AT
CHAPEL HILL

Collaborative Studies Coordinating Center
Department of Biostatistics
School of Public Health

CB# 8030, Suite 203, NCNB Plaza
The University of North Carolina at Chapel Hill
Chapel Hill, N.C. 27514-4145

M E M O R A N D U M

TO: ARIC Principal Investigators, Study and Data Coordinators

FROM: ARIC Coordinating Center

DATE: October 20, 1988

SUBJECT: Updates to ARIC Manual 7; Version 1.1 approved July, 1988

==========

Enclosed is a new printing of Manual 7. It is labelled Version 1.1 because none of the changes (except for the description of inversion of tubes, see page 15) were substantive in nature. Version numbers are changed to the next whole number when they contain major procedural changes. In this case, however, an entire new document was
printed since there were small changes on almost half of the pages.

The following changes (typed in CAPS) have been made to Version 1.0.

Page 2, Section 1. Spelling corrections.

Page 2, Section 2.3. RECERTIFICATION TAKES PLACE ANNUALLY AND IS AUTHORIZED BY THE CENTRAL HEMOSTASIS LABORATORY. (Correction)

Page 3, Line 3. ... Appendix II. (Correction)

Page 5, Line 3. THE SPECIAL ANTICOAGULANT MATERIAL IS PROVIDED BY THE CENTRAL HEMOSTASIS LABORATORY ON A QUARTERLY BASIS. (Correction)

Page 15, Section 3.5., Line 1. NOTE DATE AND TIME OF VENIPUNCTURE ON VENIPUNCTURE FORM. (Correction)

Page 15, Line 2., TO INVERT TUBES, HOLD THE TUBE HORIZONTAL TO THE FLOOR. SLOWLY TIP THE BUTT END DOWN WHILE WATCHING THE AIR BUBBLE RISE TO THE STOPPER. (1st INVERSION) WHEN THE BUBBLE REACHES THE STOPPER, THE TUBE SHOULD BE AT APPROXIMATELY A 22 DEGREE ANGLE TO THE FLOOR WITH THE CENTER OF THE TUBE AT THE FULCRUM. NOW, LOWER THE STOPPER END WHILE WATCHING THE BUBBLE FLOAT TO THE BUTT. AGAIN, THE TUBE SHOULD BE AT A 22 DEGREE ANGLE TO THE FLOOR WITH THE CENTER OF THE TUBE AT THE FULCRUM. (2nd INVERSION) LOWER THE BUTT END AGAIN WHEN THE BUBBLE REACHES THE BUTT. (3rd INVERSION) INVERT EACH TUBE EIGHT TIMES. EIGHT INVERSIONS SHOULD TAKE 13-15 SECONDS. (Correction)

ARIC032:man7new.mmo:10-18-88:ccp

Page 15, Line 12. DRAW TUBE #1 (13 ml RED AND GRAY TOP). GENTLY INVERT 8 TIMES. (Add) NOTE: ALL REFERENCES OF 11 ml CHEMISTRY TUBE #1 HAVE BEEN CHANGED TO 13 ml RED AND GRAY TOP.

Page 17., Figure 3., Sample Processing Flow Sheet time ranges changed.

Page 21, Section 4.3.2, #8. FILL ONE YELLOW MICROSAMPLE TUBE TO THE TOP. THEN, DIVIDE THE REMAINING PLASMA EVENLY BETWEEN THE OTHER TWO YELLOW ALIQUOT TUBES. (Addition)

Page 21, Section 4.3.2, #9. USING THE AUTOMATIC PIPET, REMOVE EXACTLY 0.5 ml OF PLASMA FROM ONE OF THE YELLOW ALIQUOT TUBES AND PLACE IT ON THE TOP OF THE CENTRICON UNIT. (Correction)

Page 22, Section 4.4. Paragraph 3. IF TUBES 1 AND 3 CANNOT BE SPUN IMMEDIATELY AFTER THE 30 MINUTE TIMER GOES OFF, REMOVE THEM FROM THE HEAT HOCK AND PUT THEM INTO THE MELTING ICE BATH. (Correction)

Page 23, Section 4.6, Line 4. SAMPLES MUST BE PLACED INTO THE FREEZER WITHIN 90 MINUTES FROM VENIPUNCTURE TIME. (Addition)

Page 24, Section 5.1.1 and 5.1.2, Line 4. PLACE THE BAG IN THE CENTRAL CHEMISTRY LABORATORY ... (Correction)

Page 29, Section 6.1. Last line. APPENDIX VI. (Correction)

Page A-2, Supplies provided by the Central Laboratories. 5 ml disposable syringes deleted.

Page A-2, Supplies to be provided by the field centers. (Catalog numbers added.)

Page A-2, Equipment purchased and maintained by the field centers. AEROSOL COVERS added.

Page A-15, Blood Drawing Processing Certification or Recertification. Format revised. Section C. # 11 (added) Tourniquet released as soon as flow starts in last tube."

Page A-18, ARIC QUALITY CONTROL PHANTOM PARTICIPANT AND NON-PARTICIPANT ID FORM, added.

ARIC PROTOCOL

Manual 7

Blood Collection and Processing

For Copies, Please Contact
ARIC Coordinating Center
Department of Biostatistics
CB #8030, Suite 203, NCNB Plaza
The University of North Carolina
Chapel Hill, NC 27514-4145

Version 1.0: June 1987

Version 1.1: July, 1988

FOREWORD

This manual entitled, Blood Collection and Processing, is one of a series of protocols and manuals of operation for the Atherosclerosis Risk in Communities (ARIC) Study. The complexity of the ARIC Study requires that a sizeable number of procedures be described, thus this rather extensive set of materials has been organized into the set of manuals listed below. Manual 1 provides the background, organization, and general objectives of the ARIC Study. Manuals 2 and 3 describe the operation of the Cohort and Surveillance Components of the study. Detailed Manuals of Operation for specific procedures, including reading centers and central laboratories, make up Manuals 4 through 11. Manual 12 on Quality Assurance contains a general description of the study's approach to quality assurance as well as specific protocols for each of the study procedures.

ARIC Study Protocols and Manuals of Operation

MANUAL	TITLE
1	General Description and Study Management
2	Cohort Component Procedures
3	Surveillance Component Procedures
4	Pulmonary Function Assessment
5	Electrocardiography
6	Ultrasound Assessment
7	Blood Collection and Processing
8	Lipid and Lipoprotein Determinations
9	Hemostasis Determinations
10	Clinical Chemistry Determinations
11	Sitting Blood Pressure and Postural Changes in Blood Pressure and Heart Rate
12	Quality Assurance and Quality Control

Manual 7. Blood Collection and Processing

TABLE OF CONTENTS

1. PURPOSE

The Atherosclerosis Risk in Communities study (ARIC) is a multidisciplinary study designed to measure risk factors for atherosclerosis and heart disease. It is a prospective study which will sample a large, normal population and then follow it for an extended period of time. In the course of the study, it is expected a portion of the participants will develop atherosclerosis and heart diseases or other related problems. The stored blood samples and other information on these cases and matched (age, sex and other relevant criteria) controls will be extensively analyzed to determine whether there are statistically significant differences between the two groups in any of the study parameters which may have predisposed the affected participants to heart disease, stroke and vascular disease.

Nationally there are four field centers involved. Each of these field centers collects blood and processes it for shipment to three central laboratories which each perform specialized tests on the blood samples. The central laboratories include: a Central Chemistry laboratory at University of Minnesota in Minneapolis; a Central Lipid laboratory at Baylor College of Medicine in Houston and a Central Hemostasis Laboratory at University of Texas Medical School in Houston.

The Central Chemistry laboratory performs general blood chemistry tests on the participant serum samples. The Central Lipid laboratory evaluates the lipid profiles of the participant including general tests for lipid content as well as other more specialized lipoprotein profiles. The Central Hemostasis laboratory evaluates various blood coagulation factors including tests for platelet activation and natural inhibitors of blood clotting as well as more general tests of the hemostatic system. In addition to the central laboratories, each of the field centers has its own hematology laboratory which evaluates hematological parameters.

The foundation on which all of these tests is based is the blood samples that are collected and processed by the technologists at each of the field centers. Probably the most important step (and potentially the most variable) is the collection and processing of the blood samples. Laboratory tests can be repeated, but if the blood sample itself is not correctly drawn and processed, the laboratory results may be precise but may not be valid. In a study such as ARIC which may involve more than 40,000 samples over an extended period of at least 7 years, even a small amount of variability can add up to a statistically significant difference. It is important that the study measures true differences between cases and controls rather than variability in blood drawing procedures. The ARIC study depends on the field center technologists who perform the blood drawing and sample processing. It is important that these people be not only well trained and competent at drawing and processing the blood, but are also willing to take pride and responsibility in their work.

2. PREPARATION

2.1 General

The ARIC study involves the collection of approximately 57 ml of blood from participants. Since the study depends on the voluntary return of participants over an extended period of time, every effort must be made to make the entire procedure as easy and painless as possible both for the participants and the technologists. The technologist must remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The best way to achieve this is for the technologists to be thoroughly knowledgeable about all aspects of the procedures, including: participant contact, the set up of the tubes, the order of tube collection, the handling of each tube, the final destination and purpose of each sample and the potential causes of variability with each tube.

2.2 Participant Contact

It should be stressed that this study requires the voluntary cooperation of the participants over a period of at least 6 years. These people are donating both time and blood 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. A total of 8 tubes of blood of various sizes is collected. The smallest tubes contain less than a teaspoon (3 ml), while the largest tube contains slightly over 2 teaspoons (11 ml) of blood. Any participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is less than 4 tablespoons although it may look like more. The technologist may also assure participants that they donate 9 times as much blood (450 ml) when they donate a pint of blood.

2.3 Staff Certification Requirements

The blood drawing and preparation are performed by two certified ARIC technicians. The technologists complete a standardized training course taught by supervisory personnel from the Central Hemostasis Laboratory. Each technician must complete the training and pass both written and practical exams before becoming ARIC certified. Recertification takes place annually and is authorized by the Hemostasis Laboratory. The technicians and the clerk should be properly attired in a white lab coat. Any long hair should be tied back.

2.4 Blood Collecting Trays and Tubes

At the beginning of the day, two trays are prepared for each participant. One tray holds the vacutainer tubes used in the blood collection. The other tray holds the various colored plastic tubes which contain the final serum and plasma aliquots which are frozen and sent to the central

laboratories for analysis. Both of these sets of tubes should be pre-labeled with the appropriate code numbers for the participant of that day. A list of equipment, suppliers, and vendors is provided in Appendix II.

2.4.1 Blood Collection Tray

First, the technicians are trained to organize and prepare the blood collection tray. The tray itself should be made of hard plastic which is unbreakable and can be easily cleaned. The tray has ten individual compartments which are filled with the following supplies as illustrated in Figure 1.

- A test tube rack to hold the eight blood collection tubes which are drawn from each participant. These tubes are described in detail in the next section.
- Three sterile, disposable 21 gauge butterfly needles.
- A plastic vacutainer tube guide.
- Three vacutainer Luer slip adaptors to connect the butterfly.
- Sterile alcohol swabs.
- Gauze sponges.
- A tourniquet.
- Bandages ("Band Aids").
- A styrofoam ice water bath filled with ice and water approximately 10 minutes before blood drawing.
- A stopwatch.

2.4.2 Blood Collection Tubes

About 57 ml of blood are drawn from each participant using eight vauctainer tubes. Samples from these eight blood collection tubes are used in approximately 40 different biochemical and hematological assays. It is important that the technicians know more than just the arrangement of the blood collection tubes and the sequence of tube collection. They should also be familiar with the purpose of each tube, the type of anticoagulant in each tube and possible sources of error in the handling of each tube. These tubes are organized in the test rack in the following sequence:

Tube #1 is an 13 ml red and gray stoppered tube that is to be filled with 11 ml of blood. This tube does not contain anticoagulant. After drawing, the blood is allowed to clot at room temperature for 30 mintues. After 30 minutes, the tube is centrifuged and the serum is removed, frozen and stored for weekly shipment to the Central Chemistry lab in Minnesota. One potential problem with the processing of this tube is that one of the tests it is used for is a blood glucose determination. If the serum is allowed to remain in contact with the red cells for longer than 30 minutes, the serum glucose levels can be falsely decreased.

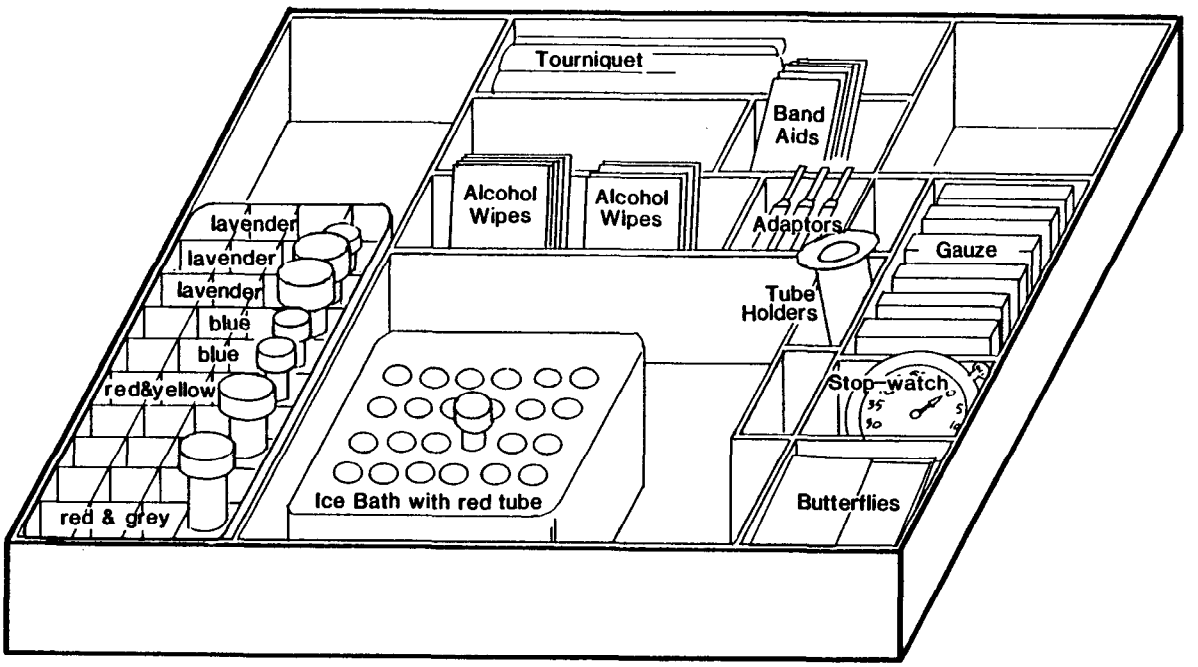


Figure 1. Blood Sample Collection Tray

Tube #2 is a 7 ml siliconized red stoppered tube which is injected with 0.7 ml of special anticoagulant by the field center technologists. The special anticoagulant mixture is provided by the Central Hemostasis laboratory on a quarterly basis. The mixture is kept at 4° C at all times. Tube #2 is filled with 6.3 ml of blood. The plasma from this tube is sent to the Central Hemostasis lab for measurement of unstimulated levels of platelet and coagulation factors which may be present in the circulating blood. Since the plasma in this tube is used to measure unstimulated levels of these factors, it is extremely important that any potential in vitro activation be avoided. Less than flawless venipuncture can stimulate both the platelets and coagulation systems and even a small amount of activation can cause a large false positive elevation in these factors. For the same reasons, this tube is also chilled in an ice water bath for at least 10 minutes prior to sample collection, then immediately mixed after collection and returned to the ice bath. After centrifuging, the sample is filtered immediately to remove any remaining platelets. Until the plasma is filtered, the remaining platelets can still cause a false elevation of some of the factors. After the blood is filtered, a portion of it is recentrifuged over a special molecular filter to remove molecules that could break down in the freezing process and cause false levels of other coagulation factors. The technologist should be aware of all of these potential pitfalls in the collection and processing of this tube in particular.

Tube #3 is a 7 ml red and yellow stoppered tube that is filled with 7 ml of blood. This tube contains silica particles to activate the clotting system and the platelets. The serum from this tube is sent to the Central Hemostasis lab for the measurement of various activated platelet and coagulation factors. It is critical that this tube be filled with 7 ml of blood, mixed well and transferred to a 37° C heat block immediately after collection. A delay of even a few minutes at room temperature can cause an irreversible decrease in the activation of these factors.

Tubes #4 and #5 are 4.5 ml blue stoppered tubes contain 0.5 ml of 3.8% sodium citrate as an anticoagulant. Each tube is filled with 4.5 ml of blood. The plasma from these tubes is sent to the Central Hemostasis lab for assay of some of the more generally measured coagulation factors and inhibitors. Since some of these factors are unstable, it is important that these tubes be kept cold in a refrigerated centrifuge until they are aliquotted into their respective tubes. Of all of the tubes which are immediately centrifuged, these tubes are aliquotted last and the rack containing these aliquots are transferred to the refrigerator immediately after they are aliquotted.

Tubes #6 and #7 ml lavender stoppered tubes contain the anticoagulant, EDTA. After being drawn, place the tubes in the ice water bath until you have completed drawing the last participant. Tubes are then immediately placed in the centrifuge for a 10 minute spin. The plasma from these tubes is sent to the Central Lipid lab at Baylor College of Medicine in Houston.

Tube #8 is a small 3 ml lavender stoppered tube contains the anticoagulant EDTA. The tube remains at room temperature until the last participant is drawn. A hematology requisition is then completed, attached to the tube and the sample is stored in the refrigerator until being sent to the hematology lab at each field center. This tube is used to assay for a standardized set of hematology tests.

2.5 Blood Collection Tubes: Labeling and Set-up

Eight tubes are drawn in the following sequence:

Tube #1: 13 ml red and gray stoppered tube
Tube #2: 7 ml siliconized red stoppered tube
Tube #3: 7 ml red and yellow stoppered tube
Tube #4: 4.5 ml blue stoppered tube
Tube #5: 4.5 ml blue stoppered tube
Tube #6: 10 ml lavender stoppered tube
Tube #7: 10 ml lavender stoppered tube
Tube #8: 3 ml lavender stoppered tube

Strips of prenumbered adhesive labels for each vacutainer tube, the Centricon® tube, each plastic microsample storage tube, the specimen bags and packing list are attached to the initial data collection forms. Apply labels to the blood collection tubes for each participant just prior to blood collection. Arrange the set of tubes in a test tube rack, one rack per participant (Figure 1). Check the identifying information on the form and label to make sure that the specimen belongs to the participant identified on the labels. The labeling of tubes for aliquots of specimens to be sent to the central laboratories can be done by a clerk working side by side with the venipuncture technician. The chance of mislabeling is minimized when only one subject's specimens are handled at a time.

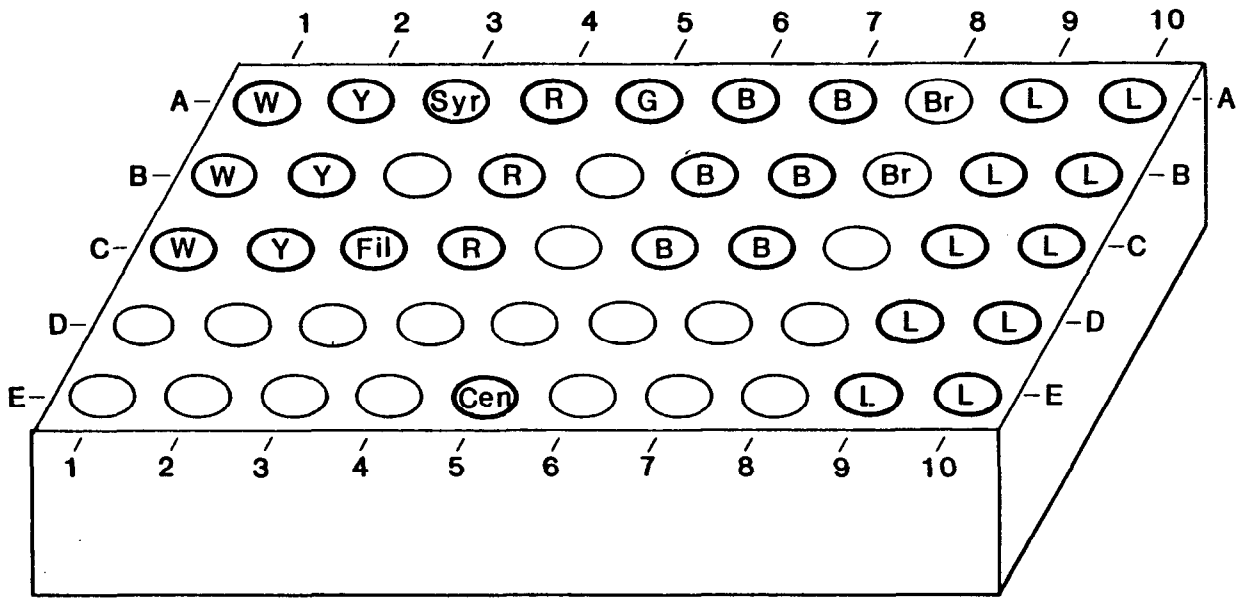
A number of ARIC participants are randomly selected by the Coordinating Center and asked to donate duplicate samples for analysis. Specifically duplicate samples are obtained from approximately 7% of the participants for each of the three central laboratories. Duplicate samples are assigned their own ID number and shipped to the designated central laboratory one week later.

2.6 Sample Aliquot Tubes: Labeling and Set-up

The technoloigst prepares a tray of the plastic freezer tubes which contains the final samples to be shipped to the central labs for each participant. These sample tubes are color coded so each type of sample tube or preparation has a correspondingly colored freezer tube. The plastic tubes are positioned in separate rows in an order corresponding to the order of sample drawing. The technicians should be trained to organize the tray for the sample processing and aliquotting as follows:

2.6.1 Sample Tray

The tray itself should be a flexible sponge test tube rack which will fit tubes from 10-16 mm in diameter (see Figure 2). The tray has 5 horizonal rows and 10 vertical rows. The veritical rows are numbered 1-10 from left to right. The horizontal rows are lettered A,B,C,D,E from top to bottom.



W = White sample tube
Y = Yellow micro sample tube
R = Red micro sample tube
G = Green micro sample tube
B = Blue micro sample tube
L = White vial with lavender screw caps
Br = White vial with brown screw caps

Syr = Syringe
Fil = Millipore Filter
Cen = Centricon 30

Figure 2. Blood Sample Storage Tray

2.6.2 Organization

The technicians need the following supplies for each sample tray:

3 - 12x75 mm clear white polypropylene test tubes
3 - 1.5 ml yellow polypropylene microsample tubes
3 - 1.5 ml red polypropylene microsample tubes
1 - 1.5 ml green polypropylene microsample tube
6 - 1.5 ml blue polypropylene microsample tubes
12 - 2 ml white polypropylene screw top vials
10 - lavender screw caps for 2 ml vials
2 - brown screw caps for 2 ml vials
1 - Millipore Millex HA-4 filter
1 - 5 ml syringe
1 - Centricon® 30 molecular filter
6 - plastic transfer pipets

The colored plastic sample aliquot tubes are labeled with the participant ID number and arranged in the sample tray in the following order:

Row 1: a white 12x75 mm polypropylene test tube in wells A,B, and C
Row 2: 3 yellow sample tubes in wells A-C
Row 3: The Millipore filter in well C, and the 5 ml syringe in well A
Row 4: 3 red sample tubes in wells A-C.
Row 5: one green sample tube in well A and the Centricon® filter in well E
Row 6: 3 blue sample tubes in wells A-C
Row 7: 3 blue sample tubes in wells A-C
Row 8: 2 white screw cap vials in wells A and B
Row 9: 5 white screw cap vials in wells A-E
Row 10: 5 white screw cap vials in wells A-E

2.7 Preparation for Specimen Collection

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

1. Check to make sure the blood collection tray is properly equipped. Every item on the checklist must be ready before proceeding.

2. Check that each vacutainer tube is properly labeled with the appropriate participant number.

3. Check that the sample processing tray is properly equipped. Every item on the checklist must be ready and in its proper position.

4. Check that each sample aliquot tube and the Centricon® 30 filter unit are labeled with their appropriate participant identification number.

5. Perform quality control (Q.C.) check on centrifuge temperature (4°C ± 2°C).

6. Perform Q.C. check on heating block (37°C ± 1°C).

7. Perform Q.C. check on refrigerator temperature (4°C ± 2°C).

8. Perform Q.C. check on freezer temperature (-70°C ± 10°C).

Approximately 10 minutes before scheduled participant arrival:

1. Fill styrofoam ice bath 3/4 full with crushed ice.

2. Fill styrofoam ice bath with cold water.

3. Place Tube #2 (7 ml red top) in ice bath to chill.

At participant arrival:

1. Check that the ID number on the tubes matches the participant ID.

2. Check that duplicate tubes are prepared and labeled, where requested by the Coordinating Center.

3. VENIPUNCTURE

3.1 Precautions for Handling Blood Specimens

All specimens are handled as potentially infectious for laboratory workers. Transmissions of the infectious agents associated with hepatitis and the acquired immunodeficiency syndrome (AIDS) via "needlestick" skin punctures have been documented.

Where feasible, wear disposable plastic gloves when collecting and processing specimens. Alternatively, wash hands thoroughly with disinfectant soap prior to leaving the work area. Skin cuts or abrasions should be covered.

If the phlebotomist accidentally sustains a contaminated needle stick, the wound is thoroughly cleansed with soap and water. The ARIC physician is notified to order the analysis of the participant's serum for possible hepatitis. Needles are also stored in a locked cabinet when the clinic is closed.

Use 0.1% sodium hypochlorite (household bleach) to clean up any spills of blood, plasma, or serum. Use this solution to clean up all laboratory work surfaces at the completion of work activities.

Dispose of all needles and tubing in puncture-resistant containers for safe disposal.

Do not perform any pipetting by mouth; especially of any blood, serum, or plasma.

Avoid formation of potentially infectious aerosols by careful pipetting and centrifugation.

All used vacutainer tubes and blood products are to be placed in biohazard bags for disposal.

3.2 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, heating block, refrigerator and freezer should be nearby.

3.3 Participant Preparation

Informed consent must be obtained by the receptionist (see ARIC Manual 2) before drawing blood. This procedure is followed to ensure that the subjects understand the purpose of blood drawing and the possible complications of venipuncture. A standard informed consent has been

prepared for this study. With regard to laboratory procedures, the consent statement informs study subjects that there is a small risk of bruising at the spot on the arm where the blood is taken, and that about four tablespoons of blood are drawn. The consent statement also informs study subjects that a copy of the test results is sent to their physicians, and that they will be contacted if clinically important tests are abnormal.

The ARIC Venipuncture Form is completed (see appendix III).

The subject is asked whether he/she has a bleeding disorder before the blood is drawn. If such a disorder is present, ask the subject whether he/she has had blood drawn previously and if so, whether he/she had any problems with excessive bleeding or bruising at the venipuncture site. If the participant has a history of venipuncture problems, the participant should be sampled only if approved by a physician.

Blood drawing is to be standarized to the sitting position. It is difficult to standardize the length of time that a subject is in the sitting position prior to venipuncture, but to the extent that it is feasible, this should be attempted.

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 which 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. If the participant is concerned about the venipuncture, he/she may be reassured to know such care is taken. 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 technologist who draws the blood and their general attitude and competence.

If the participant is nervous or excited, the technologist briefly describes the procedure, e.g., "I am going to be drawing about 3 tablespoons of blood. This blood will be used in tests for lipids and cholesterol and blood clotting factors. We hope to be able to use the results of these tests to predict who might have a greater risk of heart attacks."

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."

3.4 Venipuncture

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow).
A tourniquet is used to increase venous filling. It makes the veins more prominent and easier to enter. PRECAUTIONS WHEN USING A TOURNIQUET: The tourniquet should be on the arm for the shortest time possible. Never leave the tourniquet on for longer than two (2) minutes. To do so may result in hemoconcentration or a variation in blood test values. If a tourniquet must be applied for the preliminary vein selection, it should be released and reapplied after a wait of two minutes. If the patient has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.

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

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

3. If a velcro tourniquet is used, adhere the tubes to each other.

Identify vein: Palpate and trace the path of veins several times with the index finger. Unlike veins, arteries pulsate, are more elastic, and have a thick wall. Thrombosed veins lack resilience, feel cord-like, and roll easily. If superficial veins are not readily apparent, have the participant close his fist. Lowering the extremity over the arm of the chair will allow the veins to fill to capacity. Identify the best available vein.

Cleanse the venipuncture site.

1. Remove alcohol prep from its sterile package.

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

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

4. If venipuncture becomes difficult, the vein may need to be touched again with your hand. If this happens, the site is cleansed again with alcohol.

Assemble the butterfly-vacutainer set.

1. Attach the Luer adaptor to the vacutainer holder.

2. Attach the Luer end of the butterfly needle set to the the Leur adaptor.

3. Place the #1 red and gray stoppered tube in the vacutainer holder being careful not to break the vacuum.

Perform venipuncture.

1. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches (2.5 or 5.0 cm) below the venipuncture site.

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

3. 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.

4. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.

5. Start a timer to measure the flow rate of blood into the first blood collection tube. If the flow rate in the tube is so slow that blood does not fill the first collection tube within 50 seconds, stop the blood collection and repeat on the other arm. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw.

6. Remove the tourniquet after blood is flowing into the second tube. Once the draw has started, do not change the position of the tube until it is withdrawn from the needle. During the procedure, do not allow the contents of the tube to contact the stopper.

7. 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.

8. 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 vacutainer and attach another without removing needle from vein.

9. When the blood flow ceases, remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted (if necessary).

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

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

2. If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.

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

4. The same technician should not attempt a venipuncture more than twice.

To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into needle box.

Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.

Bandaging the arm.

1. Under normal conditions,

   a. Slip the gauze pad down over the site, continuing mild pressure.

   b. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.

2. If the patient continues to bleed,

   a. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.

   b. Wrap a gauze bandage tightly around the arm over the pad.

   c. Tell the patient to leave the bandage on for at lease 15 minutes.

PRECAUTIONS - WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD DRAWING.

1. Have the person remain in the chair, if necessary have him/her sit with head between knees.

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

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

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

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

6. If the person faints, use smelling salts to revive.

7. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member, who will advise you on further action.

3.5 Blood Mixing During Venipuncture

Note date and time of venipuncture on the Venipuncture Form.

To invert tubes, hold the tube horizontal to the floor. Slowly tip the butt end down while watching the air bubble rise to the stopper. (1st inversion) When the bubble reaches the stopper, the tube should be at approximately a 22 degree angle to the floor with the center of the tube at the fulcrum. Now, lower the stopper end while watching the bubble float to the butt. Again, the tube should be at a 22 degree angle to the floor with the center of the tube at the fulcrum. (2nd inversion) Lower the butt end again when the bubble reaches the butt. This is the third inversion. Invert each tube eight times. Eight inversions should take 13-15 seconds.

Start stopwatch.

Draw Tube #1 (13 ml red and gray top). Gently invert 8 times. Place the tube in a rack at room temperature. Note and record the amount of time it takes for the tube to fill with blood. If this is greater than 50 seconds, the blood flow is not adequate and the venipuncture must be repeated.

Draw Tube #2 (7 ml red top). Gently invert 8 times then immediately replace in ice bath.

Draw Tube #3 (7 ml red and yellow top). Invert 8 times and immediately place in 37°C heating block. Start a timer to indicate when the 30 minute incubation period ends.

Draw Tube #4 (4.5 ml blue top). Invert 8 times then place in ice bath.

Draw Tube #5 (4.5 ml blue top). Invert 8 times then place in ice bath.

Draw Tube #6 (10 ml lavender top). Invert 8 times then place in ice bath.

Draw Tube #7 (10 ml lavender top). Invert 8 times then place in ice bath.

Draw Tube #8 (3 ml lavender top). Invert 8 times then replace in rack at room temperature.

Finish venipuncture.

4. BLOOD PROCESSING

Processing of the various blood samples is divided into 3 stages. Attention should be paid to the condition at which the sample tubes are kept prior to centrifuging and aliquotting. See Figures 3-5.

4.1 Stage One: Immediate Processing

At the conclusion of venipuncture, tube #1 and tube #8 are incubating at room temperature. Tube #3 is incubating at 37°C. Tubes #2, #4, #5, #6, and #7 are in the ice water bath.

Tubes #2, #4, #5, #6, and #7 are removed from the ice bath and placed in the centrifuge cups.

Tubes #2, #4, #5, #6, and #7 are centrifuged at 3,000 x g for 10 minutes.

Tubes #1 and #3 remain incubating at their respective temperatures.

Wait for centrifuge to come to a complete stop. Proceed to stage 2 processing.

Tube #8 is sent directly to the local field center hematology laboratory.

4.2 Operating the Centrifuge

Turn power switch on and cool the centrifuge to 4°C.

If centrifuge is not pre-cooled, close and lock the cover, set the temperature control at 4°C, set the speed at 1,000 rpm, set the timer control for 15 minutes, and push the run button. Allow the centrifuge to come to a complete stop before continuing.

Balance the load.

1. Place opposite shields containing filled vacutainers on the balance.

2. To the lighter centrifuge shield, add water (or 50/50 water/alcohol) around the vacutainers, no closer to the top of the container than one-quarter inch.

3. Add the same solution to the other centrifuge shield until the two are balanced.

Load the rotor, being careful to place balanced shields directly opposite each other.

Verify the TEMPERATURE control is set at 4°C. Set the SPEED control at 3,000 x g (4,000 - 4,200 rpm depending on the centrifuge). Set the TIMER to 10 minutes. Set the BRAKE control to the MAX position.

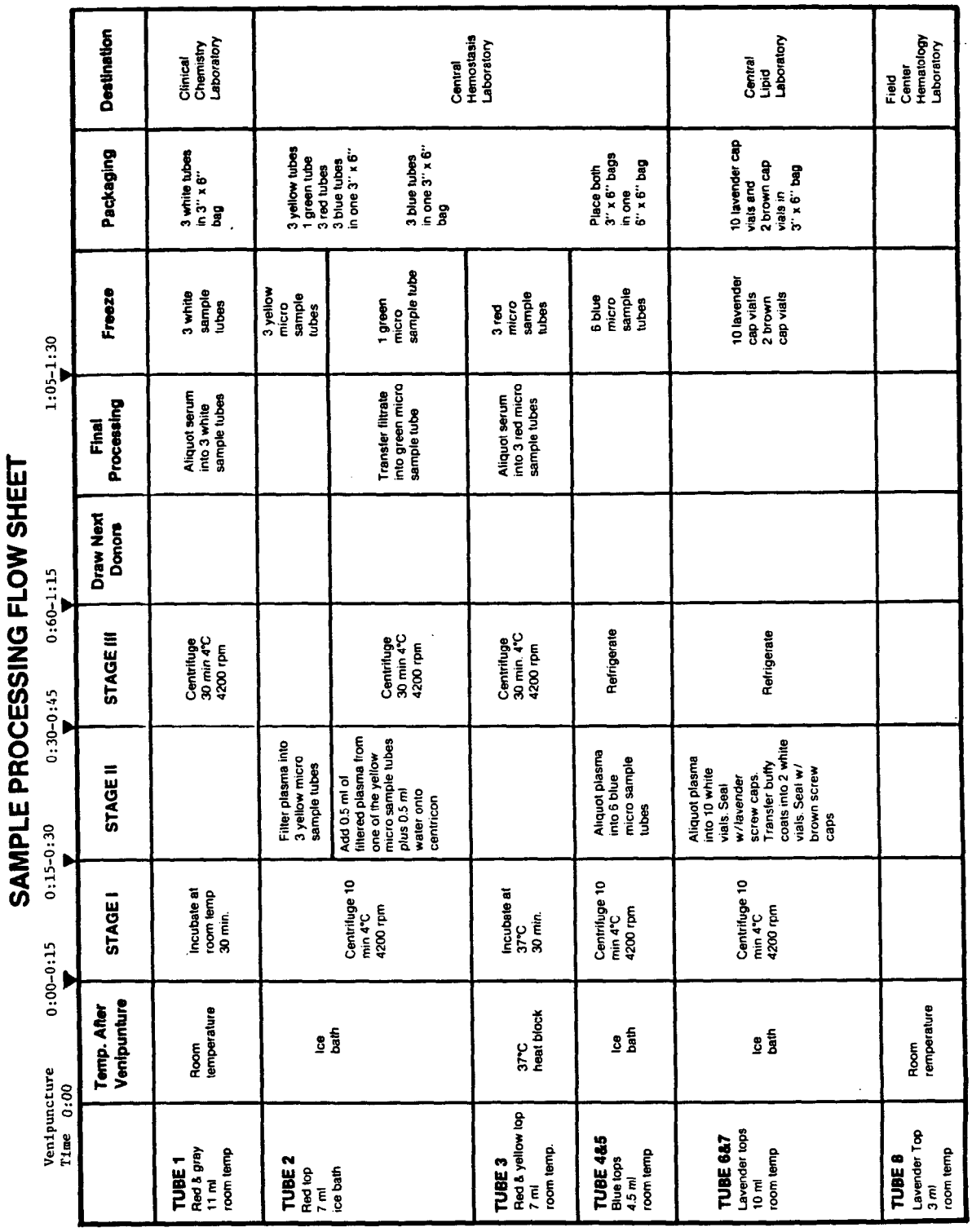


Figure 3. Sample Processing Flow Sheet

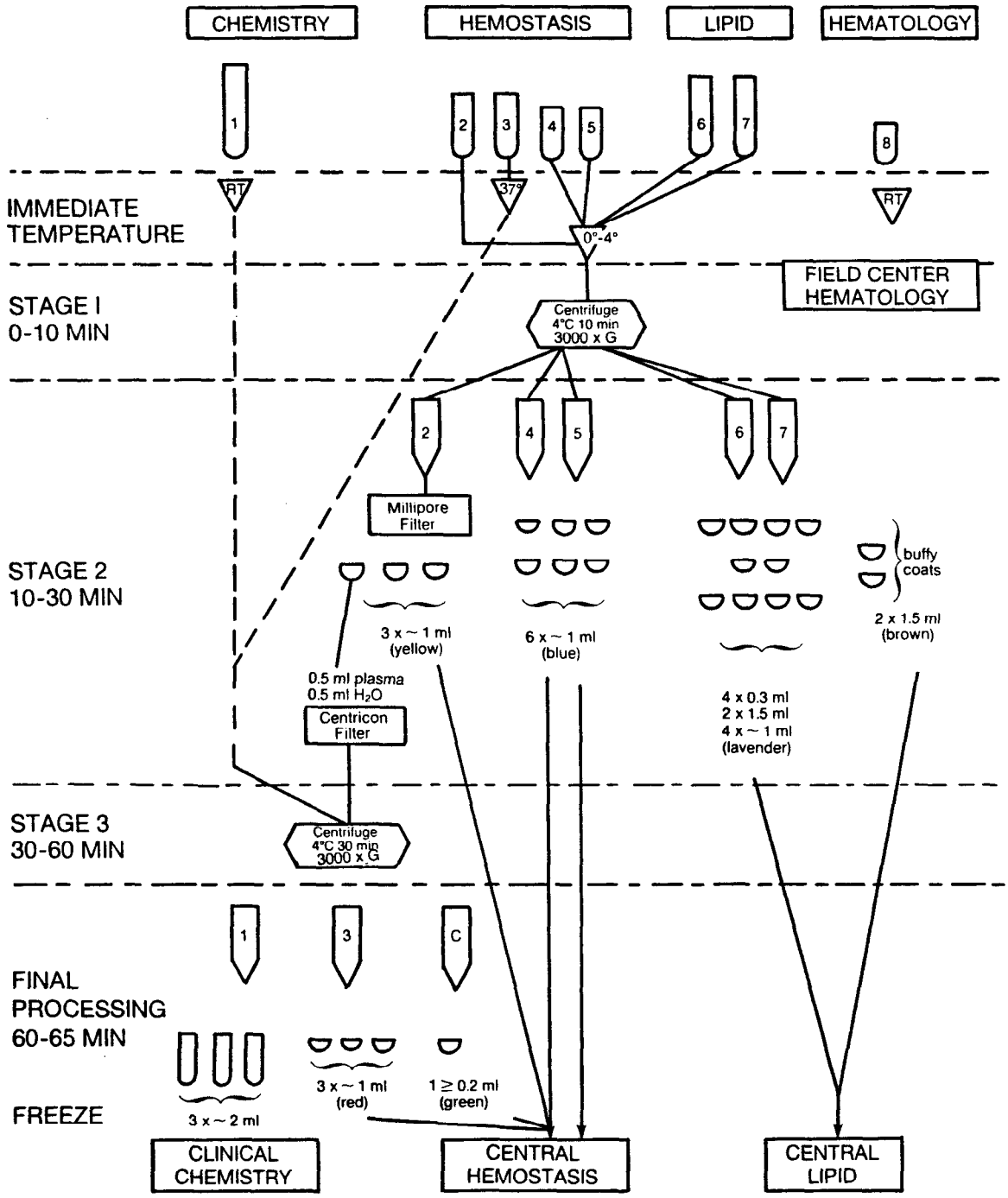


Figure 4. Blood Sample Processing Sequence (for legend, see Figure 5)

Sample Processing Sequence

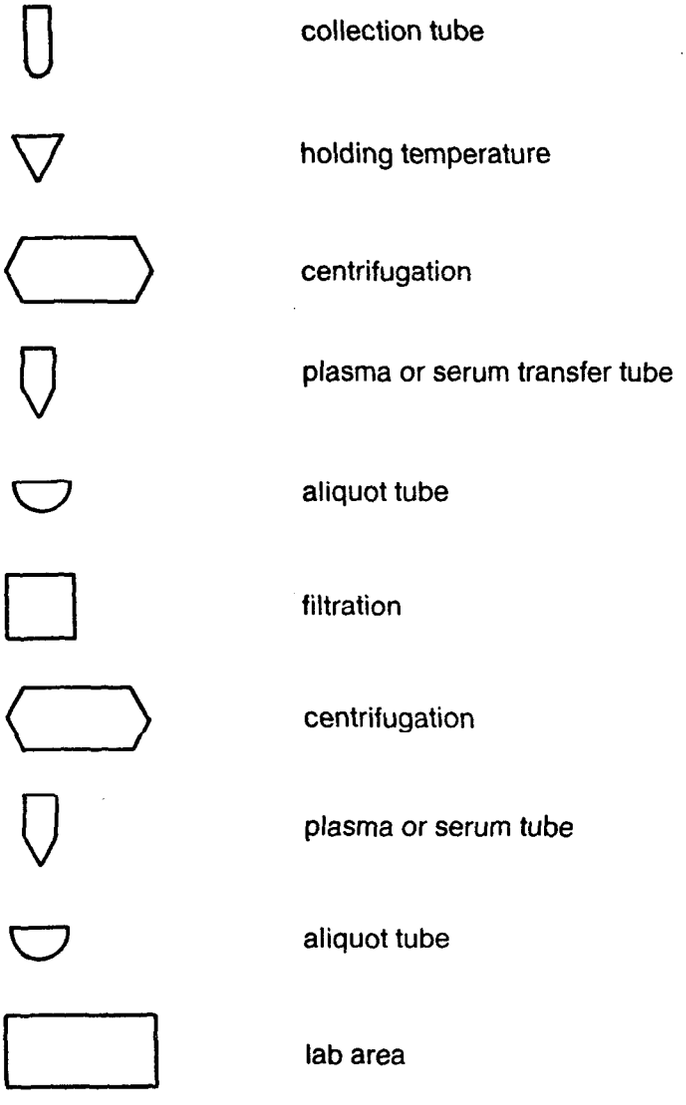


Figure 5. Blood Sample Processing: Legend for Figure 4

Close cover and lock.

Push the RUN button to begin the run cycle. Record the time the samples were placed in the centrifuge in the TIME SPUN section on the Venipuncture Form.

The centrifuge will stop automatically at the prescribed time and the stop indicator will light. Remove samples one at a time.

Consult the Centrifuge Service manual for other guidelines and for troubleshooting.

4.3 Stage Two:

Approximately 15 minutes after venipuncture.

4.3.1 Lavender Stoppered Tubes (Tubes #6 and #7)

1. Remove lavender stoppered tubes (#6 and #7) from centrifuge. Allow red top (#2) and blue top tubes (#4 and #5) to remain temporarily in refrigerated centrifuge.

2. Put tubes #6 and #7 in wells 8D and 8E of the sample preparation tray which contains sample tubes labeled with the corresponding participant number. Remove the stoppers.

3. Using a plastic transfer pipet, and being careful not to disturb the red or white cell layers, remove the clear plasma supernatant. Inspect for hemolysis, then transfer plasma from one lavender top tube into a white 12 x 75 mm plastic test tube. Place tube in well 7E. Using the automatic pipet, transfer 0.3 ml of plasma from the test tube in well 7E into each of four white screw top vials in wells 9A-9D. Using a plastic transfer pipet, fill the two sample tubes in wells 9E and 10E with approximately 1.5 ml of plasma to within one centimeter of the top of the vial.

4. Repeat the aliquotting process, dividing the plasma from the remaining lavender collection tube, plus any extra plasma from the previous tube, equally into the four white screw top vials in wells 10A-10D. To allow for expansion during freezing, do not fill any vials more than 3/4 full.

5. Fasten the lavender screw caps onto the white screw top vials in rows 9 and 10 and allow them to remain in the sponge rack.

6. Using a plastic transfer pipet, gently remove the white cell layer from each of the lavender blood collection tubes and transfer approximately 1.5 ml of cells from each tube into the white screw top vials in wells 8A.

7. Fasten the brown screw caps onto the white screw cap vials and allow them to remain in the sponge rack.

8. Restopper the sample collection tubes and discard them in a biohazard waste bag.

4.3.2 Red Stoppered Tube (Tube #2)

1. Remove the red stoppered tube from the refrigerated centrifuge. Place it in well 2E in front of the yellow sample aliquot tubes.

2. Pull out the plunger of the 5 ml syringe which is in well 3A.

3. Attach the male Luer connector of the syringe barrel to the female Luer connector of the Millipore HA-4 filter in well 3C.

4. Remove the stopper from the red top tube. Using a plastic transfer pipet, remove all of the plasma and transfer it to the syringe barrel which is attached to the Millipore filter. Be careful not to disturb the underlying buffy coat or red cells.

5. Position the lower part of the Millipore filter over the first yellow sample tube (well 2A).

6. Replace the plunger of the syringe. Filtered plasma will start to flow from the bottom.

7. Slowly depress the plunger of the syringe to continue filtering the plasma directly into the yellow sample aliquot tubes.

8. Fill one yellow microsample tube to the top. Then divide the remaining plasma evenly between the other two yellow aliquot tubes. Fill each of the 3 yellow aliquot tubes with approximately 1 ml of filtered plasma.

9. Using the automatic pipet, remove exactly 0.5 ml of plasma from one of the yellow aliquot tubes and place it on the top of the Centricon^{R} unit. Add exactly 0.5 ml of distilled water to the plasma in the Centricon unit.

10. Fasten the caps of the yellow aliquot tubes and allow them to remain in the sponge rack.

11. Leave the Centricon^{R} 30 in the sponge rack. It is saved for further processing.

12. Restopper the blood collection tube and discard it into a biohazard waste bag.

4.3.3 Blue Stoppered Tubes (Tubes #4 and #5)

1. Remove the two blue stoppered tubes from the refrigerated centrifuge. Place the tubes in wells 6E and 7E in front of the blue sample aliquot tubes. Remove the stoppers.

2. Using a plastic transfer pipet, transfer the plasma from one of the blue top tubes, in approximately equal aliquots, into each of the three blue sample aliquot tubes placed in the wells behind it. Repeat this process with plasma from the other blue top tube.

3. Fasten the caps on the sample aliquot tubes and replace them in the sponge sample tray.

4. Replace the stoppers on the blue top tubes and discard them in a biohazard bag.

Place the Centricon® 30, which contains 1 ml of plasma into the centrifuge to be spun.

Place the entire sponge sample tray with all of the aliquot tubes into the 4°C refrigerator.

Proceed to Stage 3 processing.

4.4 Stage Three

Stage three begins approximately 30 minutes after venipuncture.

When the 30 minute timer goes off, remove the 7 ml red and yellow stoppered tube (Tube #3) from the heat block.

If tubes 1 and 3 cannot be spun immediately after the 30 minute timer goes off, remove them from the heat hock and put them into the melting ice bath.

Place the red and gray top tube and the red and yellow top tube in the centrifuge with the Centricon® 30.

Centrifuge the tubes at 3,000 x g for 30 minutes.

If another participant is scheduled to be drawn, the tubes may be left spinning in the centrifuge until the next venipuncture is finished.

4.5 Final Processing

When the centrifuge has come to a complete stop, remove the sponge sample rack from the refrigerator.

4.5.1 Red and Gray Stoppered Tube (Tube #1)

1. Remove the red and gray top tube from the centrifuge and place it in well 1E of the sponge test tube rack in front of the three white test tubes.

2. Remove the stopper from the tube. Use a plastic transfer pipet to aliquot the serum equally into the three white tubes. Each of the tubes is filled to at least half full.

3. Place caps on the test tubes.

4. Replace the stopper on the blood collection tube and discard it in a biohazard waste bag.

Red and yellow top tube (Tube #3)

1. Remove the tube from the centrifuge and place it in well 4E in front of the red microsample tubes. Remove the stopper.

2. Use a transfer pipet to divide the serum equally into the three microsample tubes. Each tube should be approximately 3/4 full. Fasten the caps and replace the tubes in the sponge racks.

3. Replace the stopper on the blood collection tube and discard it in a biohazard waste bag.

Centricon^{R} 30

1. Remove the Centricon^{R} 30 from the centrifuge and place it in well 5E in front of the green microsample tube.

2. Carefully remove the upper half of the Centricon^{R} unit to expose the filtrate in the lower sample cup. Place the upper half of the unit vertically in well 3A of the sponge rack.

3. Use a plastic pipet to transfer all of the filtrate in the lower sample cup into the green microsample tube. There will only be approximately 300 µl of filtrate. Fasten the cap and replace the tube in the sponge rack.

4. Discard all parts of the Centricon^{R} unit in a biohazard waste bag.

4.6 Freezing

When all of the blood collection tubes have been aliquotted into their respective microsample tubes and the microsample tubes have been replaced in the sponge rack, the entire rack is placed upright in the -70°C freezer for a minimum of 30 minutes. Samples must be placed into the freezer within 90 minutes from venipuncture time. Samples must be thoroughly frozen before packaging them for storage and shipping. Record the time that the samples are placed in the freezer on the Venipuncture form.

5. STORAGE AND SHIPPING

5.1 Packaging

Each participant's blood samples are packaged in freezer storage bags corresponding to the final destination of the tubes.

1. Label four 3" x 6" storage bags and one 6" x 6" storage bag with the appropriate participant number.

2. Remove the sponge sample tray with the corresponding participant specimens from the -70°C freezer. Packaging is done quickly after this point to avoid thawing of the specimens.

5.1.1 Central Chemistry Laboratory

Place the three 12 x 75 mm white tubes into a prelabeled 3" x 6" storage bag. Check again to make sure all tubes are numbered and the number corresponds to the participant number on the bag. Press the air out of the bag and seal. Place the bag in the Central Chemistry Laboratory styroforam box in the -70°C freezer and follow the storage directions described in Section 5.2.

5.1.2 Central Lipid Laboratory

Place the ten white screw top vials with lavender screw cap and the two white vials with brown screw caps into a prelabeled 3" x 6" storage bag. Again check to verify that tubes and bag are numbered correctly. Press the air out of the bag and seal. Place the bag in the Central Lipid Laboratory styrofoam box in the -70°C freezer and follow the storage directions described in Section 5.2.

5.1.3 Central Hemostasis Laboratory

1. Place three of the blue sample tubes into a prelabeled 3" x 6" storage bag. Press the air out of the bag and seal.

2. Place the remaining ten sample tubes into a prelabeled 3" x 6" storage bag. The bag should contain 3 red, 3 yellow, 1 green, and 3 blue sample tubes. Press the air out of the bag and seal.

3. Place the 3" x 6" bag containing the three blue tubes into the larger 6" x 6" bag. Place the 3" x 6" bag containing the remaining ten multicolored tubes into the same 6" x 6" bag. Press the air out of the larger outer bag and seal. Place the bags in the Central Hemostasis Laboratory styrofoam box in the -70°C freezer and follow the storage directions in Section 5.2.

5.2 Storage

Three boxes are placed in the -70°C freezer for temporary storage prior to their shipment to the three central laboratories. These boxes are labeled CHEMISTRY, LIPID and HEMOSTASIS respectively.

The 3" x 6" bag containing the 3 white 12 x 75 mm tubes is placed in the CHEMISTRY box.

The 3" x 6" bag containing the 10 lavender and 2 brown tubes is placed in the LIPID box.

The 6" x 6" bag containing the remaining thirteen tubes is placed in the HEMOSTASIS box.

All bags remain in their boxes until shipment to their respective labs.

5.3 Shipping

The samples remain in their styrofoam boxes at -70°C until they are shipped. All frozen sera collected and stored within the last work week are shipped to their respective clinical laboratories on Monday by overnight courier. (Minneapolis uses a day courier service for clinical chemistries as both the field center and the Central Chemistry Laboratory are in the same city.) Samples can be shipped on Tuesday if the field center is closed on Monday, but the contact person at each central laboratory must be notified that the shipment will arrive one day later than usual. There is no minimum shipping requirement; frozen samples are shipped weekly regardless of the number of specimens that have been frozen and stored within the last collection period.

5.3.1 Packaging Instructions

The bags of frozen sera sample for clinical chemistry, hemostasis and lipids are packed and shipped in the same sytrofoam boxes which have served as storage containers in the field center freezers. See Figure 6. Packaging instructions are as follows:

1. Place a 2" layer (approximately 2 lbs.) of dry ice on the bottom of the styrofoam box.

2. Put half of the bags of sample tubes into a 1 gallon zip lock bag and seal. Place this bag in the styrofoam box on top of the dry ice.

3. Layer another 2 lbs. of dry ice on top of and around the sample bags.

4. Put the remaining sample bags into a second 1 gallon zip lock bag, seal, and place this bag on top of the dry ice.

5. Layer another 2 lbs. of dry ice on top of and around the sample bags.

6. Place packing material on top of the dry ice to fill the box.

7. Place the paper shipping forms on top of the packing material. The shipping forms with instructions are shown in the Appendix.

8. Seal the box tightly with strapping tape.

9. Address the box and place it in the -70°C freezer to wait for pick up.

5.3.2 Mailing Instructions

Shipping containers with frozen sera are sent to the respective central laboratories by day or overnight courier to ensure receipt within 24 hours and the empty sytrofoam containers are returned to the field centers by UPS.

5.3.2.1 Clinical Chemistry Laboratory

Shipping containers to the Clinical Chemistry Laboratory are addressed as follows:

ARIC Central Chemistry Laboratory
University of Minnesota Hospital
Receiving Unit K/E
425 East River Road
Minneapolis, MN 55455
Telephone: (612) 626-5031

Field Centers in Hagerstown, Jackson, and Winston-Salem ship the specimens by Federal Express with a guaranteed delivery within 24 hours. The Minneapolis field center uses a local one-day courier service.

5.3.2.2 Hemostasis Laboratory

To reduce shipping costs, the central Hemostasis Laboratory serves as a central processing center for both hemostasis and lipids specimens if the two boxes can be taped together and sent as one package. Hemostasis and lipid laboratory specimens, however, are packed separately in their individually labeled styrofoam shipping containers as described above. ID labels for each shipping container are placed on a side of the container so that they cannot be seen until the tape is removed and the boxes are separated at the Hemostasis Laboratory. The Federal Express shipping label for the double container to the Central Hemostasis Laboratory (or for a single insulated box with only serum for the Hemostasis Laboratory) is addressed as follows:

Central Hemostasis Laboratory
University of Texas Medical School
6431 Fannin
Houston, TX 77030
Telephone: (713) 792-5813

Upon receipt of the double package, Hemostasis Laboratory personnel separate the two boxes, process the hemostasis samples as described in Manual 9 (Hemostasis Laboratory) and deliver the lipids sera to the ARIC Central Lipid Laboratory for processing (Manual 8, Lipid Laboratory).

If the overnight courier does not permit the taping of the two specimen boxes together, the hemostasis serum is sent to the Central Hemostasis Laboratory and the lipid serum is sent to the Central Lipid Laboratory.

5.3.2.3 Central Lipid Laboratory

Shipping containers to the Central Lipids Laboratory are addressed as follows:

Central Lipid Laboratory
Atherosclerosis Clinical Laboratory
Methodist Hospital, Mail Station F701, Rm. F756
6565 Fannin
Houston, TX 77030
Telephone: (713) 790-4351

6. QUALITY CONTROL

6.1 Venipuncture and Equipment Records

Quality control procedures performed in ARIC central laboratories are addressed in Manuals 8-10. One component involves evaluation at the coordinating center of monthly mean values for each technician. This is informative because field centers select representative subsamples for examination each month. In the field centers there are two different aspects of quality control. One is the daily or monthly record of the performance of the equipment. This is most easily kept as a check sheet with the daily of monthly records. The other aspect of quality control is the record of the venipuncture which is part of each participant's records. This record documents how long it takes to fill the standard 11 ml vacutainer tube during the blood collection. It also shows the number of attempts it takes to get a good venipuncture and the initials of the technologist who performs it. This record provides needed assurance that the blood was drawn in a standardized manner and that the equipment was functioning properly. Quality control is the best documentation that samples in each of the four field centers are being drawn and processed identically. Differences in the way the samples are collected or processed could potentially create a statistically significant difference in assay results, which could make the laboratory results unusable. It is very important that the quality control records of the procedures and the equipment be properly maintained.

For the equipment, daily records should be kept on all refrigerators and freezers. The heating block must be maintained at 37°C ± 1°C or else assay values could be affected. A daily record of the heating block temperature is also vital. The temperature of the refrigerated centrifuge must be recorded daily. In addition, the actual speed of the centrifuge needs to be checked and recorded monthly with a tachometer. Sample Quality Control sheets are enclosed in this manual (see Appendix VI).

6.2 Quality Control Duplicate Blood Samples

As part of the quality control program for laboratory determinations from blood samples (clinical chemistry, hemostasis, lipids and hematology), duplicate specimens are sent to the laboratories, with one half of each specimen pair sent under the participant's regular ARIC I.D., and the other half under a Quality Control Phantom Participant (Q.C.) I.D. The Q.C. I.D.s are not distinguishable from other ARIC I.D.s so that this will form a blinded external quality control program monitoring measurement variability.

To reduce the burden upon ARIC respondents, no one respondent is asked to contribute sufficient extra blood to make a complete set of duplicates for all laboratories. Instead, extra blood is drawn from several respondents and sent out under the same Q.C. I.D. For data analysis, results on each laboratory measurement are matched to the appropriate participant results.

ARIC Field Centers should draw two full sets of quality control duplicates are drawn each week, so that for each blood variable, approximately 7% of all ARIC participants have duplicate measures. Note that a Q.C. duplicate is considered "complete" at the end of a week, even if not all of the samples were drawn. All Q.C. samples are stored an extra week at the field centers and then sent to the central laboratories with a regular shipment.

The plan for processing blood samples from ARIC participants calls for processing blood tubes in cycles, with blood from one or two participants in each cycle. To reduce the risk of confusing which Q.C. tube matches which real participant, Q.C. blood samples are drawn from only one member of each pair of participants whose blood is processed at the same time. Each day is devoted to a fixed laboratory. Draw a Q.C. set from only one participant in each pair of ARIC participants whose blood is processed simultaneously. For example, on Monday draw Tube 1 (chemistry); on Tuesday, draw Tubes 2 & 3 (hemostasis); on Wednesday, Tubes 4 & 5 (hemostasis), on Thursday, draw tubes 6 & 7 (lipids); on Friday, draw Tube 8 (hematology) and draw any tubes that might have been missed earlier in the week due to holidays, no-shows, upsetting the schedule, or other reasons.

6.2.1 Weekly Blood Q.C. Sample Checklist

The ARIC Field Center venipuncture technicians maintain a weekly checklist posted in their work area of the Q.C. samples to be drawn during the week. As each sample is drawn and processing completed, it is checked off. On Friday morning, this checklist is consulted to see if there were any additional samples needed to make up the complete set of Q.C. samples. An example of the checklist is given below.

Weekly Blood Q.C. Sample Checklist

Week:

Day	Tubes	Laboratory	Sample 1	Sample 2
Monday	1	Chemistry	_____	_____
Tuesday	2, 3	Hemostasis	_____	_____
Wednesday	4, 5	Hemostasis	_____	_____
Thursday	6, 7	Lipids	_____	_____
Friday	8	Hematology	_____	_____
Friday	Make-up any missed above			

6.2.2 Preparation for Drawing and Processing Q.C. Samples

Blood-drawing Tubes: Each morning the blood-drawing technicians prepare extra blood-collection tubes for the samples to be drawn that day. Each tube is labelled with one of the Q.C. I.D.s to be used that week. In addition, the technicians may wish to mark Q.C. tubes (other than Tube 8) "Q.C." in a clearly visible fashion, to reduce the chance that these tubes might be mixed up with the regular blood collection tubes during processing. (Since Tube 8 goes to the Hematology Lab, it should not be marked as Q.C.). The Q.C. Tube #2 (7 ml red top) must be kept in the ice bath until use, as is the regular participant Tube #2. The other Q.C. tubes are set in the same rack used to hold the regular blood collection tubes, in a separate row from the other tubes.

Sample Aliquot Tubes: Each morning a separate foam block is prepared for each set of Q.C. blood tubes that the technician plans to draw that day. The foam block contains all the aliquot tubes needed to process the day's quality control samples. The tubes in each block are labelled in advance with one of the Q.C. I.D.s being used that week. Care must be taken during processing that the labels on the sample aliquot tubes match the label on the Q.C. blood collection tubes. Since only one Q.C. set is drawn in each blood collection cycle, only the foam block with I.D.s for that set is out in the work area at that time.

Centrifuge Balancing: Processing the extra Q.C. blood tubes requires extra work balancing the centrifuge. Each morning, the technician prepares a fourth centrifuge shield with the appropriate tubes filled with water to balance the expected weight of the Q.C. tubes to be drawn that day. This allows quickly adding the shield containing the Q.C. tubes and the balancing shield of water-filled tubes to the centrifuge without delaying the processing of the real participant tubes in the centrifuge.

6.2.3 Drawing and Processing Q.C. Blood

Selecting Participants for Q.C. Blood Draw: Normally, the Q.C. samples are drawn from the first member of each pair of participants whose blood is being processed simultaneously. Based upon the size of their veins, the difficulty of drawing the blood, and the apprehension a participant shows about the blood draw, the venipuncture technician may need to forego the drawing of the Q.C. tube from the first, and draw from the second member instead. Occasionally, it may happen that a Q.C. sample cannot be drawn from either member of a pair.

Order of Q.C. Tubes in Relation to Regular Blood Collection: The Q.C. Tube #2 follows immediately after the real Tube #2 in the draw. The other Q.C. tubes may be added at the end of the blood draw without harming the measurements. This procedure is followed to cause the least disruption of the collection of the regular blood samples. If the blood flow falls off at the end of the draw, so that it would be difficult to obtain the extra Q.C. tubes, a different participant is used to get this blood. A NEW NEEDLE STICK SHOULD NOT BE DONE JUST TO GET MORE BLOOD FOR Q.C.

Processing and Freezing Q.C. Blood: Q.C. blood samples are processed along with the regular blood samples. At certain points, the Q.C. blood samples

must wait for processing until the regular blood samples have completed a particular step. For example, at Stage 2 of processing, Q.C. samples are not taken out of the refrigerated centrifuge until after the regular tubes #4 and #5 have been aliquotted into sample vials and put in the refrigerator. After processing is completed for each Q.C. blood collection tube, the sample aliquot tubes are put into the -70C° for freezing (for a minimum of 30 minutes). After the samples are thoroughly frozen, they are put into a freezer storage bag and put into the freezer box corresponding to the destination of these tubes.

Since hemostasis tubes are collected from more than one participant, the samples from the first hemostasis tubes collected are still bagged as soon as they are frozen. A small holding box is set up in the freezer for bags with incomplete sets if hemostasis samples. When a full set is completed, these bags are taken out of the holding box, zipped open and all the hemostasis tubes for a particular Q.C. I.D. rebagged together and put into the regular hemostasis freezer box.

Filling out the Venipuncture Form for Q.C. Blood: Only one Venipuncture form is filled out for each set of blood samples for one Q.C. I.D. Information on the venipuncture attempts and filling time (questions 5 and 6) pertain to the participant contributing tubes 2 and 3, and the rest of the form pertains to the participant contributing tubes 4 and 5. To prevent the Hemostasis Laboratory from matching Q.C. and real participant tubes by checking to see which tubes have exactly the same time of blood drawing on the venipuncture forms, the blood drawing technicians randomly add or subtract 5 minutes to the time when the blood draw began when filling out the Q.C. sample's venipuncture form.

Logging the Match between Q.C. and Regular ARIC IDs and Reporting These to the Coordinating Center: The Q.C. Phantom Participant's folder is kept in the blood drawing area while the phantom I.D. is being used to draw Q.C. blood tubes. In the folder is the ARIC Quality Control Phantom Participant Form which is used to keep track of the match between Q.C. and regular ARIC I.D.s for the various blood tubes that are put together to make up a Q.C. "phantom" set. A sample copy is shown in Appendix VI. At the top of the log sheet is a space for the Q.C. Phantom Participant's I.D. As participants donate blood to make up a Q.C. set, labels with their I.D.s are added to the line corresponding to the tubes donated. This step must be done immediately after completing drawing blood from that participant, to minimize the chance of recording the wrong I.D. One such form is recorded for each Q.C. I.D. used. As soon as the full set of tubes is completed for each phantom participant (or at the end of the week, if any set is incomplete), the Q.C. phantom particpants' folder with this form is given to the receptionist (or other person designated by the Study Coordinator). When all repeatability studies for the phantom are completed, the folder is processed like other participants' folders, except that the Q.C. phantom participant form is sent to the Coordinating Center and the field center keeps a photocopy of this form in the phantom's folder. Each regular participant's folder also holds an ARIC Participant Blood Q.C. Log Form to indicate whether any blood was drawn from that person for quality control, the tube(s) drawn, and the Q.C. I.D. to which they are matched. This sheet is filled out at the venipuncture station while the participant is there. A sample is shown in Appendix VI.

7. TRAINING PROCEDURES

7.1 Audiovisual Training Tape

After the technologists have studied this manual, they view the slides with the cassette on blood collection. These slides review all of the procedures involved in the tray preparation, the techniques of venipuncture and sample collection, the blood processing, and the separation and destination of the tubes. Technologists with questions go back to the procedures manual or replay the slides and tape.

7.2 Technologist Practice and Evaluation

After reviewing the manual and the training tape, the technologists are ready to prepare their own blood collection trays and sample preparation trays. Technologist sets up 3 sets of trays and should be able to answer questions on what the tubes are for, the order of drawing, the incubation temperatures, the incubation and centrifuging times, the final number of sample tubes, the correct tube colors and the labs where each of the sample tubes are sent.

Once the blood processing supplies have been prepared, the technologist proceeds to a mock drawing and processing of samples. Mock venipuncture is performed with the butterfly needle and vacutainer system. A piece of latex tubing with a knot in one end leading to a glass of water is used as a target vein. A "venipuncture" is collected in the correct order, then placed at their proper positions and temperatures. The sample is processed from start to finish exactly as if real blood were being used. Each technologist performs a minimum of two mock draws from beginning to end. Although the mock draws take time, they provide hands-on experience and allow the technologist to become comfortable with the procedures before proceeding to live participants.

When the technologists are thoroughly capable of handling the entire process with dummy tubes, they watch the procedure being demonstrated with a live volunteer (possibly even themselves). Watching a live demonstration they are able to judge the ease or difficulty of some of the procedures.

At this point the technologists are ready to practice on live participants. The technologists practice at least once with just one participant at a time and again process the blood entirely by themselves from start to finish. If the technologists do not feel comfortable, they can always go back and repeat the process with dummy tubes. When the technologists have successfully practiced with a live participant, they are fitted into a team where blood is drawn from two participants, and two samples are processed at the same time. If volunteers are available, it may be beneficial to repeat this several times. Any questions or problems that the technologists have must be solved before the technologists actually proceed to drawing the ARIC participants. Before the technologists draw blood from any ARIC participant, they must take and pass the practical and written tests

included in the evaluation portion of this manual. After passing the tests and depending on the written evaluation of their instructor, they may proceed either to drawing blood from the ARIC participants as part of a team, or to do more practice on live volunteers.

7.3 Clerk Training

The best way for the clerk or technologists to learn the forms and procedures is to go through each of them step by step. The clerk should carefully read and understand the Participant Information Form. Examples of forms with correct and incorrect responses are compared and the clerk should recognize inappropriate responses. At this point, the clerk fills out a practice form making appropriate responses for himself, or for a fictional participant.

After becoming familiar with the participant information forms, the clerk learns to label the sample collection and freezing tubes. The trays for blood collection and for sample processing are set up by the field center technologists. Every tube in every tray must have a label attached with the appropriate participant code number. This number must be on all forms, results, tubes and shipping packages leaving the field center. The clerk needs to know the destination of each form, tube, and sample. A checklist is kept with the material from each participant, and the clerk needs to know what each item is on the list.

Before assisting in the blood collection, the procedure should be explained to the clerk. The clerk also reviews the training slides on the blood collection and processing. He or she should be familiar with the whole procedure even though he or she will not be actively participating in most of the process. The clerk is shown how to gently mix the tubes of blood. He or she is also taught which tubes are placed in the 37°C heat block and which are placed (replaced) immediately in an ice bath. The clerk should is also shown how to time the filling of the first blood collection tube and where to record that time on the participant's Venipuncture form. Once the first participants have been drawn, the clerk or technologist makes sure that the blood drawing stations are set up for the next set of participants. A checklist is placed at each station to facilitate the preparation and completeness of each station.

After viewing the training slides, the clerk watches at least two sets of two volunteers donate blood. The clerk may need to become accustomed to the sight of real blood before actually participating in the blood collection. When he or she has watched several series of blood drawings, the clerk is taken through a mock drawing procedure at least three different times. When he or she is familiar with the mixing and placement of the tubes, he/she should participate in at least one practice set of two participants. Once he or she is comfortable with the mixing and placement of tubes, the pace required, and the preparation of the drawing stations, he or she is ready to assist in the actual drawing procedure. If the field center does not have a clerk available to help during the blood collection and processing, the technologists must be able to perform the clerk's duties as well.

8. FIELD CENTER HEMATOLOGY SERVICES

8.1 Clinical Significance

Quantitation of the formed elements of the blood (erythrocytes - RBCs, leucocytes - WBCs, and platelets) is important in the ARIC study primarily so that the associations of the formed elements with atherosclerosis and its clinical manifestations can be studied. The association of elevated WBC count with cardiovascular incidence requires confirmation. These determinations are also of value in recognizing preclinical disorders (e.g., anemia, leucocytosis, and thrombocytopenia) which may require the ARIC participant's referral to his usual source of care for further medical evaluation.

8.2 Principles of Quantitative Hematologic Determiantions (1)

Procedures for counting circulating blood cells, whether manual or automated, all involve a sequence of (1) diluting the blood specimen, (2) aliquotting the diluted specimen into a measured volume, and (3) counting the cells in that volume.[1]

All hospital-based and independent laboratories now use automated instruments to count blood cells. These instruments work on either of two basic principles. In the first type of instrument employing electronic particle counting (e.g., the Coulter counter, Coulter Diagnostics, Hialeah, FL) blood cells pass through an aperture through which an electrical current is passed. The change in electrical resistance caused by the cell's passage is counted as a voltage pulse. Combinations of aperture size and threshold/window discrimination of voltage pulse height allow distinctions between erythrocytes, leucocytes, and platelets. The second type of instrument (e.g., Hemalog H-6000, Technicon Corporation, Tarrytown, NY) uses light-scattering from cells flowing through a counting chamber. Scattered light is detected by a photomultiplier tube, and cell number and size are evaluated as voltage pulses.

Automated hematology analyzers directly measure the cell counts for total RBCs, WBCs, and platelets. Total hemoglobin (Hb) is measured by the formation of hemiglobincyanide (HICN). The hematocrit (Hct) is calculated from the measurement of RBCs and either the calculated erythrocyte mean cell volume (Coulter Counter) or pattern of light-scattering (Hemalog H-6000). The hematocrit, as calculated by these automated analyzers, may differ from the hematocrit as determined directly by centrifugation ("Packed cell volume"). These differences are usually not significant in normal subjects with correct handling of samples.

Three red-cell indices are calculated by automated hematology analyzers: (1) the mean cell volume (MCV), (2) the mean cell hemoglobin (MCH), and (3) the mean cell hemoglobin concentration (MCHC). The following formulas are used in these derivations:

(1) $$MCV\ (\mu^3 \text{ or } fl) = \frac{Hct \times 1000}{RBCs\ (10^6/\mu l)}$$

μ = micrometer
fl = femtoliter
μl = microliter

(2) $$MCH\ (pg) = \frac{Hb\ (g/L)}{RBC\ (10^6/\mu l)}$$

pg = picograms
g/L = grams per liter

(3) $$MCHC\ (g/dL) = \frac{Hb\ (g/dL)}{Hct}$$

g/dL = grams per deciliter

These indices are clinically useful in recognizing and classifying various types of anemias. If the primary erythrocytic measurements (Hct, RBC count Hb) are normal, these indices will also be essentially normal.

8.3 General Operation of Field Center Hematology Studies

In contrast to the other types of laboratory determinations in the ARIC study which are performed at a central laboratory (e.g., coagulation, lipids, and chemistry), hematology procedures use specimens collected in EDTA which cannot be shipped to distant sites without jeopardizing sample stability and reducing reliability.

Each ARIC Field Center uses a local reference laboratory to perform the routine hematology procedures specified by the ARIC protocol. These laboratories are responsible for prompt specimen pickup, analysis, and result reporting. Although whole-blood specimens collected in EDTA are stable for up to 24 hours at 4°C, it is desirable that ARIC specimens collected in the morning at the Field Center be analyzed that day by the reference laboratory. Specimens collected by the Field Centers in the afternoon are analyzed promptly after storage at 4°C. (EDTA is the only acceptable anticoagulant for samples to be analyzed for cell counts. [Heparin produces variable artifacts of cell size.]

The professional staff at each Field Center periodically review the performance of the laboratory performing ARIC hematology studies, particularly in terms of the laboratory's quality control program for automated hematology. This review is carried out annually or semiannually.

8.4 Calibration and Interlaboratory Standardization

Each field center utilizes the services of one or more local hematology laboratories. Jackson uses the hematology laboratory at the University of Mississippi Medical Center seven days a week. The Jackson laboratory runs all ARIC hematology specimens on one instrument. Washington County uses the services of two hematology laboratories; the Hagerstown Medical Laboratory to process samples during the week; the Washington County Hospital Hematology Laboratory on weekends. Both laboratories process samples on a Coulter S + IV instrument. The Minneapolis field center sends blood samples to one hematology laboratory which uses two instruments, a Coulter S + III and a Coulter S + IV. Forsyth County began using one

hematology laboratory and changed to a second (Roche-Biomedical Laboratories, Inc.) in September 1987, which uses a Technicon H-6000 for processing ARIC samples. A technical summary of type of instruments, calibration and quality control is provided in Table 1. Three of the four laboratories use the same type of hematology analyzer with similar calibration procedures (as shown in Table 1), thus reducing problems with interlaboratory standardization. Standardization of the processing of hematology specimens, however, remains problematic as illustrated in the following article by RA Savage.

No stable reference materials are available for standardizing cell counts.

> The International Committee for Standardization in Hematology and the College of American Pathologists have both recognized that an automated hematology calibrator material that possesses the physical and chemical characteristics of fresh whole blood and is of sufficient stability to be analyzed by reference methods and distributed to hospital and outpatient laboratories for calibration purposes is not now available nor is likely to appear because of the technical inadequacies of surrogates for white blood cells and platelets and to the compromises in matrix composition that are necessary in rendering the product stable for long-term analysis.[2]

Table 1. ARIC Field Center Hematology: Technical Summary

Field Center	Instrument	Calibration	Quality Control[1]
Jackson, MS	Coulter S + IV	S-Cal	CAP Survey Patient Samples
Washington County, MD	Coulter S + IV	S-Cal	CAP Survey Patient Samples
Minnesota, MN	Coulter S + III Coulter S + IV	S-Cal	CAP Survey CDC Survey State of Minnesota
Forsyth County, NC	Technicon H-6000	Fisher Computrol	CAP Survey Medicare Survey Staff Samples

[1]CAP Survey - College of American Pathologists Survey
CDC Survey - Centers for Disease Control Survey

Laboratories currently use stabilized materials prepared by the manufacturers of automated hematology analyzers to calibrate their instruments. Thus, interlaboratory standardization in this area depends upon the widespread use of the same calibrator.

8.5 Precision

Precision of cell counts within the laboratory relies upon (1) replicate determinations performed on the same specimen over a 24-hour period, (2) use of stabilized cell suspensions, (3) calculation of a "moving average" of all patient results, or (4) some combination of two or more of the preceding quality control methods (3). The precision (expressed as coefficient of variation (CV)) of routine automated hematology assays and the minimum bias which can be detected by either replicate assays of fresh blood or serial assays of stabilized blood are summarized in Table 2 (modified from reference 4). Precision is better (and thus minimum detectable bias is less) for erythrocyte than for leucocyte or platelet counts.

Table 2. Precision of Routine Automated Hematology Assays

Routine Assay (CV)	Type of Whole Blood Control	Minimum Detectable Bias (%)		
		Day	Week	Month
1. Cyanmethemoglobin (1%)	Fresh	2		
	Stabilized	2	2	2
2. RBC, by automated (<1%) counter	Fresh	2		
	Stabilized	2	4	7
3. Hct or MCV by automated (<1%) counter	Fresh	2		
	Stabilized	2	4	7
4. WBC by automated (2%) counter	Fresh	6		
	Stabilized	6	8	8
5. Platelet count by (4%) automated counter	Fresh	12		
	Stabilized	12	15	15

(Modified from Reference 4)

Markedly abnormal hematology results are to be telephoned back to the Field Center, according to the criteria specified in ARIC Manual 2.

8.6 Accuracy of Automated Hematology Procedures

Interlaboratory comparability of these data can also be evaluated by mailed proficiency-testing samples in programs operated by external organizations. All the laboratories selected by the Field Centers participate in the hematology proficiency survey of the College of American Pathologists (CAP). Table 1 also shows the other quality control procedures used by each laboratory.

The major interlaboratory variable in automated hematology which these external proficiency programs have identified is that of the material used for calibration. As indicated previously, three of the four laboratories participating in the ARIC Field Center hematology studies use the same material.

8.7 Reporting of Results

The laboratories performing automated hematology for the ARIC Field Centers have the responsibility for reporting results formatted (either manually or electronically) for incorporation into the ARIC data base. Eight data elements are included as hematology results in this data base:

1) Total hemoglobin (Hb)
2) Erythrocyte (RBC) count
3) Leucocyte (WBC) count
4) Platelet count
5) Hematocrit
6) Mean cell volume (MCV)
7) Mean cell hemoglobin (MCH)
8) Mean cell hemoglobin concentration (MCHC)

8.8 References

1. Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. Philadelphia: W.B. Saunders, 1985, pp. 586-601.

2. Savage RA. Calibration bias and imprecision for automated hematology analyzers. An evaluation of short-term bias resulting from calibration of an analyzer with S-Cal. Am J Clin Pathol 84: 186-190, 1985.

3. Lappin TRJ, Farrington CL, Nelson MG, Merrett JD. Intralaboratory quality control of hematology. Comparison of two systems. Am J Clin Pathol 72: 426-431, 1979.

4. Bull BS. The use of patient values, calibrator and control materials in the routine laboratory. In Advances in Hematological Methods: The Blood Count. Van Assenfeldt OW, England JM, eds. Boca Raton: CRC Press, 1982, pp. 217-227.

Appendix I. Tests to be Performed

Central Chemistry Laboratory (ARIC Manual Vol. 10)

Glucose
Creatinine
Insulin
Total protein
Albumin
Uric acid
Urea nitrogen
Calcium
Phosphorus
Magnesium
Sodium
Potassium

Central Hemostasis Laboratory (ARIC Manual Vol. 9)

Activated PTT
Fibrinogen
Factor VII
Factor VIII C
Von Willebrand Factor - Antigen
Fibrinopeptide A
Fibrinopeptide Bβ (1-42) & (15-42)
Beta-thromboglobulin
Platelet Factor 4
Thromboxane B2 (in serum)
Tissue Plasminogen Activator
Protein C
Antithrombin III

Central Lipid Laboratory (ARIC Manual Vol. 8)

Total Cholesterol
Total Triglycerides
HDL Cholesterol
HDL_2
HDL_3
LDL Cholesterol (calculated)
Lipoprotein Lp (a)
Apolipoprotein AI
Apolipoprotein B

Field Center Hematology Laboratory (ARIC Manual Vol. 7)

Hematocrit
White Blood Cell Count
Platelet Count

Appendix II. Equipment and Supplies

Supplies provided by the central laboratories

1. Special anticoagulant mixture to be provided by Hemostatis Lab.
2. "Millipore$_R$" filters.
3. "Centricon$_R$" filters.

Supplies to be obtained by field centers

Supplier	Catalog No.	Description	Approx. No. per Week
		Microsample tubes are interchangeable priced at $29.40 per 1,000.	
Sarsdtedt	72.690.477	Red Micrsoample Tubes 500/pk	90
"	72.690.478	Yellow Microsample Tubes 500/pk	90
"	72.690.475	Blue Microsample Tubes 500/pk	180
"	72.690.476	Green Microsample Tubes 500/pk	30
"	65.716.008	Lavender Screw Caps 500/pk	300
	65.716.009	Brown Screw Caps 500/pk	60
	72.609	2 ml White Screw Cap Vials 500/pk	360
S/P	T1226-12	White 5 ml tubes 1,000/pk	90
"	T1226-32	Caps for 5 ml tubes 1,000 pk	90
Fisher	11-676-21	Styrofoam boxes multipurpose biomailers (for mailing to Central Lipid & Central Chemistry Labs)	2
Fisher	03-530	Frozen Sample Shipper (Styrofoam boxes for mailing to Central Hemostatis Labs)	1

Miscellaneous Supplies

Abbott	4492	Butterfly Needles 40/box	45
S/P	B3035-12	Luer Adaptors BD #7226 100/pk	30
"	B3062-Swab	Alcohol Swabs 2,000/cs	45
"	B-3063-5	Gauze Sponges 200/pk	90
"	B3062-191	Band Aids 100/pk	45
"	B3060-1	Tourniquets	
"	B3035-4	Vacutainer Tube Holders 10/pk	
"	PB214-12	Transfer Pipets 500/pk	210
"	B1210-11	Freezer Bags 3" X 6" 250/pk	120
"	B1210-12	Freezer Bags 6" X 6" 250/pk	30
"	S9221	Sponge Tube Rack	
"	S9546-23G	Hypodermic Needles 23GA 100/pk	5
Unspecified	Vendor	Syringes 1 ml	1
Rainin	RT-200	Pipet tips 100-1000 µl	90
		Dry Ice approximately 6 lbs. per shipping box	25 lbs.

Supplier	Catalog No.	Description	Approx. No. per Week
		Vacutainer Tubes 100/pk	
S/P	B2970-33	Serum Separator Red/Gray BD#6512	30
"	B2980-52	Red Stopper 7 ml BD#6431	30
"	B2979-53	Clot Activator Red/Yellow BD#6422	30
"	B2994-94	Na Citrate, Blue BD#6418	60
"	B2991-54	EDTA-Lavender 10 ml BD#6457	60
"	B2991-65	EDTA-Lavender 3.5 ml BD#6458	30

Small Equipment Items

Rainin	P-1000	Automatic Adjustable Pipet 100-1000 µl
S/P	C6548-3	Digital Stopwatch
"	B2922-1	Blood Collection Trays
"	T2050-1	Thermometers -20°C - +110°C
"	B1796-Balance	Balance Harvard (Ohaus 1550SD)
"	H2025-1	Dry Heating Block - Heater
"	H2027-4	Dry Heating Block - Tube Adaptor
"	C6510-1	Timer - 3 channel digital

Equipment purchased and maintained by field centers

1. Table-top refrigerated Centrifuge
   Beckman Accuspin FR
   AH4 Swining Bucket Rotor
   Tube adaptors

2. Freezer (-70°C)
   Revco ULT 1186D (10.9 cubic ft., upright)

3. Refrigerator with crushed ice maker

4. Aerosol Covers

APPENDIX III

O.M.B. 0925-0281
exp. 7-31-89

ARIC VENIPUNCTURE FORM

ID NUMBER: [ ][ ][ ][ ][ ][ ][ ] CONTACT YEAR: [0][1] FORM CODE: [V][E][N] VERSION: A 11-01-86

LAST NAME: [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ] INITIALS: [ ][ ]

INSTRUCTIONS:
This form should be completed during the participant's visit. ID Number, Contact Year, and Name must be entered above. Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost box. Enter leading zeroes where necessary to fill all boxes. If a number is entered incorrectly, mark through the incorrect entry with an "X". Code the correct entry clearly above the incorrect entry. For "multiple choice" and "yes/no" type questions, circle the letter corresponding to the most appropriate response. If a letter is circled incorrectly, mark through it with an "X" and circle the correct response.

VENIPUNCTURE FORM (VENA screen 1 of 2)

A. BLOOD DRAWING

1. Do you have any bleeding disorders?.....YES Y
NO N
DON'T KNOW D

2. Date of blood drawing:..... [ ][ ]-[ ][ ]-[ ][ ]
month day year

3. Time of blood drawing:..... [ ][ ]:[ ][ ] AM A
PM P

4. Was blood drawn before the snack?.....YES Y
NO N

5. Number of venipuncture attempts:..... [ ]

6. Filling time of tube 1:..... [ ][ ] seconds

7. Code number of phlebotomist completing this section:..... [ ][ ][ ]

B. BLOOD PROCESSING

8. Time specimen tubes 2,4-7 were spun:..... [ ][ ]:[ ][ ] AM A
PM P

9. Time specimen tubes 1,3 were spun:..... [ ][ ]:[ ][ ] AM A
PM P

VENIPUNCTURE FORM (VENA screen 2 of 2)

10. Was the specimen visibly hemolyzed?.....YES Y
NO N

11. Time specimen was placed in freezer:... [ ][ ]:[ ][ ] AM A
PM P

12. Comments: _____

C. ADMINISTRATIVE INFORMATION

13. Code number of technician processing the blood:..... [ ][ ][ ]

VENIPUNCTURE FORM INSTRUCTIONS

11-01-86

A. BLOOD DRAWING

1. Do you have any bleeding disorders?.....YES Y
   NO N
   DON'T KNOW D

2. Date of blood drawing:..... [ ][ ]-[ ][ ]-[ ][ ]
   month day year

3. Time of blood drawing:..... [ ][ ]:[ ][ ] AM A
   PM P

4. Was blood drawn before the snack?.....YES Y
   NO N

5. Number of venipuncture attempts:..... [ ]

6. Filling time of tube 1:..... [ ][ ] seconds

7. Code number of phlebotomist completing this section:..... [ ][ ][ ]

B. BLOOD PROCESSING

8. Time specimen tubes 2,4-7 were spun:..... [ ][ ]:[ ][ ] AM A
   PM P

I. GENERAL INSTRUCTIONS

The Venipuncture Form should be completed during the participant's clinic visit to record the results of that procedure. Both technicians must be certified and should have a working knowledge of the ARIC Blood Collection and Processing Manual of Operations. Both technicians should also be familiar with and understand the document titled "General Instructions for Completing Paper Forms", prior to completing this form. ID Number, Contact Year, and Name should be completed as described in that document, prior to the arrival of the participant.

II. DETAILED INSTRUCTIONS FOR EACH ITEM

1. If the participant has a bleeding disorder, consult with the field center physician, physician assistant or nurse practitioner before proceeding with the venipuncture. If the participant does not know whether he/she has a bleeding disorder, offer the explanation, "If you had a bleeding disorder you would have symptoms like excessive nose bleeds, or very easy bruising, or problems with bleeding after tooth extractions, or any type of surgery." If the participant is still unsure, consult with field center medical personnel before going on.

2. Note the date of blood drawing on the form. Code in numbers using leading zeroes where necessary to fill all boxes. For example, May 3, 1986, would be entered as:

3. Note the time of completion of blood drawing on the form. Fill in the boxes using leading zeroes where necessary and indicate AM or PM.

4. Check the participant's Itinerary sheet, or ask the participant, to determine if blood is being drawn before the clinic snack.

5. The same technician should not attempt a venipuncture more than twice.

6. Start a timer to measure the flow rate of blood into the first blood collection tube. If the flow rate in the tube is so slow that blood does not fill the first collection tube within 50 seconds, stop the blood collection and repeat on the other arm. If blood is flowing freely, the butterfly needle can be taped to the donor's arm for the duration of the draw.

7. The phlebotomist who performed the blood drawing procedure must enter his/her code number in the boxes provided.

8. Note the time that the specified tubes were placed in the centrifuge. Fill in the boxes using leading zeroes where necessary and indicate AM or PM.

9. Time specimen tubes 1,3 were spun:..... ☐☐:☐☐ AM A / PM P

9. Note the time that the specified tubes were placed in the centrifuge. Fill in the boxes using leading zeroes where necessary and indicate AM or PM.

10. Was the specimen visibly hemolyzed?.....YES Y / NO N

11. Time specimen was placed in freezer:... ☐☐:☐☐ AM A / PM P

11. Note the time that the samples were placed in the freezer. Fill in the boxes using leading zeroes where necessary and indicate AM or PM.

12. Comments: _____

12. Include any clarifications or other information relevant to the assays being performed.

C. ADMINISTRATIVE INFORMATION

13. Code number of technician processing the blood:..... ☐☐☐

13. The technician who processed the blood must enter his/her code number in the boxes provided.

Appendix IV. ARIC Shipping Forms

1) Instructions for Use

There are two types of shipping forms, a face sheet and contents sheet. Both forms are computer generated and are included in every shipment sent. The contents sheet is composed of an original and three copies. The original and the first copy are sent to the central laboratories. The second copy is kept by the field center and the third is sent to the Coordinating Center. The central laboratories acknowledge the receipt of the specimens by returning a self-addressed postcard and a copy of the contents sheet to the field center.

The top half of the face sheet is completed by the field center.

a. The full address of the field center and the laboratory are preprinted on the forms.

b. Date and time shipment was packed and sealed at the field center.

c. An eight character shipping batch number constructed as follows: ARabnnnn, where

"AR"	is the two character study code for ARIC
a	is a one character ARIC agency code for the sending agency, defined below
b	is a one character ARIC agency code for the receiving agency, defined below
nnnn	is a sequential shipping batch number, counting all batches shipped from "a" to "b" since the beginning of the project

Example:
ARJL0003 represents the third shipment of blood specimens from Jackson to the Lipid Lab.

ARIC Agency Codes

Field Centers:	F	Forsyth County, NC
	J	Jackson, MS
	M	Minneapolis Suburbs, MN
	W	Washington County, MD
Central Agencies:	C	Clinical Chemistry Laboratory
	D	ECG Computer Center-Halifax
	E	ECG Reading Center-Minneapolis
	H	Hemostasis Laboratory
	L	Lipid Laboratory
	N	Program Office, NHLBI
	P	Pulmonary Function Reading Center
	U	Ultrasound Reading Center
	Z	Coordinating Center

d. Total number of participant samples enclosed. The technician confirms this total by both counting baggies and adding the numbers in the "specimen ID" column on the contents sheets.

e. Number of contents pages attached. The number varies depending on the number of samples in the shipment.

f. Other Remarks. Anything peculiar about the shipment is included here.

g. Initials of the person packing and filling out the shipping form.

The bottom part of the face sheet is completed by the central laboratory personnel upon arrival of the shipment.

a. Time and date shipment arrived.

b. Comments on condition of the total shipment; e.g., shipment totally thawed.

c. Initials.

The second shipping form is the contents sheet. Each central laboratory has its own specific contents sheet. More than one contents sheet may be included in each shipment depending on the number of specimens enclosed. The number of pages attached and each page number are filled in at the top of the contents page. This form is filled out as the specimens are collected and stored. In this case, the form must is checked against the specimens when packaged for shipment.

a. Specimen ID#. The ID number is entered in these spaces. This is most easily accomplished by attaching one of the adhesive Participant ID number labels in the space provided. It is suggested that a second person check these ID's against the vials' ID's to correct any errors.

b. Color vial. The color of the appropriate vial(s) is indicated here. It is especially important when shipping the multiple vials to the Central Hemostasis and Central Lipid laboratories.

c. Number vials. The number of that same size and colored vial with the same ID # is indicated; e.g., there are 3 red vials sent to the Hemostasis laboratory.

d. Comments. Optional. To be completed by the laboratory indicating specific problems per ID#; e.g., tube broken, thawed.

e. Arrived. To be completed by the central laboratory for inventory purposes and acknowledgement of receipt.

ARIC SHIPPING FORM

Face Sheet

TO: _____
_____
_____
_____

FROM: _____
_____
_____
_____

FIELD CENTER TIME : DATE

ARIC BATCH NUMBER AR ___ ___ ___ ___ ___ ___

REPORTING PERIOD:

STARTING DATE:

ENDING DATE:

TOTAL NUMBER SPECIMENS ENCLOSED:

NUMBER OF CONTENTS PAGES ATTACHED:

Other remarks concerning shipment contents: _____
_____
_____

Initials: _____

****

ARRIVE AT LABORATORY TIME : DATE

Comments on condition of total shipment on arrival: _____
_____
_____

Initials: _____

ARIC SHIPPING FORM
CENTRAL HEMOSTASIS LABORATORY
UNIVERSITY OF TEXAS MEDICAL SCHOOL
6431 FANNIN
HOUSTON, TX 77030

SPECIMEN ID	COLOR	NO. OF VIALS	FIELD CENTER COMMENTS	CONDITION ON ARRIVAL
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			
	BLUE			
	RED			
	YELLOW			
	GREEN			

ARIC SHIPPING FORM
DR. WOLFGANG PATSCH
ATHEROSCLEROSIS CLINICAL LABORATORY
METHODIST HOSPITAL, MAIL STATION F701, ROOM F756
6565 FANNIN
HOUSTON, TX 77030

SPECIMEN ID	COLOR	NO. OF VIALS	FIELD CENTER COMMENTS	CONDITION ON ARRIVAL
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			

ARIC SHIPPING FORM
CENTRAL CHEMISTRY LABORATORY
UNIVERSITY OF MINNESOTA HOSPITAL - RECEIVING UNIT K/E
425 EAST RIVER ROAD
MINNEAPOLIS, MN 55455

SPECIMEN ID	COLOR	NO. OF VIALS	FIELD CENTER COMMENTS	CONDITION ON ARRIVAL
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			
	WHITE			

CHECKLIST
BLOOD DRAWING STATION

Stopwatch

Alcohol Wipes

Gauze Squares

Vacutainer Holder

Butterfly Adaptor

Butterfly Needles

Tourniquet

Band Aids

Ice water bath

1	Red and Gray Top 13 ml tube - labeled with donor ID number
1	Red and Yellow 7 ml tube - labeled
1	Red 7 ml tube with liquid anticoagulant - labeled in ice water bath
2	Blue 4.5 ml tubes - labeled
2	Lavender 10 ml tubes - labeled
1	Lavender 3 ml tube - labeled

Appendix V. Checklists and Logs

ARIC
DAILY TEMPERATURE RECORD

DATE M D Y	Htg. Block	Freezer	Refrig	Room	Initials	DATE M D Y	Htg. Block	Freezer	Refrig	Room	Initials

Appendix VII. Certification

ARIC BLOOD DRAWING PROCESSING CERTIFICATION OR RECERTIFICATION

CENTER _____

DATE _____ TIME _____

CERTIFYING SUPERVISOR _____ ID NUMBER ___/___/___

TECHNICIAN _____ ID NUMBER ___/___/___

		SATISFACTORY	UNSATISFACTORY	COMMENTS
A.	Equipment, environment			
1.	Isolated room, professional environment	____		_____
2.	Equipment, forms, supplies adequate (needles, vacutainers, bandaids, alcohol swabs, gauze, tourniquet, ice bath, ammonia inhalents, butterfly needles, butterfly adapter, syringes)	____		_____ _____ _____
B.	Equipment			
1.	Equipment, supplies adequate	____		_____
2.	Equipment working correctly, centrifuge at 4°C	____		_____
3.	Daily record of refrigerator, freezer, and room temperatures up to date	____		_____ _____
4.	Biohazard labels available	____		_____
5.	Other _____	____		_____
C.	Phlebotomy Procedure			
1.	Label checked	____		_____
2.	Participant prepared, procedure explained	____		_____
3.	Time and date participant last ate queried and recorded	____		_____
4.	Hepatitis or AIDS queried and recorded	____		_____

		SATISFACTORY	UNSATISFACTORY	COMMENTS
5.	Bleeding disorders queried and recorded	____		_____
6.	Needle, adapter, vacutainer prepared	____		_____
7.	Tourniquet applied properly	____		_____
8.	Vein palpated, cleansed, and dried	____		_____
9.	Venipuncture technique	____		_____
10.	Tubes filled in proper order and inverted 8 times	____		_____
11.	Tourniquet released as soon as flow starts in last tube	____		_____
12.	Total tourniquet time within 2-minute limit	____		_____
13.	Vacutainers filled	____		_____
14.	Stasis obtained, bandaged	____		_____
15.	Needle disposed	____		_____
16.	Tubes labeled	____		_____
17.	Form completed accurately	____		_____
18.	Other _____	____		_____
D.	Processing			
1.	Vacutainers labeled	____		_____
2.	Tubes: correct temperatures	____		_____
3.	Centrifuge balanced	____		_____
4.	Centrifuge operation	____		_____
5.	Aliquotting equipment ready, vials labeled and organized, biohazard labels available	____		_____
6.	Proper specimen volumes in respective vials	____		_____

		SATISFACTORY	UNSATISFACTORY	COMMENTS
7.	Sealing of vials			
8.	Completion of Phlebotomy Form page 2 (F, N, E code)			
9.	Freezer organization and storage (separate tray for each lab)			
10.	Time constraints observed throughout procedure (90 min. maximum drawing to freezing)			
11.	Disposal of blood tubes and contaminated equipment			
12.	Other _____			
E.	Shipping			
1.	Analysis of CBC within 12 hours			
2.	Knowledge of shipping schedule for each lab			
3.	Dry ice available			
4.	Supplies adequate (specimen trays, insulated shipping containers, forms)			
5.	Specimens for other labs packed properly (container, sufficient dry ice, forms, return envelope)			
6.	Specimens remain frozen while being packed			
7.	Storage vial held until proper shipping time			
8.	Paper work completed			
9.	Other _____			

ARIC QUALITY CONTROL PHANTOM PARTICIPANT AND NON-PARTICIPANT ID FORM

Form Code: PNPA Record Type: 028

Note: This form should be sent to the Coordinating Center: (1) the same week it is filled out for a non-participant's ID, or (2) within two weeks of the first entry for a QC phantom.

Phantom Participant
ID Number _____ Contact Year: _____

This ID is for (circle one): P A QC Phantom Participant
N An ID used for a Non-Participant

Date ID Assigned: ____/____/____ ID of Person Assigning ID: _____

Venipuncture Phantom QC Log

Tube	Matching Participant ID	Date Drawn (Mo/Day/Yr)	Technician ID
1	_____	__/__/__	_____
2 & 3	_____	__/__/__	_____
4 & 5	_____	__/__/__	_____
6 & 7	_____	__/__/__	_____
8	_____	__/__/__	_____

Anthropometry Phantom QC Log

Procedure	Matching Real Participant ID	Date of Meas. (Mo/Day/Yr)	Technician ID
Measurement Group D (triceps skinfold, subscapular skinfold, wrist breadth)	_____	__/__/__	_____
Measurement Group G (waist girth, hip girth, unadjusted sitting height, stool height)	_____	__/__/__	_____

PRACTICAL EXAM FOR ARIC BLOOD DRAWING TECHNICIAN

1. Place the following 20 blood collection tubes in the correct order for the venipuncture: 4-11 ml red and gray tops; 3-7 ml red and yellow tops; 3-7 ml red tops with anticoagulant; 3-5 ml blue tops; 3-10 ml lavender tops and 4-3 ml lavender tops.

2. Specify which of these tubes go into the heating block, and for how long?

3. Specify which tube(s) go into an ice bath. How long before collection and how long after collection should the tubes remain on ice?

4. Remove the appropriate tubes from the tray, balance them and place them in the centrifuge. How long should they spin? At what speed?

5. Set up a sponge tray with the appropriate number, color and order of 10 of each color microsample tubes (white, red, yellow, green, blue, lavender).

6. Place the collection tubes in front of their respective colored sample tubes. Describe what further processing is required of each collection tube before it is aliquotted into its respective sample tube.

7. Divide the colored sample tubes and place them in bags according to their final destination.

8. Describe the quality control for each piece of equipment.

SAMPLE
WRITTEN EXAM

1. What anticoagulant is in the 10 ml red and yellow stoppered tube?
   a) 3.8% sodium citrate
   b) A special antiplatelet, enzyme inhibitor cocktail
   c) EDTA
   d) None of the above

2. Which tube(s) contains a special mixture of enzyme inhibitors and antiplatelet anticoagulants?
   a) The 13 ml red and gray top
   b) The 7 ml red top
   c) The 7 ml red and yellow top
   d) The 4.5 ml blue tops

3. What temperature should the red and yellow top tube be kept at after the blood collection?
   a) 37°C
   b) Room temperature
   c) 0°C (ice water bath)
   d) -20°C

4. The contents of which tube(s) are the most sensitive to differences in venipuncture?
   a) The 7 ml red top
   b) The 7 ml red and yellow top
   c) The 4.5 ml blue tops
   d) The 10 ml lavender tops

5. Which tube(s) needs to be filtered to remove remaining platelets from plasma?
   a) The 3 ml lavender top
   b) The 4.5 ml top
   c) The 7 ml red top
   d) None of the above

6. Which tube is drawn last?
   a) A 3 ml lavender top
   b) A 4.5 ml blue top
   c) A 7 ml red and yellow top
   d) An 11 ml red and gray top

7. Which tube(s) contain unstable factors that must be kept cold while being processed?
   a) The 3 ml lavender top
   b) The 4.5 ml blue top
   c) The 7 ml red and yellow top
   d) The 13 ml red and gray top

8. What type of studies will the 10 ml lavender top tubes be used for?
   a) Chemistry
   b) Lipid
   c) Coagulation
   d) Hematology

9. What lab does the serum from the 13 ml red and gray top tube go to?
   a) Clinical Chemistry laboratory
   b) Central Lipid laboratory
   c) Central Hemostasis laboratory
   d) Field Center Hematology laboratory

10. Which tube is sensitive to incubation temperature and time?
   a) The 13 ml red and gray top
   b) The 7 ml red and yellow top
   c) The 7 ml red top
   d) All of the above

True or False

11. The factors being analyzed from the Clinical Chemistry tube are stable for up to 2 hours at 37°C?

12. The 7 ml red and yellow top tube must be put into a 37°C heating block within 1 minute after being drawn?

13. The 7 ml red top tube has a special anticoagulant to prevent enzymatic breakdown of lipids?

14. The frozen serum samples in the red microsample tubes are sent to the Clinical Chemistry laboratory?

15. The Central Lipid laboratory samples are less sensitive to venipuncture technique than the Hemostasis Laboratories.