Jackson Heart Study Protocol

Manual 9

Central Laboratory and Specimen Repository
Specimen Collection and Processing

Visit 1

Version 1.0

February 15, 2001

For Copies, Please Contact:

Jackson Heart Study Coordinating Center
Jackson Medical Mall
350 W. Woodrow Wilson Dr.
Jackson, MS 39213

FOREWORD

This manual is one of a series of protocols and manuals of operation for the Jackson Heart Study (JHS). The complexity of the JHS requires that a sizeable number of procedures be described, thus this rather extensive list of materials has been organized into the set of manuals listed below. Manual 1 provides the background, organization, and general objectives of the JHS Study. Manuals 2 and 10 describe the operation of the Cohort and Surveillance Components of the study. Detailed Manuals of Operation for specific procedures, including those of reading centers and central laboratories, make up Manuals 3 through 9 and 11.

JHS Study Protocols and Manuals of Operation

MANUAL	TITLE
1	General Description and Study Management
2	Cohort Component Procedures
3	Family Study
4	Blood Pressure
5	Electrocardiography
6	Echocardiography
7	Ultrasound Assessment
8	Pulmonary Function Assessment
9	Specimen Collection and Processing
10	Morbidity and Mortality Classification Manual
11	Data Management System

Manual 9. Central Laboratory and Specimen Repository Specimen Collection and Processing

Table of Contents

Manual 9. Central Laboratory and Specimen Repository Specimen Collection and Processing

Appendices

Manual 9. Central Laboratory and Specimen Repository Specimen Collection and Processing

Tables

Figures

1.0 PURPOSE

The Jackson Heart Study (JHS) provides a framework for research into the genetic, biochemical, epidemiological, and physiological causes of cardiovascular disease in African-Americans. The study participants will include 6,500 African-Americans between the ages of 35-84 years. Female and male participants will be equally represented. Blood and urine samples and other information on these individuals will be extensively analyzed to determine how best to prevent and treat cardiovascular disease.

Jackson, MS is the site of the only Exam Center involved. The technicians at the exam center collect blood and urine specimens, process them, and ship them to the Central Laboratory at Fairview-University Medical Center in Minneapolis, MN. Specimens collected for immediate testing and specimens collected for long-term storage are both sent to the Central Laboratory.

The Central Laboratory performs general blood and urine chemistry tests, routine plasma lipid tests (cholesterol, triglycerides, and HDL-cholesterol), and other more specialized analyses. Other routine chemistry and hematology tests are performed locally. A complete list of tests performed is located in Appendix 1.

The foundation on which all of these tests are based is the blood and urine samples that are collected and processed by the technicians at the Exam Center. Probably the most important step (and potentially the most variable) is the collection and processing of the samples. Laboratory tests can be repeated, but if the sample itself is not correctly collected and processed, the laboratory results may be precise, but perhaps not reflective of the *in vivo* state. It is important that this study measures true differences between participants rather than differences in collection procedures. The JHS depends heavily on the Exam Center technicians who perform the blood and urine collection and sample processing. It is important that these people be not only well trained and competent at drawing blood and processing the blood and urine, but also willing to take pride and responsibility in their work.

2.0 PREPARATION

2.1 Participant Contact

Since the study depends on the voluntary participation of participants, every effort must be made to make the entire procedure as easy and painless as possible for them. The technicians 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 technicians to be thoroughly knowledgeable about all aspects of the procedures. The JHS involves the collection of approximately 97 mL of blood from each participant. Twelve tubes of blood are collected. Any participant who is concerned about the volume of blood should be reassured that the total amount of blood drawn is only about three ounces, although it may look like more. The technician may also assure participants that they donate five times as much blood (450 mL) when they donate a pint of blood. Participants will also be asked to collect their urine for a 24- or 48- hour period. Advise the participants to schedule this procedure during a time when they know they will be at home, such as a weekend. The technicians and the clerk should be wearing a clean laboratory coat.

As part of JHS, a 24- or 48-hour urine collection is obtained from every participant. The participant is given instructions and supplies for this procedure at the conclusion of their Exam Center visit. Containers are returned to the Exam Center at a later date.

2.2 Staff Certification Requirements

A certified JHS technician at the Exam Center performs the blood drawing and blood and urine processing. Technicians complete a training course taught by certified laboratory staff. Each technician must complete the training and pass both written and practical exams before becoming JHS-certified. Re-certification takes place annually and is authorized by supervisory personnel.

2.3 Blood Collecting Trays and Tubes

Prior to venipuncture two trays are prepared for each participant. One tray holds the Vacutainer tubes used in the blood collection. The other tray holds the various plastic microvials which contain the final serum, plasma, and urine aliquots that are sent to the Central Laboratory and local laboratory for analyses. The collection tubes and storage microvials are labeled with LABID numbers. A list of equipment, suppliers, and vendors is provided in Appendix 2.

2.3.1 Blood Collection Tray

First, the technicians 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 individual compartments, filled with the following supplies.

- A test tube rack to hold the 12 blood collection tubes drawn from each participant. These tubes are described in detail in the next section.
- Sterile, disposable 21 gauge butterfly needles
- Plastic Vacutainer tube guides
- Vacutainer Luer adapters
- Sterile alcohol swabs
- Gauze sponges

- Tourniquets
- Bandages ("Band Aids")
- Smelling salts, ice packs, and wash cloths should be readily available in the specimen collection area for patients who become faint during the blood draw

2.3.2 Blood Collection Tubes

Draw about 97 mL of blood from each participant using 12 Vacutainer tubes. Specimens from these 12 blood collection tubes are used in approximately 20 different biochemical 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:

Tubes #1 and #2 are 10-mL red and gray-stoppered tubes filled with 9.5 mL of blood. This tube does not contain anticoagulant, so it does not need to be mixed following collection. After drawing, allow the blood to clot at room temperature for 30 minutes. Following centrifugation, some of the serum is used in local testing, while the remainder is frozen and sent to the Central Laboratory. 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 much longer than 30 minutes, the serum glucose levels can be artifactually decreased.

Tubes #3 and #4 are 10-mL lavender-stoppered tubes containing the liquid anticoagulant EDTA. The whole blood from this tube is used to measure glycosylated hemoglobin. The plasma from these tubes is used for testing related to the causes of hypertension. After each tube is fully filled with blood, invert four times then place into a room temperature rack (tube #3) or ice bath (tube #4) until centrifugation. Write the number “4” on the stopper of tube #4 to differentiate it from tube #3 and tube #11.

Tubes #5 and #6 are 4.5 mL blue-stoppered tubes containing the liquid anticoagulant sodium citrate. The plasma from this tube is used for coagulation studies. After this tube is fully filled with blood, invert four times then place into a room temperature rack until centrifugation.

Tubes #7 and #8 are 8.5 mL black-and-blue-stoppered tubes containing citrate anticoagulant and ficoll hypaque cell separation media (Cell Preparation Tube or CPT). This tube is used to isolate lymphocytes for cryopreservation and, ultimately, transformation. After this tube is fully filled with blood, invert four times then place into a room temperature rack until shipment. These tubes are not centrifuged.

Tubes #9 and #10 are 8.5 mL yellow-stoppered tubes containing acid citrate dextrose (ACD) anticoagulant. This tube is used for the extraction of DNA used in genotyping analyses. After this tube is fully filled with blood, invert four times then place into a room temperature rack until shipment. These tubes are not centrifuged.

Tube #11 is a 10 mL lavender-stoppered tube containing the liquid anticoagulant EDTA. Also added to this tube is aprotinin, an anti-protease agent. This addition is performed by exam center technicians, and is described in section 2.6. In order to differentiate this tube from tubes #3 and #4 write the number “11” on the stopper. After this tube is fully filled with blood, invert four times then place into an ice bath until centrifugation.

Tube #12 is a 5 mL lavender-stoppered tube containing the liquid anticoagulant EDTA. The blood from this tube is used for hematology testing. After this tube is fully filled with blood, invert four times then place into a room temperature rack until analysis.

2.4 Blood Collection Tubes: Labeling and Set-up

Twelve tubes are drawn in the following sequence:

Tube #1 and #2: 9.5-mL red and gray-stoppered tube
Tube #3 and #4: 10-mL lavender-stoppered tube (EDTA)
Tube #5 and #6: 4.5-mL blue-stoppered tube (Citrate)
Tube #7 and #8: 8.5-mL black and blue-stoppered tube (CPT)
Tube #9 and #10: 8.5-mL yellow-stoppered tube (ACD)
Tube #11: 10-mL lavender-stoppered tube with aprotinin (EDTA/aprotinin)
Tube #12: 5-mL lavender-stoppered tube (EDTA)

Attach pre-numbered adhesive LABID labels to each Vacutainer tube and each plastic microvial prior to blood collection. Place the labels on the tubes vertically, with the letter designator at the bottom end of the tube. Arrange the set of tubes in a test tube rack. When the participant arrives for the Exam Center visit, place a LABID label on the Venipuncture form along with the participant's JHS ID number. Handle only one participant's specimens at a time so the chance of mislabeling is minimized. Each center must keep a permanent record of the matching of the JHS ID number to the laboratory ID number (e.g., participant log).

Select a number of JHS participants to donate duplicate samples for analysis. Assign duplicate samples a unique laboratory ID number and ship to the Central Laboratory one week later. This is described more completely in the Quality Control Section.

2.5 Sample Aliquot Tubes: Labeling and Set-up

The technician prepares a tray of the plastic microvials that will contain the final samples that will be assayed locally or shipped to the Central Laboratory for each participant. Each type of microvial has a corresponding color-coded screw cap that fits onto it. The technicians should be trained to organize the tray for the sample processing as follows:

2.5.1 Sample Tray

The tray itself should be a flexible sponge test tube rack that will fit tubes from 10-16 mm in diameter. The tray has 5 rows and 10 columns. The columns are numbered 1-10 from left to right. The rows are lettered A-E from top to bottom.

2.5.2 Organization

The technician needs the following supplies for each sample tray:

10 - 2.0-mL polypropylene vials (red top)
6 - 2.0-mL polypropylene vials (purple top)
3 - 2.0-mL polypropylene vials (blue top)
3 - 2.0-mL polypropylene vials (yellow top)
1 - 2.0-mL polypropylene vials (black top)
6 - 2.0-mL polypropylene vials (clear top)
2 - 5-mL polypropylene vials

Vertically label the microvials with the LABID number (letter designator at bottom) and arrange in the sample tray in the following order:

Col 1: 2.0-mL red top vials (rows A-E)
Col 2: 2.0-mL red top vials (rows A-E)
Col 3: 2.0-mL purple top vials (rows A-C)
Col 4: 2.0-mL purple top vials (rows A-C)

Col 5: 2.0-mL blue top vials (rows A-C)
Col 6: 2.0-mL yellow top vials (rows A-C)
Col 7: 2.0-mL black top vial (row A)
Col 8: 2.0 mL clear top vials (rows A-C)
Col 9: 2.0 mL clear top vials (rows A-C)
Col 10: 5 mL vials (rows A-B)

All extra labels are returned to the Central Laboratory with the weekly specimen shipment.

2.6 Preparation for Specimen Collection

Prepare for specimen collection in the following manner. In the early morning, prior to drawing blood from the 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 supply of anti-protease collection tubes. If additional tubes need to be prepared, follow these steps:
   a. Using a tuberculin syringe withdraw 0.4 mL of aprotinin from the stock bottle.
   b. Insert the needle into the stopper of a 10-mL EDTA collection tube.
   c. Allow the vacuum to draw the aprotinin into the tube.
   d. Mark the manufacturer's label on the collection tube with a yellow highlighter pen to indicate that aprotinin has been added to the tube. When the LABID label is placed onto the tube mark it with a yellow highlighter as well.
   e. The prepared tubes may be stored in the refrigerator for up to one week.

3. Check that each Vacutainer tube is properly labeled with the appropriate LABID number.

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

5. Check that each microvial is labeled with the appropriate laboratory number.

6. Perform and record quality control (QC) check on centrifuge temperature (4°C $\pm$ 2°C).

7. Perform and record QC check on refrigerator temperature (4°C $\pm$ 2°C).

8. Perform and record QC check on freezer temperature (-70°C $\pm$ 10°C).

9. Perform and record QC check on room temperature.

At participant arrival:

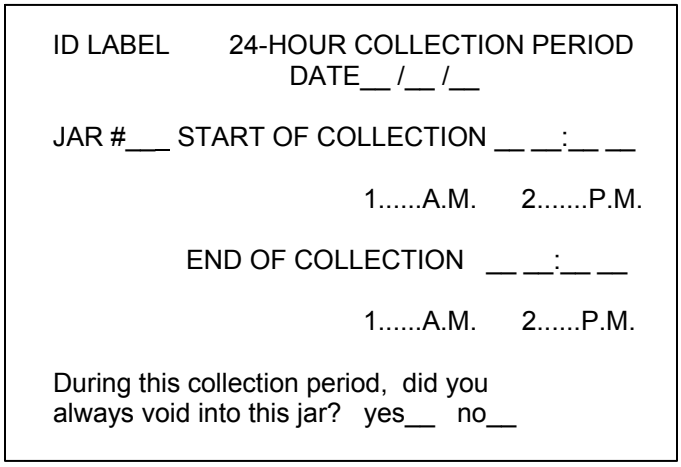
1. Place the LABID label (labeled on the collection tubes and aliquot vials) on the Venipuncture Form. Make sure the JHS ID number on the Venipuncture Form is correct.

2. Check that Quality Control tubes are prepared and labeled, if needed (see Quality Control section of this manual for details).

2.7 Urine Collection Jar Preparation

Prior to the regular clinic visit, urine collection jars (1 for a 24-hour period plus 1 extra in case the participant indicates that they have high urine output - everyone gets asked) need to be prepared for each participant. To prepare jars, place a level tablespoon of crystalline boric acid (8 gm) into each jar and put on the lid. Urine labels are then affixed to each jar. The collection label is shown below:

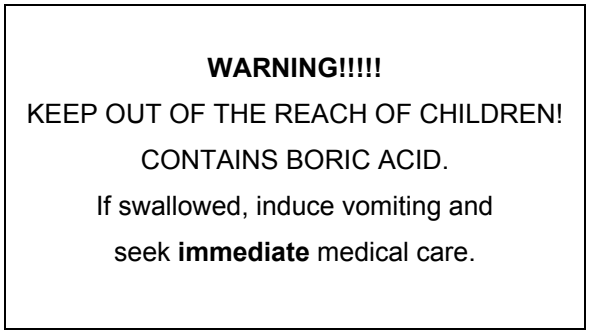
Figure 1. Urine Collection Jar Identification Labels



The collection jar labels should be marked by the technician for each 24-hour period. For the first 24-hour collection period, the word "FIRST" should be written in the blank preceding "24-HOUR COLLECTION PERIOD" for each jar given. For the second 24-hour collection period (for participants collecting for 48 hours), the word "SECOND" should be written in the blank preceding "24-HOUR COLLECTION PERIOD" for each jar given. The "JAR #" will be left blank and the participant will be instructed to complete this information as each jar is used. Thus, the participant will write in the numbers "1" or "2" as needed throughout each 24-hour collection.

In addition, an orange warning label is to be affixed to the side of the collection jar (under the collection label) to remind the participant that the collection jar contains boric acid. A sample warning label is given below.

Figure 2. Urine Collection Jar Warning Label



After the preparation of the collection jars, a collection kit should be assembled for each participant. A collection kit consists of one large carrying case containing the 1 or 2 prepared collection jars (2 or 4 for the 48-hour collection), a small empty carrying case which can hold 1 collection jar and, for women only, a plastic pan to assist in urine collection. Also, each collection jar will be placed in a plastic bag to guard against leaks. A list of items that are part of the collection kit is given below:

1. 1 or 2 prepared collection jars (2 or 4 for 48-hour group);
2. 1 or 2 plastic bags with draw-string tops (2 or 4 for 48-hour group);
3. one large carrying case;
4. one small carrying case; and
5. for women only, one plastic pan with a draw-string plastic bag.

2.8 Venipuncture Form and Urine Collection Form

Enter the participant's JHS ID Number and name on the Venipuncture form when the participant arrives for the visit. At the completion of specimen collection and processing, the entire original Venipuncture Form is kept on file at the Exam Center.

The original Urine Collection Form is also kept on file at the Exam Center.

3.0 VENIPUNCTURE

3.1 Precautions for Handling Blood Specimens

Handle all specimens as potentially infectious for laboratory workers. OSHA rules mandate that technicians must always wear disposable protective gloves when collecting and processing specimens.

Use 0.5% sodium hypochlorite (household bleach diluted 1:10) to clean up any spills of blood, plasma, or serum.

OSHA regulations require that all needles and sharp instruments be discarded into puncture resistant containers.

Avoid formation of potentially infectious aerosols when removing the rubber stoppers from Vacutainer tubes. In addition to wearing protective gloves, hold a piece of gauze over the stopper while slowly removing it from the tube.

Place all used Vacutainer tubes and blood-contaminated products in biohazard bags for proper disposal.

3.2 Phlebotomy Room

The blood drawing takes place in an isolated room or in an area where participants are separated by room dividers.

3.3 Participant Preparation

Informed consent must be obtained from the participant before drawing blood. This procedure is followed to ensure that the participants 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 participants that although there may be some minor discomfort, their blood will be drawn by trained technicians. The consent statement also states that a copy of clinically relevant test results is sent to their physicians and that they will be contacted if clinically important tests are abnormal, if so desired by the participant.

Complete the JHS Venipuncture Form with the participant.

Blood drawing is standardized to the supine position

Give the participant enough time to feel comfortable after the blood collection, as well. In many cases the most memorable part of the experience for participants will be the contact with the technicians who draw the blood and their general attitude and competence.

If the participant is nervous or excited, the technician briefly describes the procedure, e.g., "I am going to be drawing about three ounces of blood. This blood will be used in tests for lipids (or fats) and cholesterol and other chemistry tests. We hope to be able to use the results of these tests to determine some of the causes of heart disease."

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 just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood.

3.4 Venipuncture

Before applying the tourniquet, screw the Luer adapter into the plastic Vacutainer tube guide. Insert the butterfly tubing onto the adapter.

With jacket or sweater removed, have the participant lie supine with the sleeves rolled up to expose the antecubital fossa (elbow). The preferred arm to draw from is the left arm. The right arm should be used only if blood collection is not possible from the left arm. This does not mean you must stick the left arm. Only do so if an adequate vein is apparent.

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 minutes. To do so may result in hemoconcentration or a variation in blood test values. If a tourniquet must be applied for preliminary vein selection, and it remains on the arm for longer than two minutes, it should be released and reapplied after a wait of two minutes. Instruct the participant that he/she should not clench their fist prior to the venipuncture. Doing so could cause fluctuations in the results in several of the analytes being measured. Specifically, it could artifactually raise the serum potassium level. If the participant has a skin condition, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin. Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.

Identify the vein, then 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, cleanse the site again with alcohol.

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. DO NOT HAVE THE PARTICIPANT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN.

4. After blood has appeared in the butterfly tubing insert tube #1 into the plastic vacutainer tube guide. Grasp the flange of the tube guide and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle. The tube should begin filling with blood.

5. Once the draw has started, do not change the position of a tube until it is withdrawn from the needle. If blood is flowing freely, remove the tourniquet after two minutes. A tourniquet may be reapplied during the collection to spare the participant a restick, but the tourniquet must not be on for more than two minutes.

6. Keep a constant, slight forward pressure on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow.

7. Fill each Vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a Vacutainer tube fills only partially, remove the tube and attach another without removing needle from vein.

8. When the blood flow into the collection tube ceases, remove the tube from the holder. The shutoff valve covers the point, stopping blood flow until the next tube is inserted (if necessary). Tubes which require mixing (#3 through #12) should be gently inverted four times immediately following removal of the tube from the adapter, then placed into a room temperature rack or ice bath, as appropriate.

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

1. Turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.

2. 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 either arm. The same technician should not attempt a venipuncture more than twice. If a third attempt is necessary, a different phlebotomist should attempt the venipuncture.

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.

At the conclusion of the blood draw:

1. Remove the last collection tube from the Vacutainer tube holder prior to removing the needle from the participant's arm. Lightly place clean gauze over the venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard the butterfly needle, adapter and Vacutainer tube holder into a needle box. DO NOT ATTEMPT TO RECAP NEEDLES! Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.

2. If blood flow stops before collecting tube #12, repeat the venipuncture, collecting only the unfilled tubes from the previous attempt. A tourniquet may be applied in this case but should be released if possible as soon as blood flows into the first tube. As always, the tourniquet must never be on for longer than two minutes.

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 participant 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 participant to leave the bandage on for at least 15 minutes.

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

1. Have the person remain lying down with legs elevated.

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 seated or lying down until he/she feels better.

5. Have someone stay with the person to prevent them from falling and injuring themselves if they should faint.

6. Place a cold wet cloth on the back of the person's neck or on their forehead.

7. Once the episode has passed, some fruit juice may be given to the participant in order to counteract any possible hypoglycemia due to their pre-clinic visit fast.

8. 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

To invert tubes, hold the tube horizontal to the floor. Slowly tip the stopper end down while watching the air bubble rise to the butt. Now, lower the butt end slightly while watching the bubble float to the stopper (1st inversion). Invert each tube, except #1 and #2, four times. Four inversions should take 6 to 8 seconds.

1. Draw tubes #1 and #2 (10-mL red and gray top). Place the tubes in a rack at room temperature.

2. Draw tube #3 (10-mL lavender top). Invert four times and place in room temperature rack.

3. Draw tube #4 (10-mL lavender top). Invert four times and place in ice bath.

4. Draw tubes #5 and #6 (4.5-mL blue top). Invert four times and place in room temperature rack.

5. Draw tubes #7 and #8 (8.5-mL black and blue top). Invert four times and place in room temperature rack.

6. Draw tubes #9 and #10 (8.5-mL yellow top). Invert four times and place in room temperature rack.

7. Draw tube #11 (10-mL lavender top with aprotinin). Invert four times and place in ice bath.

8. Draw tube #12 (5-mL lavender top). Invert four times and place in room temperature rack.

9. Finish venipuncture.

4.0 BLOOD AND URINE PROCESSING

Processing of the various blood samples is divided into 3 stages.

4.1 Stage One: Immediate Processing

Tubes #1 and #2 remain at room temperature for thirty minutes to allow the blood to clot (blood at 4°C clots extremely slowly). Set a timer for 30 minutes as a reminder to centrifuge these tubes.

4.1.1 Whole blood for glycosylated hemoglobin

Invert tube #3 well, then remove the stopper.

Using a transfer pipet, aliquot 1 mL of whole blood into the microvial in column 7, row A.

Fasten a black screw cap onto the microvial, and move the microvial to a refrigerated test tube rack. This vial must not be frozen.

Replace the stopper on the collection tube and proceed with processing.

4.1.2 Centrifugation

Place tubes #3, #4, #5, #6 and #11 in the centrifuge trunions. Balance the centrifuge then spin these tubes at 3,000 x *g* for 10 minutes at 4ºC.

Wait for centrifuge to come to a complete stop. Remove the tubes from the centrifuge as soon as possible. Proceed to stage two processing.

4.2 Operating the Centrifuge

Refer to Centrifuge Operating Manual for specific operating and balancing instructions. In order to achieve a 3000 x *g* centrifugal force within the centrifuge, the corresponding revolutions per minute (RPM) will vary from centrifuge to centrifuge depending on radius of the centrifuge's rotor. Consult the centrifuge's operating manual for the appropriate RPM for each centrifuge.

4.3 Stage Two:

Approximately 15 minutes after venipuncture.

4.3.1 Lavender-stoppered Tube (Tube #3)

1. Remove tube from the centrifuge and put it in the sponge test tube rack holding the microvials labeled with the corresponding laboratory number. Remove the stopper.

2. Using the plastic transfer pipette, and being careful not to disturb the cell layer, remove the clear plasma supernatant from tubes #3. The pipette tip should not get any closer than one-half inch from the cells. Equally transfer the plasma into the three 2.0-mL microvials in column 3.

3. Fasten the purple screw caps onto the microvials in column 3, and leave them in the sponge rack.

4. Re-stopper collection tube #3, and discard it in a biohazard waste bag.

5. Leave the sponge rack holding the filled aliquot vials at room temperature until it is time to remove the serum from tube #1. The EDTA plasma vials must not be refrigerated. They are to remain at room temperature until placed in the freezer.

4.3.2 Lavender-stoppered Tube (Tube #4)

1. Remove tube from centrifuge. Check for number “4” on stopper. Remove the stopper.

2. Using a plastic transfer pipette, and being careful not to disturb the cell layer, remove the clear plasma supernatant from tube #4. The pipette tip should not get any closer than one-half inch from the cells. Equally transfer the plasma into the three microvials in column 4. Highlight the LABID label on these specimens with a purple marker.

3. Fasten purple screw caps onto these microvials, and transfer them to a -70ºC freezer immediately.

4. Re-stopper collection tube #4, and discard it in a biohazard waste bag.

4.3.3 Blue-stoppered Tubes (#5, #6)

1. Remove the tubes from the centrifuge. Remove the stoppers.

2. Using a plastic transfer pipette, take only about the top 2/3 of the plasma (the bottom 1/3 of the plasma contains platelets and these are to be avoided) and transfer the plasma into each of the three 2-mL microvials in column 5.

3. Fasten blue caps on the microvials and place them in the sponge rack.

4. Re-stopper collection tubes #5 and #6, and discard them in a biohazard waste bag.

Leave the sponge rack holding the aliquot vials at room temperature until tube #1 is ready.

4.3.4 Lavender-stoppered anti-protease tube (#11)

1. Remove the tube from the centrifuge. Check for number “11” on stopper. Remove the stopper.

2. Using a plastic transfer pipette, and being careful not to disturb the cell layer, remove the clear plasma supernatant from tube #11. The pipette tip should not get any closer than one-half inch from the cells. Equally transfer the plasma into the three 2.0-mL microvials in column 6. If, after leaving one-half inch of plasma on the cells, there is still too much plasma to fit in the three microvials, then that excess amount can be discarded.

3. Fasten the yellow screw caps onto the three microvials in column 6, and immediately transfer them to a -70ºC freezer.

4. Re-stopper collection tube #11 and discard it in a biohazard waste bag.

4.4 Stage Three

Stage three begins approximately 30 minutes after venipuncture.

As soon as possible after the 30 minutes timer goes off, and not longer than 45 minutes after blood collection, centrifuge tubes #1 and #2 at 3,000 x *g* for 10 minutes at 4ºC.

4.5 Final Blood Processing

1. Remove the red and gray top tubes from the centrifuge and place them in the sponge test tube rack.

2. Remove the stoppers from tubes #1 and #2. Using a plastic transfer pipette, aliquot all of the serum equally into the ten tubes in columns 1 and 2.

3. Fasten red screw caps on each of these vials.

4. Replace the stopper on the red and gray-stoppered blood collection tubes and discard them in a biohazard waste bag.

5. Place two of the microvials into a refrigerated rack for analyses in the Jackson laboratory.

6. Place the foam rack in -70ºC freezer.

4.5.1 Black and blue-stoppered tubes (#7, #8) and yellow stoppered tubes (#9, #10)

1. Each of these tube types is to be shipped to the Central Laboratory without centrifugation.

2. Shipments of tubes #7 and #8 must occur daily, and the specimens must be stored at ambient temperature.

3. Tubes #9 and #10 may be shipped within 48 hours of collection.

4.5.2 Lavender-stoppered tube (#12)

1. This tube is to be used locally for hematology testing. Centrifugation is not required.

2. Delivery of this tube to the hematology laboratory must occur daily. Store the specimen at ambient temperature until delivery.

4.6 Urine Collection and Processing

4.6.1 Background, Rationale and Hypotheses

Hypertension is one of the major risk factors for coronary heart disease. Extensive data from cross-population comparisons, clinical investigations and animal experiments suggest a strong positive association between sodium intake and blood pressure (1-12). Similarly, data from human and animal studies have recently demonstrated the importance of potassium intake in regulation of blood pressure (13-15). Clinical investigations indicate that increasing dietary potassium intake may reduce blood pressure in both normotensives and hypertensives (16-19).

Methodological problems in assessment of sodium and potassium intake have contributed to some inconsistent findings with regard to the role of these elements cardiovascular disease (20). A large scale international collaborative study, the INTERSALT study, has shown a significant positive association between 24-hour sodium excretion and blood pressure for individuals (21). Across the 52 centers of INTERSALT, median 24-hour sodium excretion was significantly related to increase in mean blood pressure with age. In addition, participants from the four centers with median sodium excretion less than 50 mmol/24 hours had low median blood pressure, low prevalence of hypertension, and little or no increase in mean blood pressure with age. Since INTERSALT encompassed a very large sample with a wide range of sodium excretion and was based on a highly standardized protocol with central training, a central laboratory and extensive quality control, these results provide important evidence to further clarify the sodium-blood pressure relationship.

More recently another study, CARDIA, has attempted to define further the role of sodium intake and the risk of hypertension later in life (22,23). Most epidemiologic studies on the relationship between sodium intake and blood pressure have been based on cross-sectional data. It remains unclear whether habitual high sodium intake during young adulthood is related to the risk of hypertension later in life. As most of CARDIA's 5100 young adults are normotensive, there was an unique opportunity

to examine the relationship between sodium intake and incidence of hypertension. Several epidemiologic studies have reported a positive association between the sodium/potassium ratio and blood pressure (24-25).

The Jackson Heart Study will provide an opportunity to further examine the relationship between sodium and potassium intake and the development of cardiovascular disease in African-Americans, Specifically, the interaction of sodium and potassium intake on blood pressure and the development of hypertension and hypertensive complications (e.g. stroke, CAD, and CHF) may be assessed. In addition, we will examine the role of chloride independent of sodium on blood pressure and cardiovascular disease.

There is evidence that urine albumin excretion is a predictor or cardiovascular disease (26,27). It is established in diabetics that elevated urine albumin excretion is a clinical predictor of diabetic kidney disease- a condition that increases cardiovascular disease 7-fold over diabetics without renal involvement (28,29). Moreover, albumin excretion has been shown to be a predicator of cardiovascular disease in non-diabetic populations (30-33). The JHS provides an opportunity to examine whether abnormal urine albumin excretion is a predicator of cardiovascular disease in African-Americans. The relationship between urine albumin excretion and the development of cardiovascular risk factors and cardiovascular disease can be examined. In addition we will be able to establish thresholds above which urine albumin to creatinine ratios are predictive of cardiovascular disease in African-Americans with and without diabetes. The latter will be of practical importance to clinicians caring for large numbers of African-American patients.

4.6.2 Method for Assessing Sodium Intake

The most commonly used methods for assessing sodium intake in epidemiologic studies are timed overnight urine collections and timed 24-hour urine collections. 24-hour specimens are considered more accurate. Several studies report that overnight urine specimens can be used to detect large differences in sodium intakes between groups, but do not accurately assess sodium intakes for individuals (34-36). Moreover, two recent studies indicate that hypertensives may excrete more sodium at night than normotensives (37,38). Based on these findings, collection of 24-hour urine specimens is preferable in the JHS.

It has been suggested that seven timed 24-hour urine specimens are needed to accurately characterize an individual's sodium intake (20). However, the collection of several specimens is likely to be very difficult in our population. In order to avoid any adverse effect of urine collection on cohort retention, we suggest collecting one 24-hour specimen on all participants and validate these results with a repeat 24-hour collection in 10% of the participants. The 10% will be randomly recruited from each sex group (n=650).

We have chosen to perform 48-hour collections in 10% of the study group for several reasons. First, as mentioned above, we want to avoid problems with cohort retention. Second, the collection of a 24-hour specimen will require several additional contacts with each participant (both verbally and physically, see below) which impacts significantly on staff time and cost. Third, formulae are currently available for computing confidence intervals for corrected simple correlation coefficients and for computing lower bounds for corrected partial correlation or regression coefficients (39,40). These correction procedures require at least two measurements. Lastly, the 650 subjects that will have 48-hour collections is a sufficiently large number to answer many of the questions to be addressed by the JHS and will provide sufficient data to validate the other collections in the study (~ 5800).

4.6.3 Urine Collection

Urine specimens would be collected during the baseline examination. One 24-hour urine collection is to be obtained on all participants, and 10% (n=650) will complete two 24-hour collections. If one of those randomly selected to complete the two collections refuses, a replacement will be randomly selected from the same sex group. Each participant will receive instructions on how to collect urine

specimens and a urine collection kit. After the participant reads the instructions, s/he would be verbally instructed by JHS staff until it is evident that collection procedures are clearly understood. The importance of providing complete collections is strongly emphasized.

The collection kit contains instruction materials, one large carrying case containing one 3-liter collection jar, a small empty carrying case and, for women only, a plastic pan or funnel to assist in urine collection. Additionally, each collection jar will be placed in a plastic bag to guard against leaks and spills. A preservative to help deter odor and avoid creatinine loss is added to the collection container. A label with the participants' ID, the date, and the beginning and ending time of the collection, and the question "During this collection period, did you always void into this jar?" will be affixed to each container. For the 10% that will be collected for 48-hours, there will be two 3-litre jars differentiated by the label "First 24-hour urine" or "Second 24-hour urine." Each label contains the participant's ID, the date, and the beginning and ending time of the collection, and the questions "During this collection period, did you always void into this jar?" The clinic provides one bottle per participant per 24-hour collection period unless the participant indicates that they have high urine output (all participants are asked). In that case, they get two bottles per 24-hour collection period.

Each participant will start the collection on an evening within 1 week of the first examination. It will be ended 24 hours after it is started. In this way, urine sodium, potassium, and chloride values will not be influenced greatly by the fast required by the JHS examination, nor will the time from serum creatinine measurement and other data collected in during the exam be too long to allow comparisons. Participants will have to be given some leeway as to when they perform the collection to minimize problems with collecting in the workplace, transportation of samples, and cohort retention. At the examination each participant will identify the period when they would prefer to perform the collection. A staff person from JHS will contact the participant within 24-hours of when the collection is to begin to remind and to verbally review the instructions.

The participant begins the collection by voiding into the toilet before s/he goes to bed. The participant then records the date and time on the label. The participant is to void all urine into the same-color coded jars until s/he is ready to go to bed the next evening. At that time, s/he should void for the last time into the jar and end the collection for the 24-hour urine specimen. Then, s/he will write the date and ending time on the label. In those that are selected for the 48-hour collection, s/he will write the date and ending time on the label and the date and beginning time on the label for the second 24-hour collection.

The participants will return the jars, or if necessary, the staff of JHS will pick-up the jars. The participant is to be carefully questioned about the completeness of the collections and whether or not s/he always answered yes to the question on completeness on the jar label. The participant is also to be asked if there is any urine missing from the collections for any reason, e.g., from spilling. If the answer is yes, s/he is to be asked if the amount is only a few drops or more than that. If the participant indicates that that amount missing is only a few drops, the collection will be considered complete. Otherwise it will be considered incomplete and will not be processed. Clinic staff must insure that each participant returns all urine jars.

4.6.4 Participant Instructions for At-Home Urine Collection

As part of JHS, a 24- or 48-hour urine collection is obtained from every participant. The participant is given instructions and supplies for this procedure at the conclusion of their Exam Center visit. Containers are returned to the Exam Center at a later date.

Proper instruction to the participant in urine collection procedures is of critical importance. The chief obstacles to successful collection, in addition to incompleteness, are failure to remember to start the first night's collection by voiding into the toilet and failure to correctly record the times of each collection period on the jar label. In instructing the participant, the staff needs to make certain that the participant clearly understands the procedures for the start of the first 24-hour collection and the

importance of accurate timing of collections. Urine collection instructions should be reviewed with the participant until it is clear that the participant understands them. The instructions are as follows:

1. Inform the participant that boric acid has been added to each jar for two reasons: (1) to control odor; and (2) to limit the breakdown of creatinine. As you point out the warning label on the side of a collection jar, make the participant aware that boric acid, if ingested, will cause nausea and vomiting, making these collection jars potentially dangerous, especially to small children. Remind the participant of their responsibility in keeping these collection jars out of the reach of children.
2. Before you go to bed on (insert date), urinate into the toilet, emptying the bladder completely. DO NOT USE A URINE COLLECTION JAR FOR THIS VOIDING.
3. Take a jar with a white label marked "FIRST 24-HOUR COLLECTION". Record the date of the evening that you start the first 24-hour urine collection on the label. Write on the label in the space provided for "START OF COLLECTION" the time you voided into the toilet before going to bed. For the first jar of the collection period, write the number "1" in the space provided for "JAR #".
4. Collect all urine passed during the night and the entire next day until bedtime. When a jar is approximately 3/4 full, start a new one by using one with a white label marked "FIRST 24-HOUR COLLECTION"; write the number "2" for the second jar space provided for "JAR #". Transfer the date and the time for "START OF COLLECTION" from the first jar to subsequent jars. Leave the "END OF COLLECTION" blank until the end of the 24-hour collection. Always remember to answer the question at the bottom of each label: "During this collection period, did you always void into the jar?".
5. On the second night of the collection period, void for the last time into a white labeled jar marked FIRST 24-HOUR COLLECTION before going to bed. DO NOT VOID INTO THE TOILET. This jar is now complete even if it is less than approximately 3/4 full. Record the time of this voiding in the space marked "END OF COLLECTION" on <u>each</u> of the jars used during the first 24-hour collection. Record this same time on a yellowed bordered label marked "SECOND 24-HOUR COLLECTION" in the space "START OF COLLECTION". Collect all urine passed during the second night and the entire next day until bedtime in the yellow bordered label jars provided for this 24-hour period. Always remember to transfer the date and the time for "START OF COLLECTION" from one jar to the next.
6. On the third night of the collection period, void for the last time into a yellow bordered label jar before going to bed. DO NOT VOID INTO THE TOILET. This jar is now complete even if it is less than approximately 3/4 full. Record the time of this voiding in the space marked "END OF COLLECTION" on <u>each</u> of the jars used during the second 24-hour collection.

After the staff member is positive that the participant is clear on these instructions and all questions have been answered, the following general instructions may be given:

A. Please refrigerate or store in a cool place all collection jars containing urine. Keep collection jars containing urine out of direct sunlight. DO NOT LEAVE URINE JARS IN A HOT CAR. DO NOT FREEZE THE URINE.

B. Do not add anything but urine to the collection jars and do not rinse them. (For women: Do not wash the Speci-Pan with soap or detergent. Rinse the Speci-Pan with water and towel or air dry.)

C. Always hold the jar while urinating (or pouring urine from the Speci-Pan to the jar) to avoid spilling.

D. Many people when having a bowel movement also involuntarily urinate. To avoid loss of urine when having a bowel movement, you should first pass urine into the collection jar, emptying the bladder completely.

E. If you need more collection jars than the number of jars given, call the clinic to make arrangements to receive extra jars.

F. Upon filling a collection jar to approximately 3/4 full answer the question at the bottom of the label on the jar: "During this collection period, did you always void into this jar?". Please be truthful. While it is very important that all collections be complete, an incomplete collection, not so labeled, is more harmful to our research than an incomplete collection known to be incomplete. While we want you to do your best, there may be a time when you simply forget to use the jar. If this occurs, please tell us.

G. We have included specific instructions for each collection period. Please read and follow them carefully.

4.6.4.1 Special Instructions for Women

There are two concerns regarding female participation in the urine collection study: (1) menstruation and (2) use of the Speci-Pan during urination.

Menstruation has no effect on the measurements of sodium, potassium, chloride, creatinine and albumin. However, some female participants may feel uncomfortable participating at this time. If this is a concern of the participant then the urine collection may be delayed until the end of her menstrual cycle. As with all participants, the technician will record the participant's start date and will contact within 24 hours of the start time to remind and review instructions. The decision on deferring the start of urine collection because of menstruation is the responsibility of the female participant and should not be interfered with or judged by the technician.

A Speci-Pan, a bowl shaped container into which the participant can urinate, will be provided to each female participant to assist in urine collection if needed. The Speci-Pan fits between the toilet seat and the porcelain rim of the toilet. After urinating into the Speci-Pan, the urine is transferred into a collection jar, carefully avoiding spills. The Speci-Pan can then be rinsed out with water and either air-dried or hand dried with a clean cloth or paper towel. The Speci-Pan can be disposed of at the end of the urine collection by the clinic or the participant.

4.6.5 Urine Processing

The receptionist should take the urine collection jug from the participant as soon as he/she arrives at the exam center. The jug must be labeled with the JHS ID number. The jug is then taken to the laboratory where it should be refrigerated until the aliquotting procedure begins.

4.6.6 Urine Aliquotting

All urine processing must take place behind a protective shield. A desktop style shield or the type worn on the head is acceptable. All other rules regarding safe blood specimen handling (Section 3.1) must be observed when processing urine.

1. Mix the complete urine specimen by tipping the jug at least four times.

2. Measure the volume of the urine by reading the graduations on the collection container. Record the volume on the Urine Collection Form.

3. Using a disposable transfer pipet, transfer approximately 2 mL of urine into each of the six microvials in columns 8 and 9 of the sponge tube rack. Fill the two large vials in column 10 to ¾ full. If the urine level is too low in the graduated cylinder to reach with a disposable pipette, pour the urine into a clean random urine container and transfer the urine from it into the microvials. If QC vials are to be collected, they can be filled at this time also. See Quality Control section below for details.

4. Screw the clear caps onto the six microvials in columns 8 and 9. Attach the appropriate caps to the large vials in column 10.

5. Place the two large vials into a refrigerated rack for local analysis.

4.7 Freezing

When all of the blood and urine specimens have been aliquotted into their respective microvials, and the microvials have been replaced in the sponge rack, the entire rack (minus the two serum specimens and two urine specimens set aside for local analyses and the whole blood specimen set aside for glycosylated hemoglobin) 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.

Once the specimens are safely stored in the freezer, the urine may be discarded. The urine can be poured down a sink with copious amounts of water, or it can be flushed down a toilet. The empty collection jug should be discarded in accordance with local biosafety guidelines.

4.8 References

1. Oliver WJ, Cohen EL, Neel JV: Blood pressures, sodium intake, and sodium related hormones in the Yanamamo Indians, a "no-salt" culture. Circulation 52: 146-151, 1975.
2. Lowenstein FW: Blood pressure in relation to age and sex in the tropics and subtropics. Lancet 1: 389-392, 1981.
3. Prior IAM, Grimley-Evans J, Harvey H.P.B. Davidson F, Lindsey M: Sodium intake and blood pressure in two Polynesian populations. New Engl J Med 279: 515-520, 1968.
4. Page LB, Damon A, Moellering RC: Antecendents of cardiovascular disease in six Solomon islands societies. Circulation 49: 1132-1146, 1974.
5. Sasaki N: The relationship of salt intake to hypertension in the Japanese. Geriatrics 19: 735-744, 1964.
6. Dahl LK: Salt and hypertension. Am J Clin Nutr 25: 231-242, 1972.
7. Dole VP, Dahl LK, Cotzias CG, Dziewiatkowski DD, Harris C: Dietary treatment of hypertension. II. Sodium depletion as related to the therapeutic effect. J Clin Invest 30: 584-595, 1951.
8. Partrey PS, Markandu ND, Roulaton J, Jones BE, Jones JC, MacGregor G: Relation between arterial pressure, dietary sodium intake, and renin system in essential hypertension. Brit Med J 283: 94-97, 1981.
9. Morgan T. Gillies A, Morgan G, Adam W, Wilson M, Garney S: Hypertension treated by salt restriction. Lancet 1: 227-230. 1978.
10. Beard TC, Gary WR, Cooke HM, Barge R: Randomised controlled trial of a no-added-sodium diet for mild hypertension. Lancet 2: 455-458, 1982.
11. MacGregor G, Markandu ND, Best FE, Elder DM, Cam JM, Sagnella GA, Squires M: Double-blind randomised crossover trial of moderate sodium restriction in essential hypertension. Lancet 1: 351-355, 1982.
12. Dahl LK, Knudsen KD, Heine MA, Leitl GJ: Effects of chronic excess salt ingestion. Modification of experimental hypertension in the rat by variations in the diet. Circ Res 22: 11-18, 1968.

13. Tobian L. Dietary sodium chloride and potassium have effects on the pathophysiology of hypertension in humans and animals. Amer Jour of Clin Nutr 65 (2 suppl): 606S-611S, 1997.

14. Fotherby MD, Potter JF. Long-term potassium supplementation lowers blood pressure in elderly hypertensive subjects. Intl Jour of Clin Practice 51: 219-22, 1997.

15. Zhou MS, Nishida Y, Yoneyama H, Chen QH, Kosaka H. Potassium supplementation increases sodium excretion and nitric oxide production in hypertensive Dahl rats. Clin and Exp Hypertension 21: 1397-411, 1999.

16. Khaw KT, Thom S: Randomised double-blind cross-over trial of potassium on blood pressure in normal subjects. Lancet 2: 1127-1129, 1982.

17. Skrabel F, Aubock J, Hortnagl H: Low sodium/high potassium diet for prevention of hypertension: probable mechanisms of action. Lancet 2: 895-900, 1981.

18. MacGregor GA, Markandu ND, Smith SJ, Banks RA, Sagnella GA: Moderate potassium supplementation in essential hypertension. Lancet 2: 567-570, 1982.

19. Limura O, Kijima T, Kikuchi K, Miyama A, Ando T, Nakao T, Takigami Y: Studies on the hypotensive effect of high potassium intake in patients with essential hypertension. Clin Sci 61: 77-80, 1981.

20. Liu K, Cooper R, McKeever J, McKeever P, Byington R, Soltero I, Stamler R, Gosch F, Stevens E, Stamler J: Assessment of the association between habitual salt intake and high blood pressure: Methodological problems. Am J Epidemiol 110: 219-226, 1979.

21. The INTERSALT Co-operative Research Group: INTERSALT: An international study of electrolyte excretion and blood pressure. Results for 24-hour urinary sodium and potassium. Brit Med J 297: 319-28, 1988.

22. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulles SB, Jacobs DR Jr., Liu K, Savage PJ. CARDIA: study design, recruitment and some characteristics of the examined subjects. Jour of Clin Epidemiol 41: 1105-15, 1988.

23. Manolio TA, Burk GL, Savage PJ, Sidney S, Gardin JM, Oberman A. Exercise blood pressure response and 5-year risk of elevated blood pressure in a cohort of young adults: the CARDIA study. Amer Jour of Hypertension 7: 234-41, 1994.

24. Langford H, Watson RL: Electrolytes and hypertension. In Epidemiology and Control of Hypertension, edited by Paul O. New York, Stratton Intercontinental Medical Book Corporation, 1975, pp 119-128.

25. Khaw KT, Rose G: Population study of blood pressure and associated factors in St. Lucia West Indies. Int J Epidemiol 11: 372, 1982.

26. Hartland A. Gosling P. Microalbuminuria: yet another cardiovascular risk factor?. Annals of Clinical Biochemistry. 36:700-3, 1999.

27. Mlacak B. Jaksic Z. Vuletic S. Albuminuria, cardiovascular morbidity and mortality in diabetic and non-diabetic subjects in a rural general practice. Family Practice. 16:580-5, 1999.

28. Mangrum A. Bakris GL. Predictors of renal and cardiovascular mortality in patients with non-insulin-dependent diabetes: a brief overview of microalbuminuria and insulin resistance. Journal of Diabetes & its Complications. 11:352-7, 1997.

29. Abuaisha B. Kumar S. Malik R. Boulton AJ. Relationship of elevated urinary albumin excretion to components of the metabolic syndrome in non-insulin-dependent diabetes mellitus. Diabetes Research & Clinical Practice. 39:93-9, 1998.

30. Bianchi S. Bigazzi R. Campese VM. Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications. Amer Jour of Kidney Diseases. 34:973-95, 1999 .

31. Borch-Johnsen K. Feldt-Rasmussen B. Strandgaard S. Schroll M. Jensen JS. Urinary albumin excretion. An independent predictor of ischemic heart disease. Arteriosclerosis, Thrombosis & Vascular Biology. 19:1992-7, 1999.

32. Jager A. Kostense PJ. Ruhe HG. Heine RJ. Nijpels G. Dekker JM. Bouter LM. Stehouwer CD. Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: five-year follow-up of the Hoorn Study. Arteriosclerosis, Thrombosis & Vascular Biology. 19:617-24, 1999.

33. Bigazzi R. Bianchi S. Baldari D. Campese VM. Microalbuminuria predicts cardiovascular events and renal insufficiency in patients with essential hypertension. Journal of Hypertension. 16:1325-33, 1998.

34. Liu K, Dyer A, Cooper R, Stamler R. Stamler J: Can overnight urine replace 24-hour urine collection to assess salt intake? Hypertension 1: 529, 1979.

35. Luft FC, Fineberg NS, Sloan RS: Estimating dietary sodium intake in individuals receiving a randomly fluctuating intake. Hypertension 4: 805, 1982.

36. Luft FC, Sloan RS, Fineberg NS, Free AH: The utility of overnight urine collections in assessing compliance with a low sodium intake diet. JAMA 249: 1764, 1982.

37. Tuomilehto J, Tanskanen A, Pietinen P: Value of overnight urines in the follow-up of people with borderline hypertension trying to reduce their salt intake. Acta Cardiol 15: 325-338, 1985.

38. Dyer AR, Stamler R, Gimm R, Stamler J, Berman R, Gosch FC, Emidy LA, Elmer P, Fishman J, Van Heel N, Civinelli G: Do hypertensive patients have a different diurnal pattern of electrolyte excretion? Hypertension 10: 417-424, 1987.

39. Rosner B, Willett WC. Interval estimates for correlations coefficients corrected for within-person variation: implications for study design and hypothesis testing. Am J Epidemiol 127: 377-386, 1988.

40. Liu K: Measurement error and its impact on partial correlation and multiple linear regression analyses. Am J Epidemiol 127: 864-874, 1988.

5.0 STORAGE AND SHIPPING

5.1 Storage

5.1.1 Frozen Specimens

Place all of the frozen serum and plasma vials from a single participant into a 5” x 8” zip-seal storage bag. Place all of the frozen urine vials from a single participant into a separate 5”x 8” zip-seal storage bag. Check again to make sure all tubes are numbered. Press the air out of the bag and seal. Place these bags in the Central Laboratory box in the -70°C freezer and do not remove it until the time of shipment. This shipment is prepared weekly.

5.1.2 Ambient Specimens

Ambient specimens (CPT and ACD tubes) are stored at room temperature until shipment. The CPT shipment is prepared daily. The ACD tubes may be shipped within 48 hours of collection.

5.1.3 Refrigerated Specimens

There are three types of refrigerated specimens: whole blood to be sent to the Central Laboratory (one vial per participant), serum to be analyzed in the local laboratory (two vials per participant), and urine to be analyzed in the local laboratory (two vials per participant). Maintain a separate rack for each vial in the refrigerator until shipment. The whole blood for the Central Laboratory is shipped twice per week (Tuesday and Thursday), and the serum and urine are delivered to the local laboratory daily.

5.2 Shipping

All frozen specimens collected and stored within the last workweek are shipped to the Central Laboratory on Monday, with the exception of Quality Control sera, as discussed in the Quality Control section below, by overnight courier. If very few participants were seen in the Exam Center during a week, two or three weeks of frozen specimens can be combined into one shipment.

Ship CPT specimens daily, even if only one participant was seen on a given day.

Ship refrigerated specimens on Tuesday and Thursday.

If there is any deviation from the regular shipping schedule contact the Central Laboratory to notify them of any changes.

Weigh all packages before shipping, if possible. It is important to record an accurate weight on the Federal Express airbill. Do not over-estimate the package weight.

5.2.1 Packaging Instructions (frozen specimens)

The bags of frozen specimens are packed and shipped in styrofoam boxes. Packaging instructions are as follows:

1. Place a layer of dry ice on the bottom of the styrofoam box.

2. Put half of the bags of specimens into the styrofoam box on top of the dry ice.

3. Layer more dry ice on top of and around the sample bags.

4. Put the remaining specimen bags into the styrofoam box on top of the dry ice.

5. Layer more dry ice on top of and around the sample bags. The amount of dry ice in the shipping box should total at least five pounds.

6. Place packing material (e.g. bubble wrap) 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 and instructions are shown in Appendix 3.

8. Seal the outer box tightly with strapping tape. Affix biohazard label to outside of box.

9. Address the box and contact Federal Express for pickup.

10. If necessary, more than one box may have to be shipped per week.

5.2.2 Packaging Instructions (ambient specimens)

1. Place ambient specimens into a foam-lined mailer. More than one mailer may be required for all of the specimens.

2. Close the mailer and place it inside a large zip-seal storage bag. Press the air out of the bag and seal.

3. Place the mailer inside a larger styrofoam shipping box. Add room temperature freezer packs and other packing material (e.g. bubble wrap) to occupy extra space.

4. Place the paper shipping forms on top of the packing material. The shipping forms and instructions are shown in Appendix 3.

5. Seal the outer box tightly with strapping tape. Affix biohazard label to outside of box.

6. Address the box and contact Federal Express for pickup.

7. If necessary, more than one box may have to be shipped per day.

5.2.3 Packaging Instructions (refrigerated specimens)

1. Place refrigerated specimens into a three-tube foam mailer system. Place the mailer in a zip-seal bag.

2. Press the air out of the bag and seal.

3. Place the mailer inside a small styrofoam shipping box. Add a previously frozen freezer pack and other packing material (e.g. bubble wrap) to occupy extra space.

4. Place the paper shipping forms on top of the packing material. The shipping forms and instructions are shown in Appendix 3.

5. Seal the box tightly with strapping tape. Affix biohazard label to outside of box.

6. Address the box and contact Federal Express for pickup. This shipment occurs on Tuesdays and Thursdays.

5.2.4 Mailing Instructions

All shipping containers are sent to the Central Laboratory by overnight courier Federal Express) to ensure receipt within 24 hours. The empty styrofoam containers are returned to the Exam Centers via UPS.

Containers shipped to the Central Laboratory are addressed as follows:

JHS Central Laboratory
Fairview-University Medical Center
Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455
Telephone: (612) 273-3318 (office)
Telephone: (612) 273-3645 (lab)
FAX: (612) 273-3489
Email: grynder1@fairview.org

6.0 QUALITY CONTROL

6.1 Venipuncture and Equipment Records

In the Exam Center there are two different aspects of quality control. One is the daily or monthly record of the performance of the refrigeration equipment and centrifuge. This is most easily kept as a check sheet with the daily or monthly records, as described below. The other aspect of quality control is the Venipuncture Form that is part of each participant's records. It shows the number of attempts it takes to achieve a successful venipuncture and the code number of the technician who performs the venipuncture. This record provides needed documentation that the blood was drawn in a standardized manner and that the equipment was functioning properly. This quality control documentation is the best evidence that all specimens in the Exam Center are being drawn and processed identically. Differences in the way the samples are collected or processed could potentially create a significant difference in assay results, which could seriously compromise the laboratory test data. 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 temperature of the refrigerated centrifuge must be recorded daily. See Appendix 4 for a sample form. In addition, the actual speed of the centrifuge needs to be checked and recorded annually with a tachometer. A sample Quality Control Checklist is enclosed in this manual (see Appendix 5). The local blood processing certifier will fill out this sheet monthly, certifying that daily checks have been performed properly and describing any problems in this area. The Monthly Quality Control Checklists should be kept in a permanent file in the Exam Center.

6.2 Quality Control Duplicate Blood and Urine Samples

As part of the overall quality control program for laboratory analyses, duplicate specimens are sent to the laboratory, with one half of each specimen pair sent under the participant's regular JHS laboratory ID number, and the other half under a Quality Control Phantom Participant (QC) laboratory ID number. The QC laboratory ID numbers are not distinguishable from other laboratory ID numbers so that this forms a blinded external quality control program monitoring measurement variability.

To reduce the burden upon JHS participants, no one person is asked to contribute sufficient extra blood to make a complete set of duplicates for all tests. Instead, extra blood is drawn from five participants and sent out under the same QC ID number. For data analysis, results on each laboratory measurement are matched to the appropriate participant results.

All QC samples are stored an extra week at the Exam Center and then sent to the Central Laboratory with a regular shipment.

Ideally the QC samples are drawn on five separate days. For example, on Monday draw Tubes 1 and 2 (chemistry); on Tuesday, draw Tubes 3 and 4 (hypertension and glycated hemoglobin); on Wednesday, draw Tubes 5 and 6 (coagulation); on Thursday, draw Tube 11 (anti-protease); and on Friday draw Tube 12 (hematology). Tubes 7 through 10 are not collected as part of the QC program. The QC urine duplicate can be collected on Thursday or Friday.

6.2.1 Weekly Blood and Urine QC Sample Checklist

The JHS Exam Center venipuncture technicians maintain a weekly checklist posted in their work area of the QC samples to be collected 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 QC samples. An example of the checklist is given below:

Figure 3. Weekly Blood QC Sample Checklist

Week of: _____

Day	Tubes	Laboratory	Sample collected?
Monday	1&2	Chemistry	_____
Tuesday	3&4	Hypertension/Gly. Hgb	_____
Wednesday	5&6	Coagulation	_____
Thursday	11	Chemistry	_____
Friday	12	Hematology	_____
Thursday or Friday	Urine	Chemistry	_____

6.2.2 Preparation for Drawing and Processing QC Samples

Blood Drawing Tubes: Each morning the blood drawing technician prepares extra blood collection tubes for the QC samples to be drawn that day. Each tube is labeled with the QC ID number to be used that week. In addition, the technician may wish to mark QC tubes "QC" in a clearly visible fashion, to reduce the chance that these tubes might be mixed up with the regular blood collection tubes during processing. The QC 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 QC blood tubes that the technician plans to draw that day. The foam block contains all the storage vials needed to process the day's quality control samples. The tubes in each block are labeled in advance with the QC ID number being used that week. Care must be taken during processing that the labels on the sample aliquot tubes match the label on the QC blood collection tubes.

On the day that the duplicate urine sample is to be collected, six extra tubes for the urine QC duplicates should be set out and labeled with the urine QC ID number. One participant per week is chosen for use as the urine QC duplicate.

6.2.3 Collecting and Processing QC Blood and Urine

Selecting Participants for QC Blood Draw: Normally, the QC samples are drawn from the first member of each group 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 QC tube from the first, and draw from another participant instead.

Order of QC Tubes in Relation to Regular Blood Collection: The QC 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 QC tubes, a different participant is used to get this blood. A NEW NEEDLE STICK SHOULD NOT BE DONE JUST TO GET MORE BLOOD FOR A QC SPECIMEN.

Processing and Freezing QC Blood and Urine: QC blood samples are processed along with the regular blood samples. After processing is completed for each QC blood collection tube, the microvials are put into the -70°C freezer (for a minimum of 30 minutes). After the samples are thoroughly frozen, they are put into a freezer storage bag. The QC samples should be kept separate from the other samples collected during the week so they are not shipped along with them.

The six urine QC samples should be placed into the freezer at the same time as their matched participant pair. As with the blood specimens, the urine samples should be kept away from the other urine specimens collected during the week so they are not included with that week's shipment.

Logging the Match between QC and Regular JHS ID's and Reporting These to the Coordinating Center: The QC Phantom Participant's folder is kept in the blood drawing area during the week the phantom ID number is being used to draw QC blood tubes. In the folder is the JHS Quality Control Phantom Participant Form which is used to keep track of the match between the QC and regular JHS specimens. A sample copy is shown in Appendix 8. At the top of the log sheet is a space for the QC Phantom Participant's laboratory ID number. As participants donate blood to make up a QC set, labels with their ID numbers are added to the line corresponding to the tubes donated. This step must be done immediately after completing drawing blood for that participant, to minimize the chance of recording the wrong ID number. One such form is recorded for each QC ID number 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 QC phantom participants' folder with this form is given to the receptionist (or other person designated by the Study Coordinator). The folder is processed like other participants' folders, except that the QC phantom participant form is sent to the Coordinating Center and the Exam Center keeps a photocopy of this form in the phantom's folder. Neither a Venipuncture Form nor Urine Collection Form is completed for the phantom duplicate.

6.3 Reporting of Results/DNA Amounts

The Central Laboratory has the responsibility for reporting results to the Exam Center as well as the Coordinating Center. All test results are transmitted to the Coordinating Center via FTP. This transmission occurs once per week. In addition, any alert result will be included in a separate manually-transmitted FAX. The following table summarizes the reference ranges and JHS alert ranges for routinely performed tests:

Table 1 JHS Laboratory Reference & Alert Ranges

Analyte	Reference Range	JHS Alert Range
[1]Sodium	136-146 mmol/L	<130, >155 mmol/L
[1]Potassium	3.5-5.1 mmol/L	<3.0, >6.0 mmol/L
[1]Glucose	74-106 mg/dL	<60, >200 mg/dL
[1]Creatinine	male: 0.7-1.4 mg/dL female: 0.5-1.2 mg/dL	>2.0 mg/dL
[1]Uric acid	male: 3.5-8.5 mg/dL female: 2.5-7.5 mg/dL	<0.5, >10.0 mg/dL
[1]Sodium(urine)	40-220 mmol/24h	NA
[1]Potassium (urine)	25-125 mmol/24h	NA
[1]Chloride(urine)	75-300 mmol/24h	NA
[1]Albumin(urine)	<25 mg/24 hours	NA
[1]Hematology panel	Hgb F: 11.7-15.7 g/dL M: 13.3-17.7 g/dL Hct F: 35-47 % M: 40-53 % Plt 150-450 x10^9/L	Hgb <7.0 g/dL Hct NA Plt <50, >1000 x10^9/L
[2]Glycosylated hemoglobin	4.3 – 6.1%	NA
[2]Chlamydia antibody	Undetectable	NA
[2]Aldosterone-urine	6 – 25 ug/24 hours	NA
[2]Endothelin I	4.0 – 9.0 pg/mL	NA
[2]Leptin	Variable	NA
[2]Lipoprotein(a)	<45 mg/dL	NA
[2]Cholesterol	<200 mg/dL*	>360 mg/dL
[2]Triglyceride	Male: 0 - 250 mg/dL	>1000 mg/dL
	Fem: 0 - 220 mg/dL	>1000 mg/dL
[2]HDL-Chol.	>35 mg/dL*	<20 mg/dL
[2]Calc. LDL-Chol.	<130 mg/dL*	>260 mg/dL
[2]Vitamin B12	223-925 pg/mL	NA
[2]Folic Acid	2-7 16.1 ng/mL	NA

[1]Test performed in local laboratory
[2]Test performed at Central Laboratory
*The National Cholesterol Education Program designates these range values as “desirable”.

Whole blood collected in ACD tubes is processed for DNA extraction in the Central Laboratory. This procedure does not generate a "result" to be reported to the exam center. However, if the total DNA yield is below the JHS threshold (100 ug), the exam center will be notified (via FAX) of this result immediately, in order to arrange specimen recollection from that individual. This recollected specimen will consist of two 8.5 mL ACD tubes. After collection the uncentrifuged ACD tubes will be shipped to the Central Laboratory under the same original conditions.

7.0 TRAINING PROCEDURES

7.1 Technician Training and Evaluation

The technician must study the JHS Specimen Collection and Processing Manual and watch several participant samples being processed. Then the technician may proceed to a mock drawing and mock processing of samples, without performing any actual venipuncture. Mock venipuncture is performed with the 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. Practice tubes are collected in the correct order, then placed at their proper positions. The sample is processed from start to finish exactly as if real blood were being used. Each technician 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 technician to become comfortable with the procedures before proceeding to live participants.

At this point the technicians are ready to practice on live volunteers. The technicians practice at least once with just one volunteer at a time and again process the blood entirely by themselves from start to finish. If the technicians do not feel comfortable, they can repeat the process with dummy tubes. If enough volunteers are available, it may be beneficial to repeat this several times. Any questions or problems that the technicians have must be solved before the technicians proceed to drawing the JHS participants. Before the technicians draw blood from any JHS participant, they must take and pass the practical and written tests included at the end of this manual. After passing the tests and evaluation of their instructor, they may proceed to drawing blood from JHS participants.

APPENDICES

Appendix 1 JHS Laboratory Tests

Traditional Risk Factors (performed on all participants):

Sodium (serum)
Potassium (serum)
Glucose (serum)
Creatinine (serum)
Uric acid (serum)
Folate (serum)
Vitamin B12 (serum)
Sodium (urine)
Potassium (urine)
Chloride (urine)
Albumin (urine)
Hematology panel (whole blood)
Glycosylated hemoglobin (whole blood)
Chlamydia antibody (serum)
Aldosterone (urine)
Endothelin I (plasma)
Leptin (serum)
Lipoprotein(a) (serum)
Cholesterol (serum)
Triglyceride (serum)
HDL-Chol. (serum)
Calc. LDL-Chol. (serum)
DNA isolation (whole blood)

Newer Risk Factors (performed on a focused subset or subsets of participants):

Insulin (serum)
Fibrinogen (plasma)
Tissue plasminogen activator (plasma)
Plasminogen activator inhibitor (plasma)
C-reactive protein (serum)
p-Selectin (serum)
e-Selectin (serum)
ACTH (plasma)
Cortisol (serum)
Estrone (serum)
Testosterone (serum)
Renin activity (plasma)
Angiotensinogen (plasma)
Homocysteine (plasma)
Vitamin B6 (plasma)
Lymohocyte cryopreservation (whole blood)
Lymphocyte transformation (whole blood)

Appendix 2 Equipment and Supplies

Supplies to be obtained by Exam Center:

Supplier	Catalog no.	Description	Usage/week
Sarstedt	72.609	Microsample Tubes 500/pk	1260
"	65.716.003	Red Screw Caps 1000/pk	450
"	65.716.002	Yellow Screw Caps 1000/pk	135
"	65.716.001	Blue Screw Caps 1000/pk	135
"	65.716.008	Purple Screw Caps 1000/pk	270
"	65.716.	Clear Screw Caps 1000/pk	225
Fisher	02-681-365	Black Screw Caps 1000/pk	45
Allegiance	B3036-4	Butterfly Needles, 21G x 3/4", BD #367250	45
"	B3062	Alcohol Swabs 2,000/cs	45
"	B3063-5	Gauze Sponges 200/pk	45
"	B3063-70	Band Aids 100/pk	45
"	B3060	Tourniquets	n/a
"	B3035-4	Vacutainer Tube Holders 10/pk	45
	BF309623	Tuberculin syringe, 27G x ½", BD #309623	5
"	P5214-12	Transfer Pipettes 500/pk	270
Sigma Chemical	A-6279	Aprotinin (protease inhibitor), 10mL	2
Obtain locally		Freezer Bags 5" x 8"	47
Obtain locally		Freezer Bags, Large	40
Allegiance	S9221-1	Sponge Tube Rack	n/a
Obtain locally		Dry Ice (approximately 9 lbs./shipment)	
Allegiance	B3062-40	PDI Ammonia Inhalant	n/a
"	U3031-51	Urine Collection Container (3L)	45
		<u>Vacutainer Tubes 100/pk</u>	
"	B2970-32	9.5 mL, SST, red/gray top, BD #366510	90
"	B2991-54	10 mL, EDTA, lavender top, BD #366457	135
"	B2991-51	5 mL, EDTA, lavender top, BD #366452	45
"	B2994-91	4.5 mL, Na citrate, BD #366415	90
"	B3003-51	8.5 mL, ACD, yellow top, BD #364606	90
"	B2973-3	8.5 mL, CPT, black/blue top, BD #362761	90
"	B2922-1	Blood Collection Trays	n/a
"	T2050-1	Thermometers -20 C - +110 C	n/a
"	1550SD*RS	Harvard Trip Balance (Ohaus 1550SD)	n/a
"	C6510-1	Timer - 3 channel digital	n/a
Polyfoam Packers	325	Styrofoam shipping box	10
"	409/410	Large 5-tube mailer/sleeve	45
"	364/365	Small 3-tube mailer/sleeve	15
"	414	Gel packs, 24 oz., +30ºF	10
UAL Med Prod	BH302CL	Biohazard labels 320/roll	10

Equipment purchased and maintained by Exam Center:

Table-top refrigerated centrifuge capable of producing 3,000 x *g*
Freezer capable of maintaining -70°C
Refrigerator 4°C

Appendix 3 JHS Shipping Forms

Serum / Plasma
JHS Central Laboratory
Fairview-University Medical Center
Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455

Contents Sheet
Frozen Specimens
Page ____ of ____
Ship date _____

Complete Sample:
8-red top microvials
6-purple top micovials
3-blue top microvials
3-yellow top microvials

LABID	SET COMPLETE? YES NO	MISSING VIALS # COLOR	COMMENTS

Serum/Plasma
JHS Central Laboratory
Fairview-University Medical Center
Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455

Contents Sheet
Ambient Specimens
Page ____ of ____
Ship date _____

Complete Sample:
2-black/blue top CPT tubes
2-yellow top ACD tubes

LABID	SET COMPLETE? YES NO	MISSING VIALS # COLOR	COMMENTS

Serum/Plasma
JHS Central Laboratory
Fairview-University Medical Center
Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455

Contents Sheet
Refrigerated Specimens
Page ____ of ____
Ship date _____

Complete Sample:
1-black top microvial

LABID	SET COMPLETE? YES NO	MISSING VIALS # COLOR	COMMENTS

Urine
JHS Central Laboratory
Fairview-University Medical Center
Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455

Contents Sheet
Frozen Specimens
Page ____ of ____
Ship date _____

Complete Sample:
6-clear top microvial

LABID	SET COMPLETE? YES	NO	MISSING VIALS #	COLOR	COMMENTS

Additional Contents Sheet
_____ Specimens
Page ____ of ____

LABID	SET COMPLETE? YES NO	MISSING VIALS # COLOR	COMMENTS

CONTENTS SHEET INSTRUCTIONS

The contents sheets list the complete inventory of specimens in a shipment. The original form is sent to the Central Laboratory with the specimen shipment, and a copy is filed at the Exam Center. More than one contents sheet may be used in each shipment, depending on the number of specimens enclosed. Use the generic “additional” page for all specimen types. The number of pages attached and each page number are filled in at the top of the contents page (e.g. "page 1 of 5"). This form is filled out at the Exam Center as the specimens are collected and stored. This form must be checked against the specimens when packed for shipment. Record the date of shipment.

The LABID number is entered in the left hand column of the contents sheet. This is most easily done by attaching one of the adhesive LABID labels in the space provided. This should be done at the time of collection. It is suggested that a second person check these IDs against the IDs on the vials to correct any errors.

The tubes comprising a complete set are listed in the upper left hand corner of the sheet. Under the category SET COMPLETE?, YES or NO should be marked for each participant to indicate whether the correct number of tubes has been shipped. If there is some deviation from the correct count, "NO" should be marked, and a description of the problem should follow in the column headed MISSING VIALS. The number of missing tubes and the color of their caps should be recorded here.

COMMENTS on the quality of the specimens upon receipt are recorded at the Central Laboratory.

Appendix 4 JHS Daily Temperature Record

JHS
DAILY TEMPERATURE RECORD

DATE Mo/Da/Yr	Freezer	Refrig	Room	Initials	DATE Mo/Da/Yr	Freezer	Refrig	Room	Initials

Appendix 5. JHS Monthly Equipment Quality Control Checklist

CENTER _____
DATE _____
TECHNICIAN _____
ID NUMBER _____

	(S)atisfactory/(U)nsatisfactory	Comments
SET UP		
1. Daily QC records		
refrigerator temperature	___	_____
centrifuge temperature	___	_____
freezer temperature	___	_____
room temperature	___	_____
2. Annual QC records		
centrifuge tachometer check	___	_____
3. Equipment and Supplies		
refrigerated centrifuge	___	_____
refrigerator	___	_____
-70ºC freezer	___	_____
stopwatch	___	_____
timer	___	_____
Vacutainer needles	___	_____
tourniquet	___	_____
Vacutainer tubes	___	_____

Appendix 6 JHS Venipuncture and Processing Procedures Certification Checklist

	VENIPUNCTURE	Satisfactory/ Unsatisfactory	Comments
1.	Labels checked	___	_____
2.	Participant prepared and procedure explained.	___	_____
3.	Venipuncture Form filled.	___	_____
4.	Tourniquet application and release	___	_____
5.	Venipuncture technique	___	_____
6.	Tube collection sequence	___	_____
7.	Inversion technique	___	_____
8.	Tube incubation location	___	_____
9.	Stasis obtained	___	_____
10.	Needle disposal	___	_____

	PROCESSING		
1.	Knowledge of centrifuge operation	___	_____
2.	Aliquotting supply set-up	___	_____
3.	Stage I tube spin	___	_____
4.	Stage II aliquotting	___	_____
5.	Stage III tube spin	___	_____
6.	Vials sealed	___	_____
7.	Final processing stage	___	_____
8.	VPT Form completed	___	_____
9.	Freezer organization	___	_____
10.	Time constraints	___	_____
11.	Disposal of contaminated supplies	___	_____

	PACKAGING AND SHIPPING		
1.	Specimens bagged	___	_____
2.	Adequate dry ice used in shipping	___	_____
3.	Shipping paperwork	___	_____

	MISCELLANEOUS		
1.	Incident Form	___	_____
2.	QC Procedure	___	_____
3.	Containers correctly labeled for shipping	___	_____

Appendix 7 Sample Exams for Certification

PRACTICAL EXAM FOR JHS BLOOD DRAWING TECHNICIAN

1. Place the following 12 blood collection tubes in the correct set-up order and location for the venipuncture: 2-10 mL red and gray tops, 2-10 mL lavender tops, 2-4.5 mL blue tops, 2-8.5 mL black and blue tops, 2-yellow tops, 1-10 mL lavender/anti-protease, 1-5 mL lavender.

2. Specify which tube(s) remain at room temperature after collection.

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

4. Set up a sponge tray with the appropriate number and order of specimen storage tubes. Indicate the colors of screw caps and the types of specimen put into these tubes.

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

6. Organize the color-capped sample tubes and prepare them for shipment.

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

SAMPLE WRITTEN EXAM

1. When handling biological specimens, what type of protective apparel must always be worn?
   _____

2. What is the recommended solution for use in cleaning an area where blood or urine has spilled?
   _____

3. If the participant arrives at the Exam Center and has not performed the timed urine collection, the next step would be:
   a) Defer the visit until a timed collection is available.
   b) Obtain a random specimen at the Exam Center.
   c) Remove participant from study permanently.
   d) Provide the participant with instructions and supplies to complete the procedure.

4. Is it acceptable for the participant to make a fist in the hand of the arm from which the blood specimen is being collected? If so, when? _____

   _____

5. During a typical week, how many JHS participants will have additional blood specimens collected to be used as part of the phantom duplicate?

   a) 5
   b) 4
   c) 2
   d) 0

6. From which tubes are the packed cells used?

   a) None
   b) All
   c) #1
   d) #2, #3, #4 and #5

7. How long should tube #1 sit at room temperature before centrifugation?

   a) 5 minutes
   b) 30 minutes
   c) 2 hours
   d) No waiting time required

8. Why is this step (un)necessary?_____

9. Which tube is drawn last?

   a) A 5 mL lavender-stoppered
   b) A 4.5 mL blue-stoppered
   c) A 10 mL red and gray-stoppered
   d) A 7 mL lavender-stoppered

10. For what type of tests will the 10-mL lavender-stoppered tubes be used?

    a) Chemistry
    b) Lipid
    c) Hypertension
    d) Special coagulation

11. When is the tourniquet removed?

    a) after tube #1 fills
    b) after the tourniquet has been attached for two minutes
    c) after all tubes fill
    d) it does not matter

12. How many JHS participants will provide duplicate QC urine samples weekly?

    a) 0
    b) 1
    c) 2
    d) 4

Appendix 8 JHS Quality Control Phantom Participant ID Form

JHS QUALITY CONTROL PHANTOM PARTICIPANT ID FORM

Note: This form should be sent to the Coordinating Center within two weeks of the first entry for a QC phantom.

Phantom Participant Laboratory ID Number (blood)_____

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

Phantom QC Log

Tube	Matching Laboratory ID	Date Collected (Mo/Day/Yr)	Technician ID
1&2	_____	____/____/____	_____
3&4	_____	____/____/____	_____
5&6	_____	____/____/____	_____
11	_____	____/____/____	_____
12	_____	____/____/____	_____
Urine	_____	____/____/____	_____

Jackson Heart Study Specimen Collection and Processing

Tube #	Stage 1 **immediate processing**	Stage 2 **15 min post-collection**	Stage 3 **30 min post-collection**	Final Stage **<90 min post-collection**	Storage
1&2 (Chemistry) 10 mL SST Red/gray top	Room temperature rack for 30-45 min		Centrifuge 10 min, 3000 x *g*, 4°C	Transfer serum equally into ten 2 mL microvials **Two to UMC (4°C)**	Store vials at -70°C Ship weekly
3 (Hypertension, Hemoglobin A1c) 10 mL EDTA Lavender top	1 mL whole blood to 2 mL microvial. Refrigerate microvial Then centrifuge tubes 3&4 10 min, 3000 x *g*, 4°C	Transfer plasma equally into three 2 mL microvials		Store at room temperature until put in freezer	Store vials at -70°C Ship weekly (Ship Tue/Thur)
5&6 (Coagulation) 4.5 mL citrate Blue top	Room temperature rack, then centrifuge 10 min, 3000 x *g*, 4°C	Transfer plasma equally into three 2 mL microvials		Store at room temperature until put in freezer	Store vials at -70°C Ship weekly
7&8 (Family Study) 8.5 mL CPT Black/blue top	Room temperature rack, do not centrifuge			Store at room temperature until shipment	**Ship daily to Central Lab (Room temp)**
9&10 (Genetics) 8.5 mL ACD Yellow top	Room temperature rack, do not centrifuge			Store at room temperature until shipment	Ship <48 hours to Central Lab (Room temp)
4&11 **4**-10 mL EDTA/ACTH **11**-10 mL EDTA/Apro Lavender top	**Ice bath**, then centrifuge 10 min, 3000 x *g*, 4°C	Transfer plasma equally into three 2 mL microvials. Freeze immediately #4 #11	Store vials at -70°C	Store vials at -70°C	Store vials at -70°C Ship weekly
12 (Hematology) 5 mL EDTA Lavender top	Room temperature rack, do not centrifuge			Store at room temperature until local analysis	Daily local hematology (CBC) testing
Urine 24-hour collection	Refrigerate container until volume measurement and aliquotting			Transfer urine equally into six 2 mL microvials **Two large vials to UMC(4°C)** **One UMC, one extra**	Store vials at -70°C Ship weekly

01/29/01