

# ALTOLD

## Laboratory Specimens and the Core Laboratory

### 1. INTRODUCTION

The Core Laboratory for LURDS is located at University of Minnesota Medical Center-Fairview in Minneapolis. The primary function of the Core Laboratory for the LURDS project is to perform testing for basic biochemistries, renal evaluations and other more specialized analyses. Although this summary primarily addresses technical issues, the quality of the specimens and the results generated when the analyses are performed rest upon excellence in following the protocols established by the LURDS study group.

### 2. PREPARATION

#### 2.1 Participant Contact

Since LURDS also depends on the voluntary participation of donors and siblings, every effort must be made to make the entire procedure as convenient as possible for them. The best way to achieve this is for the staff to be thoroughly knowledgeable about all aspects of the procedures.

The LURDS study involves the collection of approximately 65 mL of blood and a completely voided random sample of urine from each participant at every visit. A total of fourteen tubes of blood is collected per visit. Any participant who is concerned about the volume of blood should be reassured that the total amount of blood drawn is approximately four tablespoons, although it may look like more. The technician may also assure participants that seven times as much blood (450 mL) is collected when they donate a pint of blood. There is a one time only collection of an additional 10 mL of blood for DNA isolation that is usually drawn at the Baseline visit with participant consent.

#### 2.2 Supplies Provided by the Core Laboratory

The Core Lab will provide an initial shipment of supplies necessary for transport of whole blood, packed cells, plasma, serum, and urine to the Core Lab. These supplies include the plastic sample storage vials and the transport boxes needed for proper temperature storage of the specimens en route to the Core Lab. After a specimen shipment is unpacked at the Core Laboratory, the empty shipping container will be returned to the clinical site for re-use---do not discard!

Pre-numbered labels for blood collection tubes, urine containers and aliquot storage vials will be provided to the clinical centers. The Core Lab will also provide a Shipping Form (Appendix A) to be used at collection, processing and packaging of the specimens for shipment. A copy of this form must accompany each specimen shipment to the Core Lab.

After the initial shipment of labels and supplies is received, a LURDS Core Laboratory Supply Reorder form (Appendix B) must be faxed to the Core Laboratory in order to obtain more supplies.

## 2.3 Preparation for Specimen Collection and Processing

Prior to venipuncture, prepare two trays for each participant. One tray holds the blood collection tubes. The other tray holds the various plastic storage vials that will contain the final whole blood, packed cells, plasma, serum and urine aliquots.

It is important that the technicians know more than just the arrangement of the blood collection tubes and the sequence of tube collection. The blood collection and processing technicians must 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. Tubes are organized in the test rack in the following sequence:

**Two 8.5 mL red and gray-stoppered tubes (SST)** with gel separator. The serum from this tube is used for basic biochemical assays and long-term storage. After this tube is filled with blood, invert eight times and place a rack and allow it to clot at room temperature for 30 minutes. The tube is then centrifuged and the serum processed as described later.

**One 2 mL lavender-stoppered tube** containing the anticoagulant EDTA. The plasma from this tube is used for hematological studies. After the 2 mL tube is filled with blood, invert eight times then place into a room temperature rack---transfer this tube promptly to your local Hematology Lab.

**One 1.8 mL blue-stoppered tube** contains the liquid anticoagulant sodium citrate. The plasma from this tube is used for coagulation studies. After this tube is filled with blood, invert eight times then place into a room temperature rack until centrifugation.

**Three 6 mL lavender-stoppered tubes (EDTA)**. An aliquot of whole blood from one of these tubes is used for the hemoglobin A1c assay, then all three tubes are centrifuged and the plasma is used for other specialized biochemical tests and long-term storage. After the tubes are filled with blood, invert eight times then place into a crushed ice bath until centrifugation.

**Two 7 mL green-stoppered tubes** containing the anticoagulant lithium heparin. The plasma from these tubes is used for insulin, glucose and lipid measurements and long-term storage. After these tubes are filled with blood, invert eight times then place into a crushed ice bath until centrifugation.

For Iohexol GFR series:

**Five 2 mL green-stoppered tubes** containing the anticoagulant sodium heparin. The plasma from these tubes is used to measure iohexol clearance. After these tubes are filled with blood, invert eight times and place into a room temperature rack---centrifuge each tube as soon as possible.

If an additional tube is to be collected for DNA isolation:

**10 mL lavender-stoppered tube** containing the anticoagulant EDTA. The whole blood from this tube is used for DNA isolation. After the tube is filled with blood, invert eight times then place into a room temperature rack until centrifugation.

For FSIVGTT series:

**Twenty-nine 2 mL green-stoppered tubes** containing the anticoagulant sodium heparin. The plasma from these tubes is used to measure insulin and glucose. After each tube is filled with

blood, invert it eight times and place into a room temperature rack---centrifuge each tube as soon as possible after collection.

## 2.4 Specimen Identification and Labeling

**'Participant ID labels'** (Appendix C) are provided to the clinical centers in sheets of 18 labels each. This label shows the barcoded Participant ID number and has spaces to record the date and time of collection, if desired. A Participant ID label is used to label the LURDS Shipping Form, the Iohexol GFR flow sheet and any other required documentation. It is recommended that each participant's sheaf of labels be kept in his or her file folder for use at subsequent visits.

**'Lab ID labels'** (Appendix C) are used to label the blood collection tubes, the shipping form and the storage vials that contain the processed specimens. Each participant will be assigned a unique Lab ID at each visit. The Lab ID labels are not patient-specific, however, once a Laboratory ID has been designated for a patient at a given visit, all of that patient's Core Laboratory assessments will be labeled with the same barcode series for that visit. The Lab ID labels are provided in sheets of 60 labels each that contain sequences of both 'generic' Lab ID labels and 'specimen code' Lab ID labels. Note that the leading digit of the Lab IDs reflects your clinical center's numeric designation. For example, the 1000- series labels are Lab ID labels generated for the LURDS clinical site located at the University of Minnesota.

'Generic' Lab ID labels (no specimen code indicated) are used to label the blood collection tubes and the shipping form---these have the barcoded Lab ID number (accession number) and spaces to record the date and time of collection. 'Specimen code' Lab ID labels are affixed to the storage vials---the specimen code appears in the lower left corner of the label.

**Shipping Form** (Appendix A) lists the complete inventory of specimens in a shipment to the Core Laboratory. This form is filled out at the LURDS clinical center as the specimens are collected, processed and prepared for shipment. One Participant ID label and one 'generic' label with the unique Lab ID assigned to the participant for that visit are affixed to the shipping form. Visit number and collection date and time are recorded on this form as well.

## 2.5 Blood Collection Tubes and Storage (Aliquot) Vials: Labeling and Set-up

Prepare for specimen collection in the following manner, referring back to the collection and processing flow diagram (Appendix D) as needed. 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 must be ready before proceeding.
2. Create a match between the Patient ID number and the unique Laboratory ID (accession number) selected for that visit. Affix one Participant ID label and one 'generic' Lab ID label to the LURDS Shipping Form. Record the Visit number on the Shipping Form.
3. Affix a 'generic' Lab ID label to each blood collection tube. Arrange the set of tubes in a test tube rack in the order in which they will be collected.
4. Using the 'specimen code' sequence of the Lab ID labels, label the storage vials with the same Lab ID number (accession number) matched to the participant for that visit in Step 2. (Refer to Appendix C regarding proper positioning of the Lab ID label on the storage vial.)

Record the collection date and time (if required) on each vial's label and on the LURDS Shipping Form.

Approximately 10 minutes before scheduled participant arrival:

1. Prepare an ice bath by filling a container approximately 3/4 with crushed ice, then add cold water.

### **3. BLOOD COLLECTION AND PROCESSING**

#### **3.1 Venipuncture**

About 65 mL of blood is drawn from each participant using fourteen blood collection tubes. Samples from these tubes will be used for genetic and biochemical assays and retained for long-term storage.

Before applying the tourniquet, screw the Luer adapter into the plastic tube guide. Insert the butterfly tubing onto the adapter. It is also acceptable for the venipuncture to be performed using a standard 21-gauge Vacutainer needle screwed directly into the plastic tube guide.

With jacket or sweater removed, have the participant sit upright 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 (2) 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 his/her fist prior to the venipuncture. Doing so could cause fluctuations in the results of several of the possible analytes to be measured. If the participant has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin. Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.

At participant arrival:

1. Verify the match made in the previous section (Section 2.5) between the Patient ID number and the Laboratory ID number (accession number) selected for that visit.
2. Once the vein is identified, remove alcohol prep from its sterile package.
3. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.
4. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the participant when the venipuncture is performed.
5. 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. Place tube #1 into the tube holder.
2. Grasp the participant's arm firmly. Use your thumb to draw the skin taut and anchor the vein. The thumb should be 1 or 2 inches (2.5 or 5.0 cm) below the venipuncture site.
3. With the needle bevel upward, enter the vein in a smooth continuous motion.
4. Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support. **DO NOT HAVE THE PARTICIPANT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN.**
5. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle. The tube should begin filling with blood.
6. Remove the tourniquet after tube #1 fills. Once the draw has started, do not change the position of a tube until it is withdrawn from the needle. A tourniquet may be reapplied during tubes 2-14 to spare the participant a re-stick, but the tourniquet must not be on for more than 2 minutes.
7. Keep a constant, slight forward pressure (in the direction of the adapter) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow.
8. Fill each blood tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases). If a tube fills only partially, remove the tube and attach another without removing the needle from the vein.
9. 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).

All blood collection tubes should be gently inverted eight times immediately following removal of the tube from the adapter. After inversion, tubes #1-4 are placed into the room temperature rack. Tubes #5-9 are placed in a crushed ice bath. All of these tubes are to be promptly centrifuged and processed as directed so that specimens are frozen in a timely manner prior to shipment to the Core Lab.

At the conclusion of the blood draw:

1. To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle and its cap into 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 the final tube, restick the participant, 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 recollected tube. As always, the tourniquet must never be on for longer than two minutes.

Bandaging the arm:

1. Slip the gauze pad down over the site, continuing to apply mild pressure.
2. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.

### **3.2 Blood Collection**

Prepare for specimen collection in the following manner, referring back to the collection and processing flow diagram (Appendix D) as needed.

Fourteen tubes of blood are collected at each routine visit. Fill the tubes to capacity. Mix blood and preservative thoroughly. It is imperative that the specimens be processed promptly as directed.

Tubes #1 and #2 are 8.5-mL red and gray-stoppered tubes (SST). Completely fill the tubes with blood. Invert each tube eight times and place in a rack at room temperature. After drawing, allow the blood to clot at room temperature for 30 minutes. The tubes are then centrifuged and the serum is removed, frozen, and stored for shipment to the Core Laboratory.

Tube #3 is a 2-mL lavender-stoppered tube containing EDTA anticoagulant. Completely fill the tube with blood and invert it eight times, then place in a rack at room temperature. Do not centrifuge this tube---send it promptly to your local Hematology Laboratory for analysis: CBC and platelet count.

Tube #4 is a 1.8-mL blue-stoppered tube. Completely fill the tube with blood. Invert eight times and place in a rack at room temperature. The tube is then promptly centrifuged and the plasma is removed, frozen, and stored for shipment to the Core Laboratory.

Tubes #5-7 are 6-mL lavender-stoppered tubes containing EDTA anticoagulant. Completely fill each tube with blood and invert it eight times, then place into the crushed ice bath. Prior to centrifugation, a small aliquot of whole blood will be removed from one of the tubes for hemoglobin A1c analysis, then all three tubes are promptly centrifuged and the plasma is removed, frozen, and stored for shipment to the Core Laboratory.

Tubes # 8-9 are 7-mL green-stoppered tubes containing lithium heparin anticoagulant. Completely fill each tube with blood and immediately invert it eight times, then place into the crushed ice bath. The tubes are then promptly centrifuged and the plasma is removed, frozen, and stored for shipment to the Core Laboratory.

#### Iohexol GFR tubes (n=5):

This is a 5-tube series of timed collections of 2-mL green-stoppered tubes containing sodium heparin anticoagulant, one tube is collected at each timepoint. Completely fill each tube with blood and invert it eight times, then place in a rack at room temperature. Each tube is to be

promptly centrifuged and the plasma removed, frozen, and stored for shipment to the Core Laboratory. (Do not delay centrifugation and processing.)

DNA Isolation (collected one time only):

The tube to be collected for DNA isolation is a 10-mL lavender-stoppered tube containing EDTA anticoagulant. Completely fill the tube with blood and invert it eight times, then place in a rack at room temperature. The tube is promptly centrifuged and the top 2/3 of the plasma is carefully removed and discarded, then the buffy coat and red cells are removed, frozen and stored for shipment to the Core Laboratory. These packed cells are used for DNA isolation. With participant consent, this 10 mL EDTA tube is collected and processed one time only, usually at the Baseline visit.

FSIVGTT tubes (n=29, one time only for a subset of LURDS participants at the 36-month Visit):

This is a 29-tube series of timed collections of 2-mL green-stoppered tubes containing sodium heparin anticoagulant, one tube is collected at each timepoint. Completely fill each tube with blood and invert it eight times, then place in a rack at room temperature. Each tube is to be promptly centrifuged and the plasma removed, frozen, and stored for shipment to the Core Laboratory. (Do not delay centrifugation and processing.)

### **3.3 Operating the Centrifuge**

Refer to centrifuge Operator's Manual for specific operating and balancing instructions. Make certain the centrifuge is set at 4-10°C (refrigerated centrifuge). In order to achieve the recommended centrifugal force (1500 x g) within the centrifuge, the corresponding revolutions per minute (RPM) setting may vary from centrifuge to centrifuge depending on radius of the centrifuge's rotor. Consult the Operator's Manual for the appropriate speed setting (RPM) for your centrifuge.

NOTE: The centrifugation speed and time of 1500 x g for 15 minutes stated in this procedure is based on the manufacturer's recommendation for centrifugation of the citrate tube (blue stopper) using a swing-head centrifuge with the brake OFF. Citrate tubes should be centrifuged at a speed and time to consistently produce platelet-poor plasma (platelet count <10,000/uL) per NCCLS Guidelines. All other blood collection tubes requiring centrifugation may be centrifuged at the same speed and time as the citrate tube.

If using a fixed-head or high-speed centrifuge, consult your local laboratory's procedure for specific centrifugation instructions. Do not centrifuge glass tubes at forces above 2200 x g in a horizontal head (swinging bucket) centrifuge as breakage may occur. Glass tubes may break if centrifuged above 1300 x g in fixed-angle centrifuge heads.

### **3.4 Stage One - Immediate Processing**

All rules regarding safe blood specimen handling must be observed when processing specimens.

Prepare for specimen processing in the following manner, referring back to the collection and processing flow diagram (Appendix D) as needed.

*After venipuncture is completed:*

1. Tubes #1 and #2 (red and gray stopper) remain incubating at room temperature for 30 minutes from the time they are collected to allow the blood to clot. (Blood at 4°C clots extremely slowly.)
2. Tube #3 (2 mL lavender stopper) is placed in a rack at room temperature then promptly transported to your local Hematology Laboratory for a CBC and platelet count. Do not centrifuge this tube!
3. Tube #4 (blue stopper) is placed in a rack at room temperature.
4. Tubes #5 - 7 (lavender stopper) and tubes #8 - 9 (green stopper) are placed into a crushed ice bath.
5. Iohexol GFR series: after venipuncture, each tube (green stopper) is placed in a rack at room temperature; each is to be centrifuged and processed promptly.

Additional blood collections:

If a 10 mL EDTA tube is collected for DNA isolation, this is placed in the room temperature rack until processing. The 2 mL tubes collected for the FSIVGTT series are placed in a rack at room temperature; each is to be centrifuged and processed promptly.

*Preliminary Processing:*

6. Prior to centrifugation, remove tube #5 (lavender stopper) from the ice bath and invert four times for adequate re-mixing prior to pipetting. Remove the stopper and place the tube in the sample processing rack. Before pipetting, match the Lab ID label that appears on the collection tube with that on the 'HbA1c' labeled storage vial. Using a plastic transfer pipette, aliquot 0.5 mL whole blood into this vial. Tightly fasten a clear screw cap on the vial. Refrigerate this whole blood hemoglobin A1c storage vial at 4°C until shipment to the Core Laboratory within 5 days via overnight courier.

Note: In the event of a delay between blood collection and transfer of the aliquot of whole blood for hemoglobin A1c analysis to the storage vial, replace the lavender stopper and invert tube #5 ten (10) times for adequate re-mixing. Then proceed with the transfer. This will ensure a quality blood sample and an accurate hemoglobin A1c result.

7. Restopper tube #5 and retain for continued processing in the next step.

*Centrifugation:*

8. Remove tube #4 (blue stopper) from the room temperature rack and tubes #6-7 (lavender stopper) and tubes #8-9 (green stopper) from the ice bath and place them with tube #5 (lavender stopper from Step 4) into centrifuge trunions. Balance the trunions and the centrifuge, then spin these at 1500 x g for 15 minutes with the brake off (manufacturer's recommendations for speed and time in a swing-head centrifuge) at 4-10°C.
9. Wait for centrifuge to come to a complete stop. Proceed to Stage 2 processing.

### **3.5 Stage Two - Intermediate Processing**

Approximately 15 minutes after venipuncture:



1. Remove tubes #4 (blue stopper), tube #5-7 (lavender stopper) and tube #8-9 (green stopper) from the centrifuge and place in the sample processing rack. Before pipetting, match the Lab ID label that appears on the collection tubes with that on each storage vial.
2. Remove the stopper from tube #4 (blue stopper). Using a plastic transfer pipette, take off only about the top 2/3 of the plasma. (The bottom 1/3 of the plasma contains platelets and we must have platelet-free plasma.) Aspirate slowly starting at the top of the plasma. Leave a 1/2 inch layer of plasma above the red blood cells. Transfer the plasma into one 'Fibr' labeled storage vial. Tightly fasten the blue screw cap onto the vial and place it in a rack in the refrigerator. Restopper tube #4 and discard.
3. Remove the stoppers from tubes #5-7 (lavender stopper). Using a plastic transfer pipette and being careful not to disturb the cell layer, transfer 1.0 mL plasma into each of seven EDTA plasma storage vials: one labeled 'PTH', one labeled 'LPA-Hcy' and the remaining five vials labeled 'E-PSav' (saved EDTA plasma). Any excess plasma may be distributed amongst the vials. Fasten the lavender screw caps tightly onto the storage vials and place them in the same rack in the refrigerator as the vial processed in the previous step. Restopper tubes #5-7 and discard.
4. Remove the stopper from tube #8-9 (green stopper). Using a plastic transfer pipette and being careful not to disturb the cell layer, transfer 1.0 mL plasma into each of seven storage vials: one labeled 'Lip-Gluc', another labeled 'Ins' and the remaining five vials labeled 'H-PSav' (saved heparinized plasma). Any excess plasma may be distributed amongst the vials. Fasten the green screw caps tightly onto the storage vials and place them in the same rack in the refrigerator as the vials processed in the previous steps. Restopper tube #6 and discard.
5. Place all of the storage vials processed in 'Stage Two - Intermediate Processing' into the  $-70^{\circ}\text{C}$  freezer until shipment to the Core Laboratory within 5 days via overnight courier.

Additional Specimen Collections:

6. **Iohexol GFR series:** Centrifuge promptly after specimen collection. Place the tubes into centrifuge trunions. Balance the trunions and the centrifuge, then spin these at  $1500 \times g$  for 15 minutes with the brake off (manufacturer's recommendations for speed and time in a swing-head centrifuge) at  $4-10^{\circ}\text{C}$ . Before pipetting, match the Lab ID label that appears on the collection tube with that on its respective storage vial. Remove the stopper from the tube (green stopper). Using a plastic transfer pipette and being careful not to disturb the cell layer, transfer at least 1.0 mL plasma into the 'IHx 1' storage vial. Tightly fasten a green screw cap onto the vial. Process all green-stoppered tubes collected at subsequent timepoints for Iohexol determination in the same manner. When finished, restopper each empty blood collection tube and discard. Place Iohexol GFR storage vials in a rack in the refrigerator as soon as each is processed. Once all five vials have been processed, move them to  $-70^{\circ}\text{C}$  frozen storage as directed in step 5 above.
7. **If packed cells are to be retained for DNA isolation:** Centrifuge promptly after specimen collection. Place the tubes into centrifuge trunions. Balance the trunions and the centrifuge, then spin these at  $1500 \times g$  for 15 minutes with the brake off (manufacturer's recommendations for speed and time in a swing-head centrifuge) at  $4-10^{\circ}\text{C}$ . Remove the stopper from the 10 mL EDTA tube (lavender stopper). Before pipetting, match the Lab ID

label that appears on the collection tube with that on the 'DNA' labeled storage vial. Using a plastic transfer pipette and being careful not to disturb the cell layer, remove the clear plasma supernatant and discard. The pipette tip should not get any closer than one-half inch from the cells. It is important to withdraw only the plasma and none of the buffy coat (containing white cells and platelets) that forms at the cell-plasma interface following centrifugation. Aspirate slowly starting at the top of the plasma. Leave a ½ inch layer of plasma above the buffy coat-red blood cell layers. If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube under the initial processing conditions.

Next, using the same plastic transfer pipette, transfer all of the packed cells from the DNA collection tube (lavender stopper) into the 5 mL vial labeled 'DNA'. Make certain that the top portion of the cells containing the buffy coat is transferred first. Tightly fasten the blue screw cap on this vial and place it in the –70°C freezer until shipment to the Core Laboratory within 5 days. Restopper the empty blood collection tube and discard.

8. **FSIVGTT series:** Centrifuge promptly after specimen collection. Place the tubes into centrifuge trunions. Balance the trunions and the centrifuge, then spin these at 1500 x g for 15 minutes with the brake off (manufacturer's recommendations for speed and time in a swing-head centrifuge) at 4-10°C. Remove the stopper from the tube (green stopper). Before pipetting, match the Lab ID label that appears on the collection tube with that on its respective storage vial. Using a plastic transfer pipette and being careful not to disturb the cell layer, transfer at least 1.0 mL plasma into the 'GTT 1' storage vial. Tightly fasten a green screw cap onto the vial. Restopper the empty blood collection tube and discard. Process all green-stoppered tubes collected at subsequent FSIVGTT timepoints in the same manner. Place each storage vial in a rack in the refrigerator as soon as it is processed---then move vials within one hour of processing to –70°C freezer until shipment to the Core Laboratory within 5 days.

### 3.6 Stage Three – Final Processing

Approximately 30-45 minutes after venipuncture:

1. Remove Tubes #1 and #2 (red and gray stopper) from the room temperature rack and place into centrifuge trunions. Balance the trunions and the centrifuge, then spin these at 1500 x g for 15 minutes with the brake off (manufacturer's recommendations for speed and time in a swing-head centrifuge) at 4-10°C.
2. Remove tubes #1 and #2 (red and gray stopper) from the centrifuge and place them in the sample processing rack.
3. Remove the red and gray stopper from both tubes #1 and #2. Before pipetting, match the Lab ID label that appears on the collection tubes with that on each storage vial. Using a plastic transfer pipette, transfer 1.0 mL serum into each of eight storage vials: one labeled 'Elec', one 'CysC-hsCRP', one 'Chem' and the remaining five vials labeled 'SSav' (saved serum). Any excess serum can be distributed amongst the vials.
4. Tightly fasten red screw caps on each of these serum storage vials and place them directly into the –70°C freezer along with those samples processed in 'Stage Two – Intermediate Processing' until shipment to the Core Laboratory within 5 days. Restopper the empty blood collection tubes and discard.

5. Complete the LURDS Shipping Form, recording the Visit number and the date and time of collection. Make a note on the Shipping Form of any missing storage vials that correspond to uncollected specimens, noting the number of missing vials, the cap color and the 'specimen code' that appears on the Lab ID label. Attach a copy of the Iohexol GFR flow sheet (and FSIVGTT flow sheet, as needed) for this visit to the Shipping Form.

## **4. RANDOM URINE COLLECTION AND PROCESSING**

### **4.1 Urine specimen identification and labeling**

As part of the LURDS study, a random urine specimen is collected from each participant at every visit. Collect the specimen in a container labeled with the same Lab ID number (accession number) matched to the participant for that visit in Section 2.5---use one of the 'generic' Lab ID labels. Record the collection date of the random specimen on the LURDS Shipping Form. Urine collections should not be done if the participant has an active UTI, illness, or menses.

### **4.2 Urine Aliquotting**

Prepare for urine specimen processing in the following manner, referring back to the collection and processing flow diagram (Appendix D) as needed. All rules regarding safe blood specimen handling must be observed when processing urines.

1. Mix the urine specimen by tipping the container eight times. Before pipetting, match the Lab ID label that appears on the collection container with those on the urine storage vials.
2. A random void collection must have a minimum volume of 20 mL. Transfer 4 mL well-mixed urine to each of four urine storage vials: one labeled 'UTP-Alb-Cr' and three labeled 'USav' (saved urine). Be sure that the correct Lab ID, as matched to the Participant in Section 2.5 for all specimens collected at this visit, appears on these labels.
3. Tightly fasten clear screw caps on each of the urine storage vials and place them in the  $-70^{\circ}\text{C}$  freezer along with the serum and plasma storage vials processed in the previous steps. Store frozen at  $-70^{\circ}\text{C}$  until shipment to the Core Laboratory within 5 days via overnight courier.
4. Replace the lid on the urine container and bring the remainder to your local Urinalysis Laboratory for dipstick urinalysis screen (with sediment exam only if the dipstick is abnormal).
5. Complete the LURDS Shipping Form noting visit number and date and time of urine collection. Make a note of any missing storage vials that correspond to uncollected specimens.

## **5. STORAGE, PACKAGING AND SHIPPING OF SPECIMENS**

The single (one) storage vial containing 0.5 mL whole blood for hemoglobin A1c is placed into the refrigerator until shipment to the Core Laboratory. The storage vials containing serum,

plasma and urine (and packed cells) are stored in the  $-70^{\circ}\text{C}$  freezer before shipment to the Core Laboratory.

Ship all of a Participant's specimens together as a single shipment via overnight courier as soon as possible after processing. Be careful not to exceed the 5-day window for shipment to the Core Laboratory (as dictated by the prompt shipment required of the refrigerated hemoglobin A1c specimen). Shipments are to be sent Monday through Thursday, being mindful of weeks in which a holiday occurs. Shipping early in the week (Monday or Tuesday) avoids problems in transporting the specimens over weekends. Any shipment deviations or questions should be discussed directly with the Core Laboratory.

## 5.1 Completing the LURDS Shipping Form

The LURDS Shipping Form (Appendix A) lists the inventory of specimens in a shipment and is completed as the specimens are collected, processed and shipped. Both a Participant ID label and a Lab ID label must appear on the shipping form. The visit number and collection date and time must also appear on the form. This form must be checked against the specimen vials as they are packed for shipment. Be sure that the correct Lab ID as matched to the Participant in Section 2.5 for all specimens collected at this visit appears on these labels. It is suggested that a second person check the Lab ID on the form against the Lab ID on the storage vials to catch any errors.

The vials that comprise a complete specimen set are listed in the center of the form. Check off each type as it is accounted for. Under the column SAMPLE SET COMPLETE?, YES or NO should be marked to indicate whether the complete set of specimen vials has been shipped. If there is some deviation from the correct count, "NO" should be marked, and the number of missing vials, the cap color and the 'specimen code' that appears on the Lab ID label should be recorded on the form. A description of the problem should follow in the Comments section. Record the date of shipment at the bottom of the form.

Retain photocopies of the LURDS Shipping Form and the Iohexol GFR flow sheet at the clinical center. Attach the Iohexol GFR flow sheet for that visit to the original copy of the LURDS Shipping form and send both to the Core Laboratory along with the specimen shipment. (Include a copy of the FSIVGTT Form, as needed.)

## 5.2 Packaging of Refrigerated Hemoglobin A1c Specimen

The whole blood sample must be stored in the refrigerator and must be shipped to the Core Laboratory via overnight courier within five days of collection (along with the Participant's frozen samples for the visit). The whole blood sample is packed with a frozen refrigerant pack inside a small foam box with cardboard sleeve (carton) that measures 3-1/2" x 7". This assembly will be placed together with another foam box inside a single larger cardboard carton. (See Appendix E - Packaging of Specimens for Shipment to LURDS Core Lab)

NOTE: One or more refrigerant packs should be stored frozen ready for shipment, preferably inside the open foam box (from 3-1/2" x 7" shipping assembly) to avoid later difficulty in fitting the rigid pack into the foam box. Place refrigerant pack into the freezer at least one day prior to shipping---it must be frozen solid at the time of shipment.

Packaging instructions for refrigerated specimens:

1. Wrap a sheet of absorbent wadding or paper towel around the storage vial to protect it from direct contact with the frozen refrigerant pack.
2. Place the wrapped specimen into a small (3" x 5") plastic storage bag. Place an absorbent pad inside the bag and seal the bag tightly. If more than one participant's samples are to be included in the same shipment, use a separate plastic bag and absorbent pad for each participant's sample.
3. Place a frozen refrigerant pack on the bottom of the 3-1/2" x 7" foam box.
4. Place the plastic bag(s) on top of the frozen refrigerant pack. Place the lid on the foam box and insert it into its cardboard sleeve (carton) and close the tabs.
5. Place the foam box assembly into a large (13" x 15") plastic bag and seal the bag after expelling the air inside. This assembly will be included as a single shipment with another foam container holding the frozen specimens (see following section).

### 5.3 Packaging of Frozen Specimens

The samples must be stored in the -70°C freezer until shipment and must be shipped to the Core Laboratory via overnight courier within five days of collection along with the Participant's refrigerated hemoglobin A1c sample collected at the same visit. The frozen samples are packed and shipped on dry ice inside a 10" tall foam box that is placed inside a taller cardboard sleeve (carton) that is 14" high. The small foam box assembly described in Section 5.1 containing the refrigerated specimen(s) will be placed on top of the sealed 10" box within this taller cardboard carton. (See Appendix E)

Packaging instructions for frozen specimens:

1. Place all of the frozen sample storage vials from a single participant into one medium (8" x 10") plastic storage bag. Be sure that all of the vials in the bag share the same Lab ID. If more than one participant's samples are to be shipped in the same foam container, use a separate plastic bag for each participant.
2. Place an absorbent pad inside the plastic bag that contains the frozen samples and seal the bag.
3. Place a layer of dry ice inside the 10" foam box. Place the plastic bag containing the frozen vials on top of the dry ice.
4. Layer more dry ice on top of the bag. If more than one participant's samples are to be shipped in the same container, layer dry ice in between each plastic bag. Be sure to use a sufficient quantity of dry ice to fill the container, particularly during the warmer months.
5. Place the lid on the 10" foam box. To secure the lid to the box, apply a single strip of strapping tape over the top of the container---the strip should extend approximately 6 inches down each side. Do not encircle the box with strapping tape. Do not apply strapping tape along the seam where the lid meets the box. Insert the foam box down into its 14" cardboard sleeve (carton). This cardboard carton will include both this 10" foam box and the smaller 3-1/2" x 7" foam box assembly described in Section 5.1. (Appendix E)

## 5.4 Final Packaging Instructions for refrigerated and frozen specimens

1. Place the small 3-1/2" x 7" foam box assembly containing the refrigerated whole blood specimen(s) on top of the lid of the sealed 10" foam box containing the frozen samples. Be sure the small assembly is enclosed inside a large plastic bag. Add other packing material (e.g., bubble wrap or newspaper) to occupy extra space around the smaller box.
2. Place the LURDS Shipping Form with Iohexol GFR flow sheet attached (and FSIVGTT Form, as needed) on top of the packing material---check the forms again to be sure all required information appears on each form and that any missing storage vials are accounted for. Then close and seal the outer 14" cardboard carton tightly with strapping tape as shown in Appendix E.

## 5.5 Shipping Instructions

**Federal law requires that you know and follow the specific packaging instructions provided to you to be in compliance with government regulatory requirements.**

In addition to a dry ice label, IATA Regulations require that a label be affixed to the outside of shipping box that reads: **'Exempt Human Specimens'**. Affix a completed dry ice label (obtain from Federal Express) and an 'Exempt Human Specimens' sticker to outside of box. The dry ice label must indicate both the Shipper's name and address and Consignee's name and address and the total weight of the shipment in kg. Do not write within the dotted boundaries of the diamond shape on the dry ice label.

Please check your shipping boxes to ensure that they are properly labeled in order to avoid any interruption in shipment.

Shipping containers are sent to the Core Laboratory by overnight Federal Express to ensure receipt within 24 hours. After a specimen shipment is unpacked at the Core Laboratory, empty foam boxes and shipping containers will be returned to the clinical center for re-use via FedEx, UPS or the U.S. Postal Service---do not discard!

The FedEx account number for LURDS specimen shipments to the Core Lab is: **3275-9580-6**. Shipping containers to the Core Lab are addressed as follows:

Jean Bucksa 612-273-3645  
Univ of Minn / FUMC / LURDS  
420 Delaware Street, S.E., Rm L275  
Minneapolis, MN 55455-0341

Laboratory contact numbers:

Telephone: (612) 273-3645 (lab)  
FAX: (612) 273-3489

If using an online FedEx account, follow the instructions provided on the Federal Express website ([www.fedex.com](http://www.fedex.com)). Contact Federal Express (1-800-GO-FEDEX) for pickup or use the designated Federal Express pickup system at your clinical center.

If you do not have an online account, complete a FedEx airbill (see following section), then keep the Sender's copy (top copy) for your records—the package tracking number printed on the airbill is necessary to track a delayed or lost shipment. Affix the completed self-stick airbill firmly to the top of the shipping container (or insert regular airbill into a self-stick airbill pouch and affix it firmly to the shipping container). Contact Federal Express (800-GO-FEDEX) for pickup or use the designated Federal Express pickup system at your clinical center.

### Completing a FedEx Airbill

1. Field #1 ('From'): Print the date of shipment, your clinic center's address (sender's address) and phone number
2. Field #2 (Your Internal Billing Reference Information): Print '**Exempt Human Specimens**' in this field---do not omit this step!
3. Field #3 ('To'): Print the Core Laboratory's address (recipient's address) and phone number in this field:

Jean Bucksa 612-273-3645  
Univ of Minn / FUMC / LURDS  
420 Delaware Street, S.E., Rm L275  
Minneapolis, MN 55455-0341

4. Field #4a (Service): Check '**FedEx Priority Overnight**' only--do not check any other option.
5. Field #4b (Express Freight Service): Skip Field 4b
6. Field #5 (Packaging): Check '**Other**' when using a mid-size shipping container.
7. Field #6 (Special Handling): Skip Delivery options, however you must check '**Yes – Shipper's Declaration Not Required**' and check '**Dry Ice**' and record its weight.

IMPORTANT: All packages must have a completed dry ice label and an 'Exempt Human Specimens' sticker affixed to the outside of the container. Please check your shipping boxes to ensure that they are properly labeled in order to avoid any interruption in shipment.

8. Field #7 (Payment): Check the 'Recipient' box and record the FedEx Acct. No. **3275-9580-6**

Record number of **Total Packages** (1) and **Total Weight** of the shipment (very important!). It is the shipper's responsibility to record accurate weight to the nearest whole pound. Do not write units after the package weight (i.e., LBS or lbs); do not record weight using symbols such as '<'

9. Field #8 (Release Signature): Skip Field 8
10. Keep the Sender's copy (top copy) of the airbill, then affix the completed self-stick airbill firmly to the top of the shipping container.

IMPORTANT: In addition to a dry ice label, IATA Regulations require that a label be affixed to the outside of shipping box that reads 'Exempt Human Specimens'. Affix a completed dry ice label (obtain from Federal Express) and an 'Exempt Human Specimens' sticker to outside of

box. The dry ice label must indicate both the Shipper's name and address and Consignee's name and address and the total weight of the shipment in pounds. Do not write within the dotted boundaries of the diamond shape on the dry ice label.

Please check your shipping boxes to ensure that they are properly labeled in order to avoid any interruption in shipment.



**Appendix A. Shipping Form**

**LURDS SHIPPING FORM**

UMMC-LURDS  
Jean Bucksa 612-273-3645  
420 Delaware Street SE, Rm L275  
Minneapolis, MN 55455

VISIT # \_\_\_\_\_

Blood Collection Date \_\_\_\_ / \_\_\_\_ / \_\_\_\_  
Time \_\_\_\_:\_\_\_\_

Urine Collection Date \_\_\_\_ / \_\_\_\_ / \_\_\_\_  
Time \_\_\_\_:\_\_\_\_

Shipment Contents:

FROZEN VIALS

**Complete Sample Set:**

- 8 - red cap transport vials (serum)
- 1 - blue cap transport vial (citrated plasma)
- 7 - violet cap transport vials (EDTA plasma)
- 7 - green cap transport vials (heparinized plasma)
- 4 - clear cap urine transport vials (urine)
- 5 - green cap microvials (iohexol GFR series – include GFR flow sheet!)

**(one time only):**

- 1 - blue top transport vial (packed cells for DNA)

REFRIGERATED VIAL

**Complete Sample Set:**

- 1 - clear cap transport vial (whole blood)

\*\*\*\*\*

PARTICIPANT ID	LAB ID	SAMPLE SET		MISSING
		COMPLETE?		
<u>LABEL</u>	<u>LABEL</u>	<u>YES</u>	<u>NO</u>	<u># TYPE (CAP COLOR)</u>

COMMENTS:

\*\*\*\*\*

Date of Shipment: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Date of Receipt at Core Lab: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

**Appendix B. Supply Reorder Form**

**LURDS CORE LABORATORY  
SUPPLY REORDER FORM**

UMMC-LURDS  
Jean Bucksa 612-273-3645  
420 Delaware Street SE, Rm L275  
Minneapolis, MN 55455

Please complete this form and **FAX** it to the Core Laboratory at **(612) 273-3489**.

Site ID Number \_\_\_\_\_ Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Site Shipping Address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Site Phone Number: \_\_\_\_\_  
Site FAX Number: \_\_\_\_\_

Supplies provided by Core Lab:

QTY

\_\_\_\_\_ **Participant ID Labels**

\_\_\_\_\_ **Lab ID Labels**

\_\_\_\_\_ **Kits (storage vials/caps)**

(each kit contains supplies sufficient for one visit):

- 29 - 1.5 mL Microsample Tubes
- 8 - Red Screw Caps
- 1 - Blue Screw Cap
- 1 - Natural (Clear) Screw Cap
- 7 - Violet Screw Caps
- 12 - Green Screw Caps
- 4 - 4.5 mL NUNC tube, clear cap (urine specimens)
- 2 - absorbent pads
- 1 - 3" x 5" Freezer Bag
- 1 - 8" x 10" Freezer Bag (contains the above supplies)

Additional supplies:

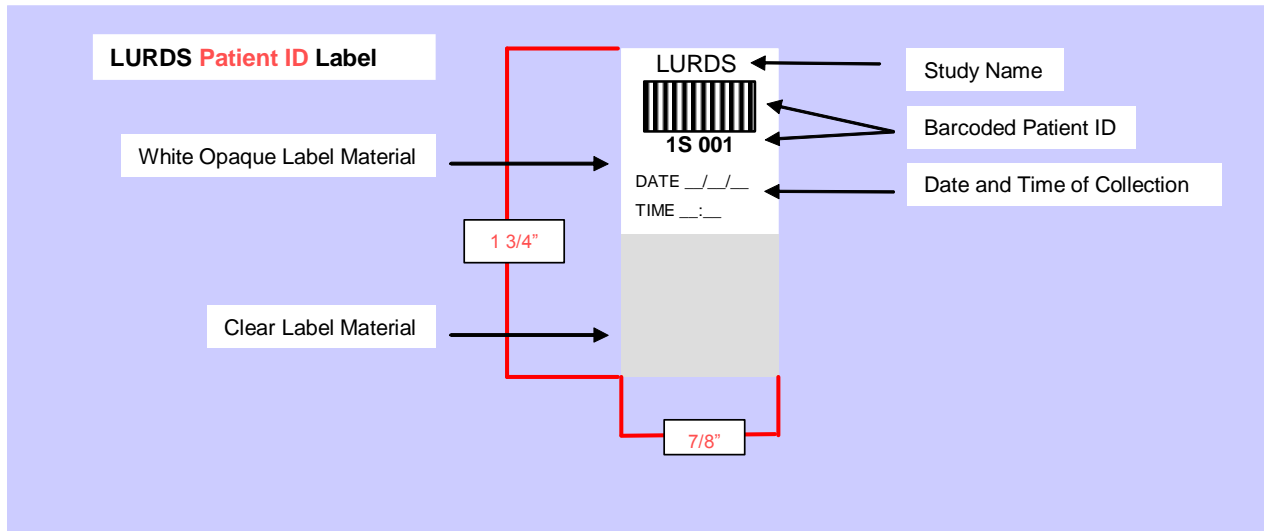
\_\_\_\_\_ Shipping supplies (packaged as a unit):  
Large foam shipping container (for frozen specimens) inside tall cardboard box  
Small foam shipping assembly with refrigerant pack (for refrigerated specimens)  
1 - 13" x 15" Freezer Bag

\_\_\_\_\_ FedEx airbills, pre-printed  
Dry ice label  
UN3373 sticker

## Appendix C. Labels

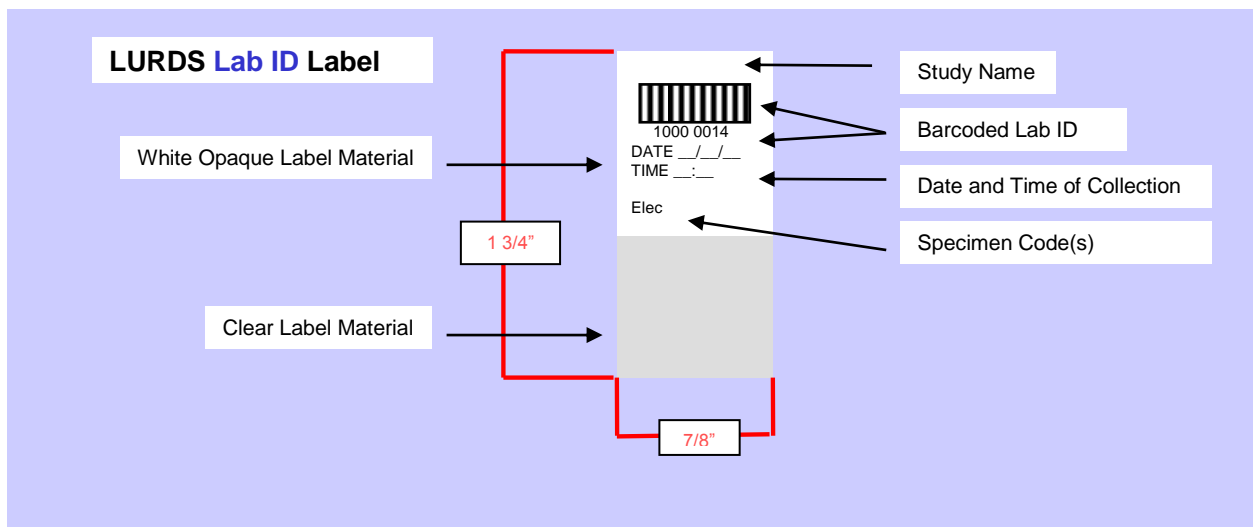
### LURDS Patient ID Labels for Blood Collection Tubes

Prior to venipuncture, the clinic staff will affix a LURDS Patient ID Label to the LURDS Shipping form, the Iohexol GFR flow sheet and any other required documentation. The original Shipping Form is to be enclosed with the specimens when shipped. Always retain a copy of the Shipping Form for your records.



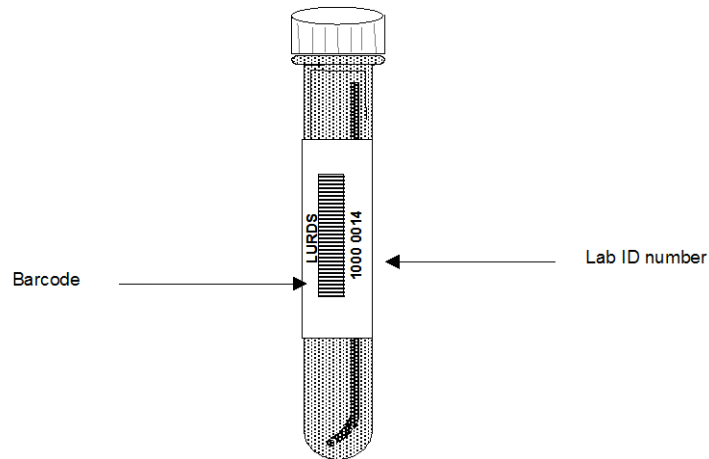
### LURDS Lab ID Labels for Storage Vials

The clinic staff will assign a unique LURDS Lab ID (accession number) to the participant for each visit. A Lab ID label is affixed to the Shipping Form and to each storage vial --- all storage vials must be labeled with the same Lab ID. The original Shipping Form must be enclosed with the specimens when shipped. Always retain a copy of the Shipping Form for your records.



## Appendix C. Labels (cont.)

### Applying the Lab ID Label to the Storage Vial



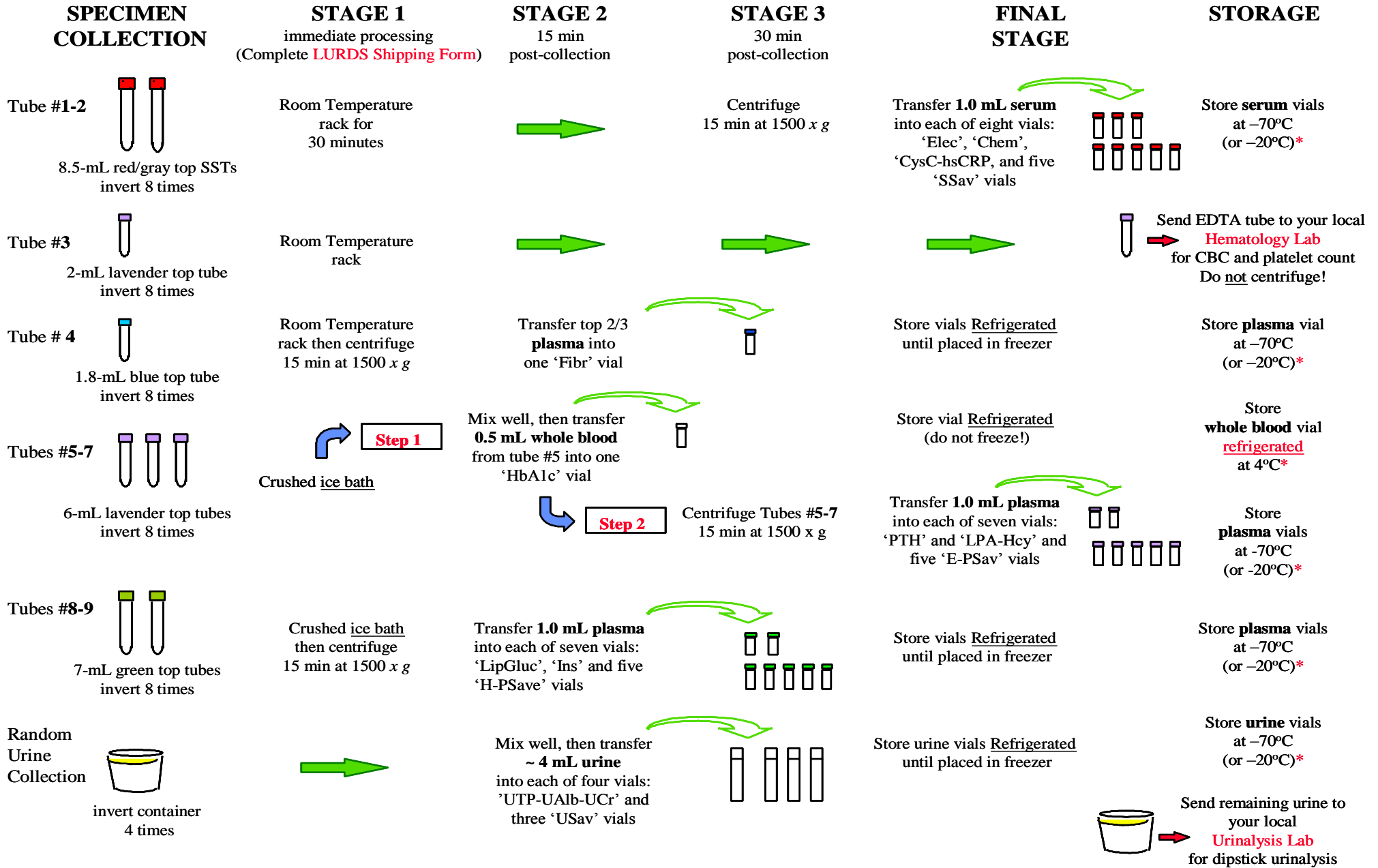
When applying the LURDS Lab ID label to the storage vial, apply the white portion of the label containing the accession number first – make sure that the leading digit of the Lab ID number (accession number) is nearest to the bottom of the vial. Then wrap the rest of the label around the vial. The clear portion of the label will overlap a portion of the white part of the label without covering any identifying information. The barcode must be straight up and down from the cap to the foot of the tube in order to be able to read with a standard barcode reader.

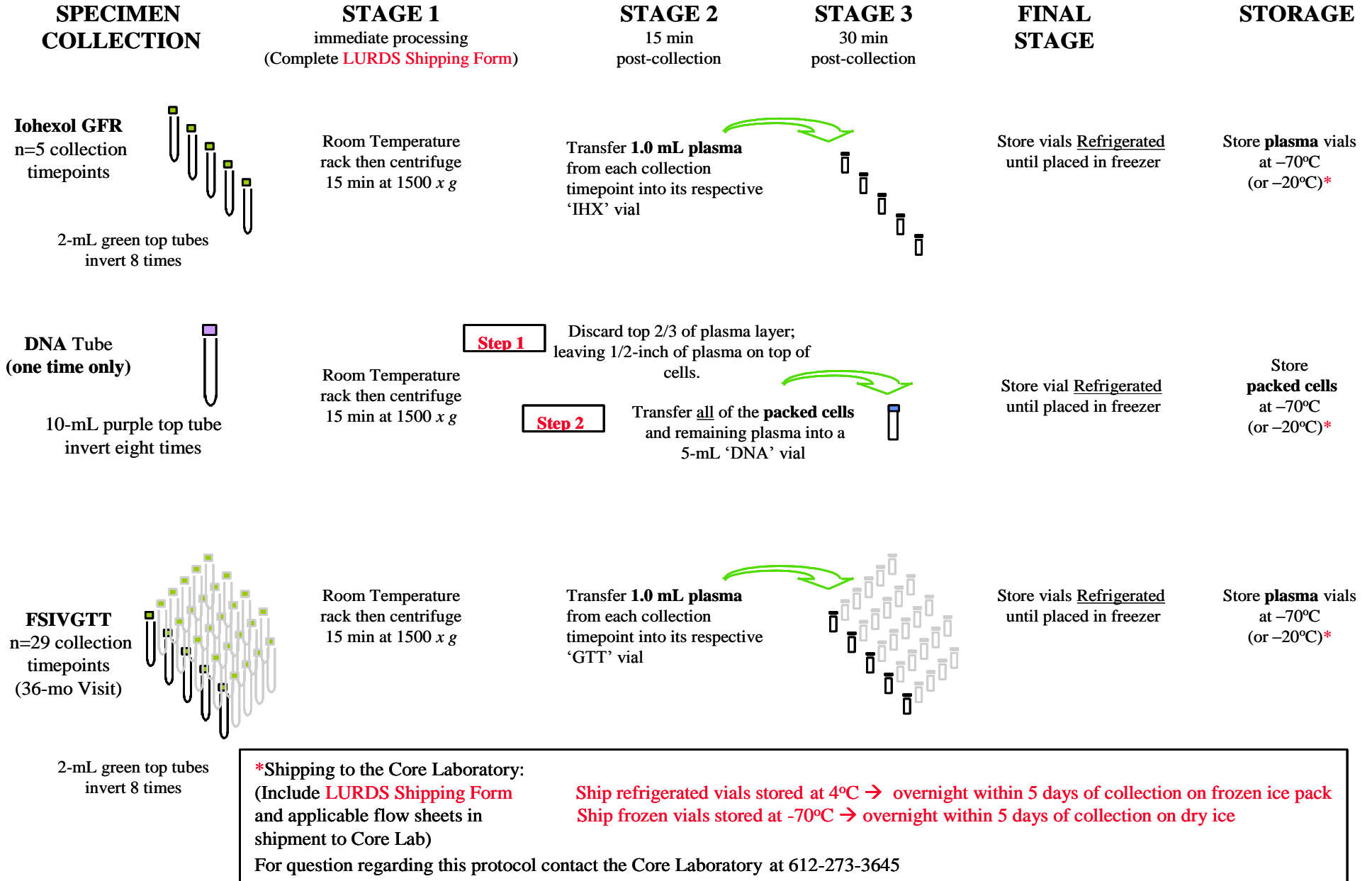
Note: These barcodes are high-density barcodes and can only be read by high-density barcode readers. The label program (Label View), which creates these barcodes, uses 128A barcode symbology.

# LURDS

Living Unrelated Donor Study

# Core Laboratory





# Appendix E. Packaging of Specimens for Shipment to LURDS Core Lab

Ship refrigerated vials stored at 4°C → within 5 days on ice pack via overnight courier  
 Ship frozen vials stored at -70°C → within 5 days on dry ice via overnight courier  
 Include LURDS Shipping Form and applicable Flow Sheets in shipment to Core Lab

For questions regarding this protocol contact the Core Laboratory at 612-273-3645

