

Jackson Heart Study Protocol

Manual 4

Central Laboratory and Specimen Repository
Specimen Collection and Processing

Visit 3

Version 2.0

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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. Manuals 2, 3 and 4 describe the Cohort Procedures, Blood Pressure and Central Laboratory and Specimen Repository components of the study. Manuals 5 and 6 comprise Electrocardiography and Magnetic Resonance Imaging studies, respectively. Manual 7 comprises Morbidity and Mortality Classification. Manual 8 articulates the quality assurance and quality control activities of the JHS Examination 3 components. Quality assurance includes activities that are designed to assure quality of data, which take place during the collection of data, while quality control relates to efforts during the study to monitor the quality of data. The Data Management System is described in Manual 9.

JHS Study Protocols and Manuals of Operation

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2	Cohort Component Procedures
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Specimen Collection and Processing**

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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 5,600 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/Clinic collect blood and urine specimens, process them, and ship them to the Central Laboratory at University of Minnesota Medical Center, Fairview 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. 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 Clinic. 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 Clinic technicians who perform the blood and urine collection and sample processing.

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 20-30 mL of blood from each participant. Three or four 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 two ounces, although it may look like more. The technician may also assure participants that they donate 15 times as much blood (450 mL) when they donate a pint of blood. Participants will also be asked to provide a random urine specimen during their visit. The technicians and the clerk should be wearing a clean laboratory coat.

2.2 Staff Certification Requirements

A certified JHS technician at the Clinic 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 three or four 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

Collect 20-30 mL of blood from each participant using three or four Vacutainer tubes. Specimens from these blood collection tubes are used in approximately eleven 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:

Tube #1 is a 10-mL red and gray-stoppered tube filled with 9.5 mL of blood. This tube does NOT contain anticoagulant, so it does NOT need to be mixed following collection. After collection, allow the blood to clot at room temperature for 30 minutes. Following centrifugation, the serum 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 values can be artifactually decreased.

Tubes #2 is a 10-mL lavender-stoppered tube containing the liquid anticoagulant EDTA. The plasma is used to measure p-selectin and stored for future testing. After each tube is fully filled with blood, invert four times then place into a room temperature rack until centrifugation. *

Tube #3 is a 3 mL lavender stoppered tube containing the anticoagulant EDTA. The whole blood from this tube is used to measure glycated hemoglobin. After the tube is filled, invert four times, then save at refrigerated temperature until shipment to the Central Laboratory. Minimum volume needed in this tube is 1 mL.

*Note: If unable to obtain tube 3, place 1 mL of well mixed WHOLE blood from tube 2 in a 1.5mL microvial and save at refrigerated temperature for the glycated hemoglobin analysis. Record this in section 8 of the venipuncture form.

2.4 Blood Collection Tubes: Labeling and Set-up

Three or tubes are collected in the following sequence:

Tube #1:	9.5-mL red and gray-stoppered tube
Tube #2:	10-mL lavender-stoppered tube (EDTA)
Tube #3:	3-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 so that the barcoded ID number is positioned vertically. Arrange the set of tubes in a test tube rack. When the participant arrives for the Clinic 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:

- 5 - 1.5-mL polypropylene vials (red top)
- 5 - 1.5-mL polypropylene vials (purple top)
- 5 - 1.5-mL polypropylene vials (clear top)

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

- Col 1: 1.5-mL red top vials (rows A-E)
- Col 2: empty
- Col 3: 1.5-mL purple top vials (rows A-E)
- Col 4: empty
- Col 5: 1.5-mL clear top vials (rows A-E)
- Col 6: empty
- Col 7: empty
- Col 8: empty
- Col 9: empty
- Col 10: empty

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 that each Vacutainer tube is properly labeled with the appropriate LABID number.
3. Check that the sample processing tray is properly equipped. Every item on the checklist must be ready and in its proper position.
4. Check that each microvial is labeled with the appropriate laboratory number.
5. Check that a urine collection container is labeled with the appropriate laboratory number.
6. Perform and record quality control (QC) check on centrifuge temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$).
7. Perform and record QC check on refrigerator temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$).
8. Perform and record QC check on freezer temperature ($-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$).
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 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 Clinic.

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

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 and collecting urine. 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 sitting (phlebotomist chair) 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.

Provide participant with a labeled urine collection container and instructions for specimen collection. If possible, obtain this specimen prior to blood collection. The urine specimen may then be processed with the blood specimens.

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 sitting 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 holder. Grasp the flange of the tube holder 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 (#1 through #3) should be gently inverted four times immediately following removal of the tube from the adapter, then placed into a room temperature rack.

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 #3, 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.

1.1 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. Collect tube #1 (10-mL red and gray top). Place the tube in a rack at room temperature.
2. Collect tube #2 (10-mL lavender top). Invert four times and place in room temperature rack.
3. Collect tube #3 (3 mL lavender top). Invert four times and place in room temperature rack.

2.0 BLOOD AND URINE PROCESSING

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

2.1 Stage One: Immediate Processing

Tube #1 remains 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 this tube.

2.1.1 Whole blood for glycosylated hemoglobin

Tube #3 is placed into the refrigerator until shipment. It is NOT centrifuged. This tube must be shipped within 48 hours of collection.

2.1.2 Centrifugation

Place tube #2 in the centrifuge trunion. Balance the centrifuge then spin these tubes at 3,000 x *g* for 10 minutes at 4°C.

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

2.1.3 Urine specimens

1. Thoroughly mix the random urine specimen.
2. Using a plastic transfer pipet, deliver 1.5 mL of urine to each of the five labeled microvials in column 5. **DO NOT OVERFILL THE URINE VIALS.**
3. Screw clear plastic caps onto each vial, and leave the sponge rack at room temperature.
4. Re-attach the screw cap to the collection container, and hold it at room temperature until all vials are safely frozen.

2.2 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.

2.3 Stage Two:

Approximately 15 minutes after venipuncture.

2.3.1 Lavender-stoppered Tube (Tube #2)

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 tube #2. The pipette tip should NOT get any closer than one-half inch from the cells. Equally transfer the plasma into the five 1.5-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 #2, 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.

2.4 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 tube #1 at 3,000 x *g* for 10 minutes at 4°C.

2.5 Final Blood Processing

1. Remove the red and gray top tube from the centrifuge and place it in the sponge test tube rack.
2. Remove the stopper from tube #1. Using a plastic transfer pipette, aliquot all of the serum equally into the five tubes in column 1.
3. Fasten red screw caps on each of these vials.
4. Replace the stopper on the red and gray-stoppered blood collection tube and discard it in a biohazard waste bag.

2.6 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 urine specimen set aside for local analyses) 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 container should be discarded in accordance with local biosafety guidelines.

3.0 STORAGE AND SHIPPING

3.1 Storage

3.1.1 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 labeled correctly. 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.

3.1.2 Refrigerated Specimens

Maintain a rack for each whole blood or 1.5 mL microvial in the refrigerator until shipment. The whole blood EDTA tube (#3) or microvial may be shipped within 48 hours of collection. .

3.2 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 Clinic during a week, two or three weeks of frozen specimens can be combined into one shipment.

Ship refrigerated whole blood EDTA specimens (tube #3) within 48 hours of collection.

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.

3.2.1 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 styrofoam box lid. The shipping forms and instructions are shown in Appendix 3.

8. Seal the outer box tightly with strapping tape. Affix UN3373 and UN1845 dry ice labels to outside of box. The UN1845 label must be filled in with the appropriate dry ice weight and addresses.
9. Address the box and contact Federal Express for pickup.
10. If necessary, more than one box may have to be shipped per week.

3.2.2 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 styrofoam box lid. The shipping forms and instructions are shown in Appendix 3.
5. Seal the box tightly with strapping tape. Affix UN3373 label to outside of box.
6. Address the box and contact Federal Express for pickup. This shipment occurs on Tuesdays and Thursdays.

3.2.3 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 Clinic via UPS.

Containers shipped to the Central Laboratory are addressed as follows:

JHS Central Laboratory
University of MN Medical Center, Fairview
Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455
Telephone: (612) 273-3645 (Julie and Vicky in the lab)
Telephone: (612) 273-3318 (Greg's office)
FAX: (612) 273-3489
Email: jidzore1@fairview.org
grynder1@fairview.org
vmakky1@fairview.org

4.0 QUALITY CONTROL

4.1 Venipuncture and Equipment Records

In the Clinic 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 Clinic 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 Clinic.

4.2 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 three 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 (except the refrigerated whole blood tube) are stored an extra week at the Clinic and then sent to the Central Laboratory with a regular shipment.

Ideally the QC samples are drawn on three separate days. For example, on Monday draw Tube 1 (chemistry); on Tuesday, draw Tube 3 (glycated hemoglobin); and on Wednesday, draw Tube 2 (special chemistries). . The QC urine duplicate can be collected on Thursday or Friday.

4.2.1 Weekly Blood and Urine QC Sample Checklist

The JHS Clinic 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 1. Weekly Blood QC Sample Checklist

Week of: _____

<u>Day</u>	<u>Tubes</u>	<u>Laboratory</u>	<u>Sample collected?</u>
Monday	1	Chemistry	_____
Tuesday	3	Gly. Hgb	_____
Wednesday	2	Special Chemistry	_____
Thursday or Friday	Urine	Chemistry	_____

4.2.2 Preparation for Drawing and Processing QC Samples

Blood Collection Tubes: Each morning the blood drawing technician prepares an extra blood collection tube for the QC sample 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, five 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.

4.2.3 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 collection 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 refrigerated whole blood QC specimen is shipped along with its mate within 48 hours of collection.

The four urine QC samples should be placed into the freezer at the same time as their matched participant specimens. 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. Send the urine QC local testing vial as usual.

Logging the Match between QC and Regular JHS ID's and Reporting These to the Coordinating Center/Data Management, Information Technology, Quality Assurance Unit.: 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 **Data Management, Information Technology, Quality Assurance (DMITQA)Unit. and the Clinic** 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. A separate JHS study ID is NOT assigned to the QC specimen set. It is NOT assigned a study ID.

4.3 Reporting of Results/DNA Amounts

The Central Laboratory has the responsibility for reporting results to the Clinic as well as the DMITQA Unit. All test results are transmitted to the **DMITQA Unit** 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
² Glycosylated hemoglobin	4.3 – 6.1%	NA
² C-reactive protein	<0.5 mg/dl	NA
² Glucose	74-106 mg/dL	<60, >200 mg/dL
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 LCL-Chol. (calculated)	<100 mg/dL*	>260 mg/dL
Urea Nitrogen		
Creatinine		
Insulin		
e-selectin		
p-selectin		

*The National Cholesterol Education Program designates these range values as “desirable”.

5.0 TRAINING PROCEDURES

5.1 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 collection 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):

- Urea Nitrogen (serum)
- Glucose (serum)
- Glycosylated hemoglobin (whole blood)
- Cholesterol (serum)
- Triglyceride (serum)
- HDL-Chol. (serum)
- Calc. LDL-Chol. (serum)
- DNA isolation (whole blood)
- C-reactive protein (serum)
- Creatinine (serum)
- e-selectin (serum)
- insulin (serum)
- p-selectin (plasma)

Appendix 2 Equipment and Supplies

Supplies to be obtained by the **Clinic:**

Supplier	Catalog no.	Description	Usage/week
Sarstedt	72.609	Microsample Tubes 500/pk	1260
"	65.716.003	Red Screw Caps 1000/pk	450
"	65.716.008	Purple Screw Caps 1000/pk	270
"	65.716.	Clear Screw Caps 1000/pk	225
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
"	P5214-12	Transfer Pipettes 500/pk	270
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
		<u>Vacutainer Tubes 100/pk</u>	
Allegiance	B2970-32	9.5 mL, SST, red/gray top, BD #366510	90
"	GR-454246B	3 mL, EDTA, lavender top, BD #366457	135
"	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., +30°F	10
UAL Med Prod	BH302CL	Biohazard labels 320/roll	10

Equipment purchased and maintained by the **Clinic:**

Table-top refrigerated centrifuge capable of producing 3,000 x g

Freezer capable of maintaining -70°C

Refrigerator 4°C

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 **Clinic**. More than one contents sheet may be used in each shipment, depending on the number of specimens enclosed. The number of pages attached and each page number are filled in at the top of the contents page (e.g. "page 1 of 5"). This form is filled out at the **Clinic** 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. The Study ID label should also be applied in this section. 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 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 | _____ | _____ |
| -70°C 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 COLLECTION TECHNICIAN

1. Place the following four blood collection tubes in the correct set-up order and location for the venipuncture: 1-10 mL red and gray top, 1-10 mL lavender top, 1-3 mL lavender top.
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. 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? _____

4. 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) 3
 - d) 0
5. From which tubes are the packed cells used?
 - a) None
 - b) All
 - c) #1
 - d) #2, #3, #4 and #5
6. 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
7. Why is this step (un)necessary? _____
8. Which tube is drawn last?
 - a) A 10 mL lavender-stoppered
 - b) An 3 mL lavender-stoppered
 - c) A 10 mL red and gray-stoppered
 - d) A 7 mL lavender-stoppered

9. For what type of tests will the 10-mL lavender-stoppered tubes be used?
- a) Special Chemistry
 - b) Lipid
 - c) Hypertension
 - d) Special coagulation
10. 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
11. 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 _____

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	_____	____/____/____	_____
Urine	_____	____/____/____	_____

