BIOSPECIMEN PROCESSING

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1. Background and rationale

The MOST 60-Month visit involves the collection of approximately 15 mL of blood from participants. The blood is collected in two types of tubes for specialized processing of different blood components. After processing, the specimens will be aliquoted into cryovials to be sent to Biomedical Research Institute (BRI) to store for later analyses.

2. Equipment and supplies

A complete supply list with ordering information for laboratory processing can be found in Appendix 4. Necessary supplies include:

- Refrigerated centrifuge (4°C)
- −70° C freezer space is required
- Preprinted specimen ID barcode labels (see Appendix 3)
- Balance tubes for the centrifuge
- Pipettes: disposable polyethylene transfer pipettes
- Rainin pipette and tips (or similar pipette filler)
- Cryovials and cap inserts (provided by Coordinating Center)
- Cryovial storage/freezer boxes 9 x 9 grid (provided by Coordinating Center)
- Lab coat and gloves
- Biohazardous waste disposal container
- Aliquot rack
- Lab mat
- 10% bleach solution
- Rubber bands
- Styrofoam shipping containers
- Absorbent pads
- Dry ice (for shipping)
- FedEx shipping labels
- TO and FROM labels

2.1 Specimen ID labels

You will be supplied with specimen ID barcode labels from the UCSF Coordinating Center to use for labeling forms, draw tubes, urine specimen cup, cryovials, and the MOST Laboratory Shipping Grid form. A sample of the barcode labels can be found in Appendix 3. Specimen labels for each participant will have a unique 5-digit specimen ID number.

Labels

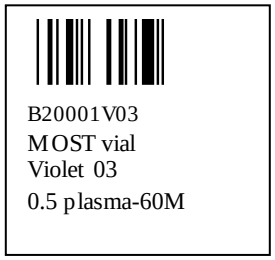
- one for the "Urine Collection form"
- one for the "Laboratory Processing form"
- two for pre-labeling the two draw tubes

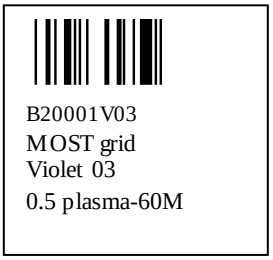
- two extras for back-up vacutainers
- one for the urine specimen cup
- 14 for cryovials
- 14 for the MOST Laboratory Shipping Grid form

There is one barcode label with the words "MOST 60M Lab Form," which is placed on the upper right hand corner of the Laboratory Processing form. Write the 5-digit specimen ID# in the boxes under the label. This process will allow the Coordinating Center to match the 7-digit participant-specific MOST ID# to a unique 5-digit specimen-specific ID# in the data system. **It is crucial that the Coordinating Center be able to match the specimen to the MOST participant in order to use the data collected from laboratory tests.**

Each of the 14 cryovials will already have a specimen ID label affixed to it. This is done the day before the clinic visit. Each cryovial label has a 2-digit extension (01 to 14) that serves as a unique identifier for each cryovial type within a specimen ID. The labels for cryovials and the MOST Laboratory Shipping Grid form have barcodes to help BRI track the repository.

On the top of the label is the barcode. The first line has the barcode information. The second line has an amalgamation of identifiers: the site ("B" or "T"), the 5-digit specimen ID# (Alabama 60-month specimen ID#s will start with 20001 and Iowa 60-month specimen ID#s will start with 70001), cap color identifier, and cryovial type extension. The third line has the study name, MOST, and if it is a cryovial label, whether the label is for the vial or grid. The fourth line refers to the cap color and number of the cryovial, or the purpose of the label. The fifth line describes the contents of the cryovial.





Each cryovial label has a duplicate label identified with the word "grid," which will be placed on a paper grid. This grid shows the contents in the cryovial storage box.

3. Safety issues and exclusion

3.1 Precautions for handling blood specimens

In accordance with the OSHA regulations on blood-borne pathogens (see copy on file in laboratory); the following laboratory safety protocol is recommended for the clinical center laboratories:

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

- 'Universal Precautions' should be followed when handling any blood or bodily fluid products.

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

- Hepatitis B vaccine must be offered to all unvaccinated technicians handling blood and documentation of vaccination or technician's declining to be vaccinated should be kept.

4. Laboratory room preparation

4.1 Preparation for processing

All items on the Laboratory Processing Checklist (Appendix 1) should be on hand before beginning processing.

Aliquot racks will be set up to correspond to each blood collection tube rack. **Rack setup is completed the previous day.** All tubes and vials are labeled with appropriate specimen ID label (see Chapter 3C, Biospecimen Collection, Appendix 2, Specimen Label (Placement on Cryovial)) and arranged in the appropriate working order *(see details in Chapter 3C, Biospecimen Collection, sections 2.1 and 2.22*).

There are a total of 36 labels. The last label identified as "END OF SET" can be discarded.

The following 20 labels will be affixed or discarded prior to the specimens arriving to the lab:

- two labels for the vacutainers
- two labels for the extra vacutainers
- one label for the "Urine Collection form"
- one label for the urine cup
- 14 labels for cryovials

The following 15 labels will be affixed in the lab:

- one label "Lab form" for the "Laboratory Processing form," (clipped to blood collection tray)
- 14 labels "MOST grid" for the MOST Laboratory Shipping Grid form

5. Detailed procedures

5.1 Processing

5.1.1 General

Personal protective equipment (non-permeable lab coats, double-gloves with at least one latex pair, splatter shields) MUST BE worn for processing.

It is possible that not all tubes will be collected due to problems with blood collection. During laboratory processing, work in the cryovial numerical order and make as many aliquots as possible while meeting the volume requirement of each cryovial. Make every attempt to fill cryovials completely. All cryovials have the capacity to hold 0.5 mL in volume.

After aliquoting the specimens, discard the vacutainers, disposable pipettes and pipette tips as biohazardous materials.

5.1.2 Description of blood collection tubes

Each draw tube is color coded (lavender or red vacutainer top) to aid in handling.

Lavender top draw tube
Draw tube #1 is a 3 to 5 mL (depending on clinical site) lavender top vacutainer containing 15% EDTA as the anticoagulant. This tube is used to collect plasma. It is very important that this tube is completely filled. If the tube is less than 2/3 full, discard the tube. After drawing the blood, the tube will have been gently inverted 10 to 15 times minimum, protected from light contamination, and placed in a "wet ice" bath.

Red top draw tube
Draw tube #2 is a 7 to 10 mL (depending on clinical site) red top vacutainer used to collect serum. This tube contains no anticoagulant, so the blood will clot to form serum. After drawing the blood, protect the tube from light contamination, and put in the tube rack at room temperature for 30 minutes. If not processed at that time, the tube should be placed in a refrigerator for up to an additional 30 minutes (processed no more than 60 minutes total after blood draw).

5.1.3 Processing of blood and urine specimens

Upon reaching the blood processing station, remove the blood drawing rack and "wet ice" bath containing the tube from the collection tray. The "wet ice" bath should contain tube #1. The rack should contain tube #2. The urine in the specimen cup should be processed immediately or refrigerated for up to 4 hours and then processed.

5.1.3.1 Plasma processing

Centrifuging and processing of draw tube #1 should take place **immediately** (maximum time of 15 minutes after collection). Plasma aliquots will be made from the lavender top draw tube. The

plasma will be aliquoted into cryovials with violet caps. The plasma (violet cap cryovials 01 to 03) will be archived for future analysis.

Aliquot	Cryovial #	Cap color	Analysis
Plasma	01 to 03	Violet	Archived

Document the time at start of EDTA plasma processing in the space provided on the top of the Laboratory Processing form.

- The EDTA lavender top tube is to be spun down in a refrigerated horizontal centrifuge at 4°C for 15 minutes at 2500 rpm.
- After centrifuging, immediately pipette and transfer 0.5 mL of the plasma from the lavender top tube into each of the three pre-labeled plasma cryovials 01 to 03 (violet caps).
- Cap the cryovials with violet caps, place in cryovial storage box, and place in –70°C freezer.
- Discard the draw tubes with only the clot remaining.
- ***Note ending time of EDTA plasma aliquoting on the Laboratory Processing form.***

5.1.3.2 Serum processing

Draw tube #2 (7 to 10 mL red top) must remain at room temperature for 30 minutes and then either be processed or placed upright in a refrigerator or wet ice bath for up to an additional 30 minutes until processed. Room temperature is 21°C (the range of 15.5°C→ 23.5°C is acceptable), 70°F (the range of 60°F→ 74°F is acceptable). The maximum allowable time before centrifugation is 60 minutes. The tube should display a clot by this time. The serum (red cap cryovials 04 to 10) will be stored for future analysis.

Aliquot	Cryovial #	Cap color	Analysis
Serum	04 to 10	Red	Archived

Document the time at start of serum processing in the space provided in the appropriate section of the Laboratory Processing form.

- The red top tube is to be spun down in a refrigerated centrifuge at 4°C for 15 minutes at 2500 rpm.
- After centrifuging, immediately pipette 0.5 mL of serum into each of the red cap cryovials 04 to 10. Once serum is centrifuged it must either be aliquoted immediately or placed in a refrigerator up to an additional 15 minutes **maximum** and then aliquoted.
- Cap cryovials with red caps, place in cryovial storage box, and place in –70°C freezer.
- Discard the draw tubes with only the clot remaining.
- ***Note the ending time of serum processing on the Laboratory Processing form.*** The total time from blood draw to end of serum processing should not exceed 90 minutes.

5.1.3.3 Urine specimen

Urine specimens will be archived for future analysis. If the urine specimen cannot be immediately aliquoted into the four pre-labeled cryovials #11 to 14, place the filled urine cup into the refrigerator until the processing can begin (maximum time four hours). Do not allow the urine to

be overly exposed to a light environment. The urine specimen is mixed by inverting the urine cup three to five times and then aliquoting 0.5 mL into each of the four cryovials.

Aliquot	Cryovial #	Cap color	Analysis
Urine	11 to 14	Clear	Archived

- Pipette 0.5 mL of urine into the clear capped cryovials #11 to 14. Note: do not overfill the cryovial.
- Cap the cryovials with clear caps, place in cryovial storage box and place in freezer.
- Discard the remaining urine.

5.1.4 Aliquots per specimen type

The following is a summary of the specimen processing. Volume indicates **aliquot size for each cryovial.** All cryovials will be 0.5 mL in size.

Plasma (3 to 5 mL EDTA tube #1)

Aliquot	Cryovial(s)	Cap color	Volume	Analysis
Plasma	3 (#01 to 03)	Violet	0.5 mL	Archived

Serum (7 to 10 mL serum tube #2)

Aliquot	Cryovial(s)	Cap color	Volume	Analysis
Serum	7 (#04 to 10)	Red	0.5 mL	Archived

Urine (urine specimen cup)

Aliquot	Cryovial(s)	Cap color	Volume	Analysis
Urine	4 (#11 to 14)	Clear	0.5 mL	Archived

The cryovials will be placed into one of three boxes with the 9 x 9 grid. The front and top of the box must be premarked. One box will be identified as **60M PLASMA**, one box will be identified as **60M SERUM**, and the third box will be identified as **60M URINE**. Write legibly with a Sharpie permanent marker. The boxes must be numbered sequentially starting with B001, then 002, etc. for Birmingham and I001, then 002, etc for Iowa. An empty green capped cryovial will be placed in the last cell of each box to allow the repository personnel to quickly orient themselves to cryovial order during specimen retrieval (see Appendix 5, MOST Laboratory Shipping Grid form).

The **PLASMA** box will contain cryovials #01 to 03 with violet caps.

The **SERUM** box will contain the cryovials #04 to 10 with red caps.

The **URINE** box will contain the cryovials #11 to 14 with clear caps.

Within each box the cryovials will be arranged sequentially by aliquot vial # (e.g., 01, then 02, and then 03; 04, then 05, etc). Each row of the box will be filled sequentially left to right from top row to bottom row. Do not leave empty spaces in the boxes. Specimens from one participant will

likely overlap into the next storage box. Boxes will have specimens from more than one participant.

5.1.5 MOST Laboratory Shipping grid form

Complete the MOST Laboratory Shipping Grid – 60 Month Visit Specimens form (download form from MOST website (https://www.keeptrack.ucsf.edu) \ Non-TELEforms \ Lab Shipping forms \ Laboratory Shipping Grid). IMPORTANT: This form must be printed on 11" x 17" paper. It will serve as the manifest for archiving the specimens. ***It is very important that the form exactly match the contents of the box, as the master database of archived specimens will be created from the information on the MOST Laboratory Shipping Grid – 60 Month Visit Specimens form.*** Make two photocopies of the completed form prior to shipping. It is okay to shrink the photocopies to fit on 8.5" x 11" paper. Keep a binder of all completed Laboratory Shipping Grid forms and mail the second copy to:

ATTN: MOST
UCSF Coordinating Center
185 Berry Street, Lobby 5, Suite 5700
San Francisco, CA 94107.

5.1.6 Freezing

Upon completion of each of the processing steps, plasma, serum, and urine specimens must be placed in labeled 9 x 9 cryovial storage boxes and immediately frozen at –70°C.

5.1.7 Completing forms

On the Laboratory Processing form, the 60-month visit bubble, MOST ID#, and Acrostic should be prefilled. If these are not prefilled, please contact the onsite study coordinator. Fill in your staff ID# and mark the bubble to indicate whether it is the First sample collection or Repeat sample collection. Check to make sure that the Bar Code Label is affixed to the form and that the 5-digit number is entered in the "Enter ID from Bar code label" boxes. Enter the "Time at start…" and "Ending time…" for each specimen that you are processing. Fill the bubble next to each cryovial that is filled, whether partially or totally. Make every attempt possible to fill vials with 0.5 mL of the specimen. If the specimen is hemolyzed, fill the bubble marked "H." If the serum is reddish in color, determine if it is hemolyzed or simply contaminated with red blood cells. One can tell the difference by recentrifuging the vacutainer tube. This will pellet any contaminating red cells and the serum will be clear. If the specimen is hemolyzed the red color will remain in the serum. Avoid partially filled tubes whenever possible, but if the tube is only partially filled, fill the bubble marked "P." If the tube is both hemolyzed and partially filled, fill the bubble marked "B." If the tube is not filled at all, only fill the last bubble, 'not filled.'

How to fill out the Laboratory Processing form for each cryovial:

Condition of tube	Indication on form
Filled, not hemolyzed	OK
Filled, hemolyzed	H (for hemolyzed)
Partially filled, not hemolyzed	P (for partial)
Partially filled, hemolyzed	B (for both)
Not filled	Not filled

The completed Initial Knee Pain and Urine Collection, Phlebotomy, Laboratory Processing forms, and the MOST Laboratory Shipping Grid – 60 Month Visit Specimens form can be set aside in a daily work folder. Make two copies of the MOST Laboratory Shipping Grid – 60 Month Visit Specimens form (okay to shrink photocopy to 8.5" x 11" paper size). One photocopy must be kept on file at the site and the other sent to the UCSF Coordinating Center. The original MOST Laboratory Shipping Grid – 60 Month Visit Specimens form must be included with the shipment of specimens to Biomedical Research Institute (BRI).

Send to BRI:

- Original of MOST Laboratory Shipping Grid – 60 Month Visit Specimens form

The 9 x 9 cryovial storage box holds 81 cryovials, but the last cryovials will be an empty green cap cryovial. The front and top of the cryovial box must be prelabeled:

60M PLASMA
B001 (or I001) – sequentially number the boxes

60M SERUM
B001 (or I001) – sequentially number the boxes

60M URINE
B001 (or I001) – sequentially number the boxes

Write legibly with a black Sharpie permanent marker. Write the type of specimen (60M PLASMA, 60M SERUM or 60M URINE) and the sequential box number on the top and front of the cryovial box.

5.2 End of the day procedures

Each site will determine which phlebotomy or lab staff are responsible for end-of-day procedures including:

- Re-stock blood collection trays with supplies.
- Label the next day's draw tubes, urine cup, and cryovials for each participant.
- Arrange draw tubes and cryovials in their proper racks.
- Clip the extra labels to the blood collection rack or aliquot rack
- Wipe down all work areas with 10% Clorox solution.

5.3 Summary of processing time limitations

From end of venipuncture to start of processing:

1. EDTA 3 to 5 mL tube	**immediately** to 15 minutes
2. Serum 7 to 10 mL tube	**30 minutes** to 60 minutes maximum
3. Urine container	immediately to 4 hours maximum (refrigerated)

After the serum has been at room temperature for 30 minutes, it must either be processed or placed upright in a refrigerator or wet ice bath for up to an additional 30 minutes **maximum** and then processed. Once serum is centrifuged it must either be aliquoted or placed in a refrigerator up to an additional 15 minutes **maximum** and then aliquoted. After aliquoting specimens, place the cryovials in a cryovial storage box, and freeze immediately at –70°C.

5.4 Shipping the blood specimens

5.4.1 General

Frozen blood and urine specimens are shipped two times a month to BRI by Federal Express overnight delivery. Do not ship on Thursdays or Fridays to avoid delivery of shipments during a weekend. Also contact the repository if you plan to ship near the date of a holiday.

Shipments to BRI are charged to your local Federal Express or UPS account number.

The shipping protocol is the mandated International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904.

Use the Specimen Shipping checklist (see Appendix 6) to prepare for shipping specimens. A sample of the Federal Express Airbill for Dry Ice Shipment is in Appendix 7 and the Dry Ice Outer Box Labeling Diagram is in Appendix 8.

5.4.2 Methods for shipping frozen specimens

The frozen blood and urine cryovials are already packaged in prelabeled freezer boxes and stored in the –70°C freezer by consecutive box number. Complete the MOST Laboratory Shipping Grid form detailing the contents of the shipment. Place an absorbent pad with a 100 mL capacity in the cryovial box. Place two rubber bands around the box so the contents do not shift during shipping. Put one or two cryovial boxes in a leak-proof zip lock bag, and put the original MOST Laboratory Shipping Grid form(s) inside a separate leak proof zip-lock bag. Keep the photocopy of the grid form(s) in a binder at the laboratory and send the second photocopy to the UCSF Coordinating Center. Pack the shipping boxes with dry ice according to the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions for shipping frozen specimens.

E-mail BRI (SPECIMENSTORAGE@aol.com) the day before you send a shipment, letting them know how may boxes they will be receiving and the FedEx airbill number. Securely tape the bottom copy of the airbill to the shipping container with scotch tape. Apply separate TO: and FROM: labels to the shipping container (in addition to the FedEx airbill). Be sure to include the name, address and phone number on the TO: label to Biomedical Research Institute (BRI) and the FROM: label from the clinical center.

6. Laboratory Processing Form

7873

Visit	MOST ID #	Acrostic	Staff ID#
O 60-month O 84-month			

Laboratory Processing O First sample collection O Repeat sample collection

Time at start of EDTA plasma processing: Hours Minutes O am O pm

Bar Code Label

Enter ID from Bar Code label:

Collection Tubes	Cryo #	Vol.	Cap	Condition of cryovial (mark only one)				
#1 EDTA plasma tube								
-plasma	01	0.5	V	O OK	O H	O P	O B	O not filled
-plasma	02	0.5	V	O OK	O H	O P	O B	O not filled
-plasma	03	0.5	V	O OK	O H	O P	O B	O not filled

Ending time of EDTA plasma aliquoting: Hours Minutes O am O pm

Time at start of serum processing: Hours Minutes O am O pm

Collection Tubes	Cryo #	Vol.	Cap	Condition of cryovial (mark only one)				
#2 Serum tube								
-serum	04	0.5	R	O OK	O H	O P	O B	O not filled
-serum	05	0.5	R	O OK	O H	O P	O B	O not filled
-serum	06	0.5	R	O OK	O H	O P	O B	O not filled
-serum	07	0.5	R	O OK	O H	O P	O B	O not filled
-serum	08	0.5	R	O OK	O H	O P	O B	O not filled
-serum	09	0.5	R	O OK	O H	O P	O B	O not filled
-serum	10	0.5	R	O OK	O H	O P	O B	O not filled

Ending time of serum aliquoting: Hours Minutes O am O pm

Urine						
-urine	11	0.5	C	O OK	O P	O not filled
-urine	12	0.5	C	O OK	O P	O not filled
-urine	13	0.5	C	O OK	O P	O not filled
-urine	14	0.5	C	O OK	O P	O not filled

H=Hemolyzed P=Partial B=Both V=Violet R=Red C=Clear

7. Quality Assurance

7.1 Training Requirements

Clinical experience with processing of blood samples is strongly recommended. Additional training should include:

- Read and study manual
- Attend MOST training session on techniques (or observe administration by experienced examiner)
- Discuss problems and questions with local expert or QC officer

7.2 Certification Requirements

__ Complete training requirements
__ Recite shipping schedule for applicable clinical center
__ Process one set of specimens from volunteer or participant while being observed by QC officer using QC checklist

7.3 Quality Assurance Checklist

Preparation

- ☐ Aliquot racks correctly set up
- ☐ Cryovials correctly labeled
- ☐ Cryovial storage boxes labeled correctly
- ☐ Empty green cap cryovial placed in last cell of cryovial storage box
- ☐ Hepatitis B vaccination given or offered to all personnel handling blood
- ☐ Non-permeable lab coats, gloves, and face shields used

Processing EDTA plasma and urine

- ☐ Time checked to ensure that tube 1 is processed within 15 minutes of completion of phlebotomy
- ☐ Urine specimen refrigerated until processed
- ☐ Note time of EDTA tube processing on data collection form
- ☐ EDTA plasma tube #1 at least 2/3 full, otherwise discarded
- ☐ Tube #1 centrifuged at 4°C for 15 min at 2500 rpm
- ☐ Aliquots immediately placed in −70°C freezer
- ☐ New pipette tips used for EDTA plasma
- ☐ Plasma correctly aliquoted in correct order
- ☐ Note ending time of EDTA processing on the form
- ☐ Urine specimen inverted 3 to 5 times before aliquoting
- ☐ New pipette or tip used for urine aliquoting
- ☐ Urine correctly aliquoted

Processing serum tubes

- ☐ Time checked to ensure that tube #2 stood at room temperature for at least 30 minutes, and if longer than 30 minutes then refrigerated or placed in wet ice bath
- ☐ Time checked to ensure that tube # 2 was processed within 60 minutes
- ☐ Tube #2 centrifuged for 15 minutes at 2500 rpm
- ☐ Centrifuge correctly balanced with water tube(s)
- ☐ Serum correctly aliquoted
- ☐ Note ending time of serum processing on the form

Freezing

- ☐ Duplicate cryovial labels placed on MOST Laboratory Shipping Grid form
- ☐ Aliquots placed in cryovial storage boxes and immediately frozen in –70°C freezer

End of day procedure

- ☐ Urine Collection, Phlebotomy, and Laboratory Processing forms reviewed for thoroughness and placed in daily work folder
- ☐ Freezer boxes correctly labeled
- ☐ MOST Laboratory Shipping Grid form checked for completeness

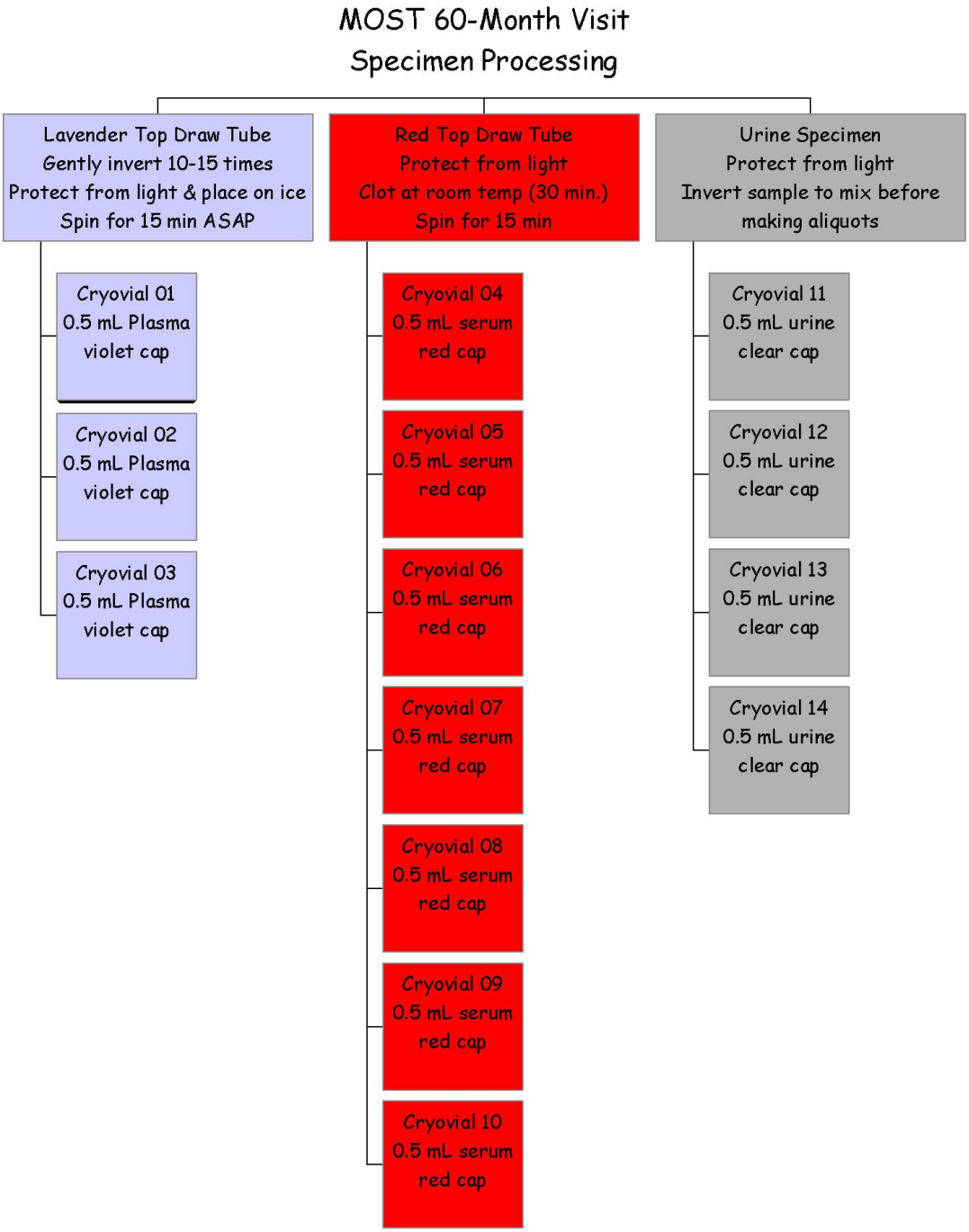
Shipment procedures – dry ice

- ☐ Freezer boxes correctly wrapped – absorbent material, rubber band, and zip-lock bag
- ☐ Styrofoam mailers correctly packed – absorbent material, dry ice, top sealed with tape
- ☐ Styrofoam mailer sealed in cardboard sleeve
- ☐ FedEx airbill correctly filled out and affixed to shipping container
- ☐ Extra scotch tape applied to bottom FedEx airbill
- ☐ TO and FROM labels with address and phone information correctly affixed to shipping container
- ☐ BRI notified by e-mail the day before shipment with FedEx airbill tracking number information

Appendix 1 Laboratory Processing Checklist

- ☐ Pipettes: disposable and pipette filler with tips
- ☐ Labeled cryovials in rack
- ☐ Lab coat and gloves
- ☐ Biohazardous waste disposal
- ☐ Refrigerated centrifuge (4°C) capable of spinning at 2,500 rpm
- ☐ Balance tubes for the centrifuge
- ☐ Tube rack in refrigerator
- ☐ 10% bleach solution
- ☐ Cryovial storage box for freezing specimens
- ☐ Freezer boxes with 9 x 9 grid
- ☐ Rubber bands
- ☐ Black Sharpie marking pen

Appendix 2 MOST Specimen Processing Schematic



Appendix 3 Cryovial Labels (page 1 of 2)

00001 ##### MOST vial Violet 01 0.5 plasma-60M	00001 ##### MOST grid Violet 01 0.5 plasma-60M
00001 ##### MOST vial Violet 02 0.5 plasma-60M	00001 ##### MOST grid Violet 02 0.5 plasma-60M
00001 ##### MOST vial Violet 03 0.5 plasma-60M	00001 ##### MOST grid Violet 03 0.5 plasma-60M
00001 ##### MOST vial Red 04 0.5 serum-60M	00001 ##### MOST grid Red 04 0.5 serum-60M
00001 ##### MOST vial Red 05 0.5 serum-60M	00001 ##### MOST grid Red 05 0.5 serum-60M

00001 ##### MOST vial Red 06 0.5 serum-60M	00001 ##### MOST grid Red 06 0.5 serum-60M
00001 ##### MOST vial Red 07 0.5 serum-60M	00001 ##### MOST grid Red 07 0.5 serum-60M
00001 ##### MOST vial Red 08 0.5 serum-60M	00001 ##### MOST grid Red 08 0.5 serum-60M
00001 ##### MOST vial Red 09 0.5 serum-60M	00001 ##### MOST grid Red 09 0.5 serum-60M
00001 ##### MOST vial Red 10 0.5 serum-60M	00001 ##### MOST grid Red 10 0.5 serum-60M

00001 ##### MOST vial Clear 11 0.5 urine-60M	00001 ##### MOST grid Clear 11 0.5 urine-60M
00001 ##### MOST vial Clear 12 0.5 urine-60M	00001 ##### MOST grid Clear 12 0.5 urine-60M
00001 ##### MOST vial Clear 13 0.5 urine-60M	00001 ##### MOST grid Clear 13 0.5 urine-60M
00001 ##### MOST vial Clear 14 0.5 urine-60M	00001 ##### MOST grid Clear 14 0.5 urine-60M
00001 ##### MOST-60M Collect Form	00001 ##### MOST-60M Lab Form

00001 ##### MOST-60M X-tra Tube Lav top 3-5 mL	00001 ##### MOST-60M X-tra Tube Red top 7-10 mL

00001 ##### MOST-60M Tube 1 Lav top 3-5 mL	00001 ##### MOST-60M Tube 2 Red top 7-10 mL
00001 ##### MOST-60M Urine Cup	END OF SET

Appendix 4 Laboratory Processing Supplies

Lab Supplies	# suggested	sample type	vendor: catalog #
Fisherbrand* Disposable Polyethylene Transfer Pipettes Use: Can be used to pipette plasma, serum, and urine volumes.	3/participant if used for plasma, serum, and urine aliquots	plasma, serum, urine	Fisher: 13-711-5A or equivalent from a different vendor
Rainin Pipette – Air-displacement Pipet-Plus® Latch-Mode™ Pipette with LTS manufactured by Rainin Instruments, LLC. Use: can be used to make all aliquots (serum, plasma, urine).	1 (purchased at baseline visit)	plasma, serum, urine	RL-1000 (volume range 100 uL – 1 mL)
Rainin Pipette Tips for Pipet-Plus® Latch-Mode™ Pipette Use: Pipette tips used in conjunction with the Rainin Pipette to measure volumes and make aliquots. A new tip must be used for each different type of matrix.	3/participant if used for plasma, serum, and urine aliquots	plasma, serum, urine	RT-L1000
Storage/Shipping Supplies	**# suggested**	**sample type**	**vendor: catalog #**
Leakproof ziplock bags (12" x 12") Use: Place cryovial storage boxes in ziplock bag before placing in shipping container.			Purchase locally
Saf-T-Pak absorbent pads (100 mL absorbent capability) Use: Place inside storage boxes	1/cryovial box		Catalog No. STP 151 or equivalent from a different vendor
Polyfoam Packer shipping containers (reusable) Use: to ship cryovial storage boxes to BRI.	4 for start-up. (Can be reused, but shipping costs may prohibit)		Catalog No. 398: (18”x11”x12”), up to twenty 2” freezer boxes. Catalog No. 346-UPS: (11.375”x8.375”x11”), up to twelve 2” freezer boxes. Catalog No. 430: (10”x17”x11”), up to five 2” freezer boxes.

Note: Educational discounts should apply.

Fisher Scientific:
Fisher HealthCare
9999 Veterans Memorial Drive
Houston, Texas 77038
1-800-640-0640
www.fishersci.com

Sigma-Aldrich Corp.
St. Louis, MO, USA
Phone: 314-771-5765
www.sigmaaldrich.com

Rainin Instrument, LLC
7500 Edgewater Drive, Box 2160
Oakland, CA 94621-0060
1-800-472-4646

Styrofoam shipping containers:
Polyfoam Packers
2320 Foster Avenue
Wheeling, IL 60090
1-800-323-7442
www.polyfoam.com

Saf-T-Pak
10807 – 182 Street
Edmonton, Alberta
Canada T5S 1J5
1-800-814-7484
www.saftpak.com

Additional Supplies:
- FedEx airbills and airbill pouches: Local FedEx office,
- Class 9 labels: Local FedEx office,
- "Diagnostic Specimens" and "Keep Frozen" labels: produced by site,
- Dry Ice: Purchase locally.

Supplies provided by the Coordinating Center:
- Cryovials and caps
- Cryovial labels
- Cryovial storage boxes

Appendix 5 Specimen Shipping Checklist

- ☐ Styrofoam Mailing Containers (2 different sizes) with outer cardboard sleeve
- ☐ *Polyfoam Packers # 398*
- ☐ *Polyfoam Packers # 346*
- ☐ *Polyfoam Packers # 430*
- ☐ Absorbent material
- ☐ Freezer boxes with 9 x 9 grids (rubber bands around box)
- ☐ Leakproof Zip-lock bags
- ☐ Packaging tape
- ☐ Dry ice (approximately 20 lbs. per box)
- ☐ FedEx Labels (provided by carrier)

Appendix 6 MOST Laboratory Shipping Grid – 60 Month Visit Specimens form

Print form on 11" x 17" paper

MOST Laboratory Shipping Grid – 60 Month Visit Specimens

SPECIMEN TYPE (MARK ONE): ____PLASMA ____SERUM ____URINE

MOST BOX #: _____

NOTE: AFFIX MOST BIOSPECIMEN LABELS TO THE GRID EXACTLY MATCHING THE SLOT LOCATION OF EACH CRYOVIAL. FILL EACH ROW OF THE BOX SEQUENTIALLY LEFT TO RIGHT FROM TOP ROW TO BOTTOM ROW. Use a separate grid form and cryovial box for each type of biological specimen – plasma, serum and urine. Number the boxes sequentially with a site identifier ("B" or "I") and a three digit number starting from one, i.e., B001, B002, etc. for Birmingham and I001, I002, etc. for Iowa City.

***Mark the visit, specimen type, and box number on the front and top of the box**, e.g., for Birmingham:"**60M PLASMA B001**," **60M SERUM B001**," and "**60M URINE B001;**" and for Iowa: "**60M PLASMA I001**," **60M SERUM I001**," and "**60M URINE I001**."*

START **TOP**

1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26	27
28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54
55	56	57	58	59	60	61	62	63
64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	**GREEN CAP Empty Vial**

BOTTOM **END**

Appendix 7 Laboratory Shipping Grid Examples

Laboratory Shipping Grid Examples for Shipping Plasma, Serum and Urine Samples to Biomedical Research Institute (BRI)

Example Grid for Shipping Samples: 60M PLASMA B001 / I001

60-Month Visit
Numbers = cryovial # (three (3) plasma violet cap cryovials per participant)

start #1 Top

Ppt #1 01	02	03	**Ppt #2** 01	02	03	**Ppt #3** 01	02	03
Ppt #4 01	02	03	**Ppt #5** 01	02	03	**Ppt #6** 01	02	03
Ppt #7 01	02	03	**Ppt #8** 01	02	03	**Ppt #9** 01	02	03
Ppt #10 01	02	03	**Ppt #11** 01	02	03	**Ppt #12** 01	02	03
Ppt #13 01	02	03	**Ppt #14** 01	02	03	**Ppt #15** 01	02	03
Ppt #16 01	02	03	**Ppt #17** 01	02	03	**Ppt #18** 01	02	03
Ppt #19 01	02	03	**Ppt #20** 01	02	03	**Ppt #21** 01	02	03
Ppt #22 01	02	03	**Ppt #23** 01	02	03	**Ppt #24** 01	02	03
Ppt #25 01	02	03	**Ppt #26** 01	02	03	**Ppt #27** 01	02	**EMPTY VIAL** **(green cap)**

Bottom end #81

Continue to next box.
Use a sharpie to mark the front and top outside cover of box:
60M PLASMA B001 (for Birmingham) and I001 (for Iowa)
Boxes must be numbered sequentially starting with B001, then 002, etc. for Birmingham and I001, then 002, etc. for Iowa.

Example Grid for Shipping Samples: 60M PLASMA B002 / I002

60-Month Visit
Numbers = cryovial # (three (3) plasma violet cap cryovials per participant)

start #1 Top

Ppt #27 03	**Ppt #28** 01	02	03	**Ppt #29** 01	02	03	**Ppt #30** 01	02
Ppt #30 03	**Ppt #31** 01	02	03	**Ppt #32** 01	02	03	**Ppt #33** 01	02
Ppt #33 03	**Ppt #34** 01	02	03	**Ppt #35** 01	02	03	**Ppt #36** 01	02
Ppt #37 03	**Ppt #38** 01	02	03	**Ppt #39** 01	02	03	**Ppt #40** 01	02
Ppt #40 03	**Ppt #41** 01	02	03	**Ppt #42** 01	02	03	**Ppt #43** 01	02
Ppt #43 03	**Ppt #44** 01	02	03	**Ppt #45** 01	02	03	**Ppt #46** 01	02
Ppt #46 03	**Ppt #47** 01	02	03	**Ppt #48** 01	02	03	**Ppt #49** 01	02
Ppt #50 03	**Ppt #51** 01	02	03	**Ppt #52** 01	02	03	**Ppt #53** 01	02
Ppt #53 03	**Ppt #54** 01	02	03	**Ppt #55** 01	02	03	**Ppt #56** 01	**EMPTY VIAL** **(green cap)**

Bottom end #81

Continue to next box.
Use a Sharpie to mark the front and top outside cover of box:
60M PLASMA B002 (for Birmingham) and I002 (for Iowa)
Boxes must be numbered sequentially starting with B001, then 002, etc. for Birmingham and I001, then 002, etc. for Iowa.

Example Grid for Shipping Samples: 60M SERUM B001 / I001

60-Month Visit
Numbers = cryovial # (seven (7) serum red cap cryovials per participant)

start #1 Top

Ppt #1 01	02	03	04	05	06	07	**Ppt #2** 01	02
03	04	05	06	07	**Ppt #3** 01	02	03	04
05	06	07	**Ppt #4** 01	02	03	04	05	06
07	**Ppt #5** 01	02	03	04	05	06	07	**Ppt #6** 01
021	03	04	05	06	07	**Ppt #7** 01	02	03
04	05	06	07	**Ppt #8** 01	02	03	04	05
06	07	**Ppt #9** 01	02	03	04	05	06	07
Ppt #10 01	02	03	04	05	06	07	**Ppt #11** 01	02
03	04	05	06	07	**Ppt #12** 01	02	03	**EMPTY VIAL** **(green cap)**

Bottom end #81

Continue to next box.
Use a sharpie to mark the front and top outside cover of box:
60M SERUM B001 (for Birmingham) and I001 (for Iowa)
Boxes must be numbered sequentially starting with B001, then 002, etc. for Birmingham and I001, then 002, etc. for Iowa.

Example Grid for Shipping Samples: 60M SERUM B002 / I002

60-Month Visit
Numbers = cryovial # (seven (7) total serum cryovials per participant-red cap cryovial)

start #1 Top

Ppt #12 04	05	06	07	**Ppt #13** 01	02	03	04	05
06	07	**Ppt #14** 01	02	03	04	05	06	07
Ppt #15 01	02	03	04	05	06	07	**Ppt #16** 01	02
03	04	05	06	07	**Ppt #17** 01	02	03	04
05	06	07	**Ppt #18** 01	02	03	04	05	06
07	**Ppt #19** 01	02	03	04	05	06	07	**Ppt #20** 01
02	03	04	05	06	07	**Ppt #21** 01	02	03
04	05	06	07	**Ppt #22** 01	02	03	04	05
06	07	**Ppt #23** 01	02	03	04	05	06	**EMPTY VIAL** **(green cap)**

Bottom end #81

Continue to next box.
Use a Sharpie to mark the front and top outside cover of box:
60M SERUM B002 (for Birmingham) and I002 (for Iowa)
Boxes must be numbered sequentially starting with B001, then 002, etc. for Birmingham and I001, then 002, etc. for Iowa.

Example Grid for Shipping Samples: 60M URINE B001 / I001

60-Month Visit
Numbers = cryovial # (four (4) total urine clear cap cryovials per participant)

start #1 Top

Ppt #1 01	02	03	04	**Ppt #2** 01	02	03	04	**Ppt #3** 01
02	03	04	**Ppt #4** 01	02	03	04	**Ppt #5** 01	02
03	04	**Ppt #6** 01	02	03	04	**Ppt #7** 01	02	03
04	**Ppt #8** 01	02	03	04	**Ppt #9** 01	02	03	04
Ppt #10 01	02	03	04	**Ppt #11** 01	02	03	04	**Ppt #12** 01
02	03	04	**Ppt #13** 01	02	03	04	**Ppt #14** 01	02
03	04	**Ppt #15** 01	02	03	04	**Ppt #16** 01	02	03
04	**Ppt #17** 01	02	03	04	**Ppt #18** 01	02	03	04
Ppt #19 01	02	03	04	**Ppt #20** 01	02	03	04	**EMPTY VIAL** **(green cap)**

Bottom end #81

Continue to next box.
Use a Sharpie to mark the front and top outside cover of box:
60M URINE B001 (for Birmingham) and I001 (for Iowa)
Boxes must be numbered sequentially starting with B001, then 002, etc. for Birmingham and I001, then 002, etc. for Iowa.

Example Grid for Shipping Samples: 60M URINE B002 / I002

60-Month Visit
Numbers = cryovial # (four (4) total urine clear cap cryovials per participant)

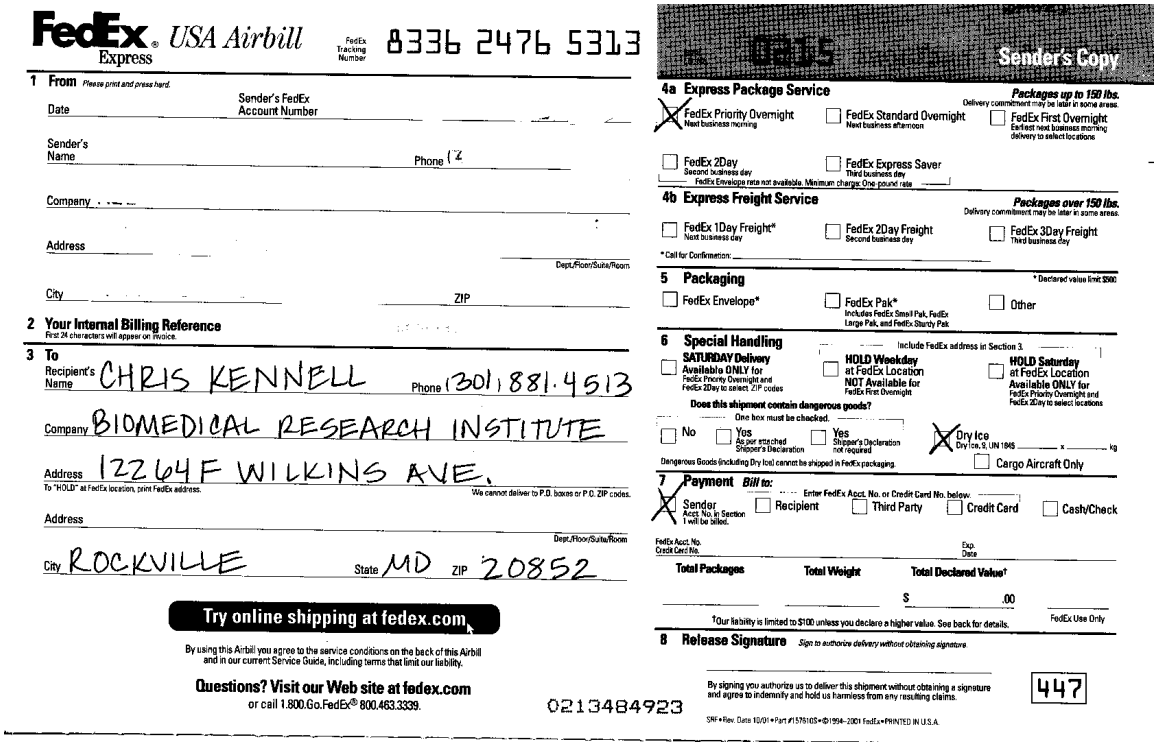
start #1 Top

Ppt #21 01	02	03	04	**Ppt #22** 01	02	03	04	**Ppt #23** 01
02	03	04	**Ppt #24** 01	02	03	04	**Ppt #25** 01	02
03	04	**Ppt #26** 01	02	03	04	**Ppt #27** 01	02	03
04	**Ppt #28** 01	02	03	04	**Ppt #29** 01	02	03	04
Ppt #30 01	02	03	04	**Ppt #31** 01	02	03	04	**Ppt #32** 01
02	03	04	**Ppt #33** 01	02	03	04	**Ppt #34** 01	02
03	04	**Ppt #35** 01	02	03	04	**Ppt #36** 01	02	03
04	**Ppt #37** 01	02	03	04	**Ppt #38** 01	02	03	04
Ppt #39 01	02	03	04	**Ppt #40** 01	02	03	04	**EMPTY VIAL** **(green cap)**

Bottom end #81

Continue to next box.
Use a Sharpie to mark the front and top outside cover of box:
60M URINE B002 (for Birmingham) and I002 (for Iowa)
Boxes must be numbered sequentially starting with B001, then 002, etc. for Birmingham and I001, then 002, etc. for Iowa.

Appendix 8 Federal Express Airbill for Dry Ice Shipment



FedEx Express USA Airbill FedEx Tracking Number 8336 2476 5313

Sender's Copy

1 From Please print and press hard.
Date
Sender's FedEx Account Number
Sender's Name
Phone
Company
Address
Dept./Floor/Suite/Room
City
ZIP

2 Your Internal Billing Reference
First 24 characters will appear on invoice.

3 To
Recipient's Name CHRIS KENNELL Phone (301) 881.4513
Company BIOMEDICAL RESEARCH INSTITUTE
Address 12264 F WILKINS AVE.
To "HOLD" at FedEx location, print FedEx address. We cannot deliver to P.O. boxes or P.O. ZIP codes.
Address
Dept./Floor/Suite/Room
City ROCKVILLE State MD ZIP 20852

Try online shipping at fedex.com

By using this Airbill you agree to the service conditions on the back of this Airbill and in our current Service Guide, including terms that limit our liability.

Questions? Visit our Web site at fedex.com or call 1.800.Go.FedEx® 800.463.3339.

0213484923

4a Express Package Service — Packages up to 150 lbs. Delivery commitment may be later in some areas.
- [X] FedEx Priority Overnight — Next business morning
- [ ] FedEx Standard Overnight — Next business afternoon
- [ ] FedEx First Overnight — Earliest next business morning delivery to select locations
- [ ] FedEx 2Day — Second business day
- [ ] FedEx Express Saver — Third business day
FedEx Envelope rate not available. Minimum charge: One-pound rate

4b Express Freight Service — Packages over 150 lbs. Delivery commitment may be later in some areas.
- [ ] FedEx 1Day Freight* — Next business day
- [ ] FedEx 2Day Freight — Second business day
- [ ] FedEx 3Day Freight — Third business day
* Call for Confirmation:

5 Packaging — * Declared value limit $500
- [ ] FedEx Envelope*
- [ ] FedEx Pak* — Includes FedEx Small Pak, FedEx Large Pak, and FedEx Sturdy Pak
- [ ] Other

6 Special Handling — Include FedEx address in Section 3.
- [ ] SATURDAY Delivery — Available ONLY for FedEx Priority Overnight and FedEx 2Day to select ZIP codes
- [ ] HOLD Weekday at FedEx Location — NOT Available for FedEx First Overnight
- [ ] HOLD Saturday at FedEx Location — Available ONLY for FedEx Priority Overnight and FedEx 2Day to select locations

Does this shipment contain dangerous goods? One box must be checked.
- [ ] No
- [ ] Yes — As per attached Shipper's Declaration
- [ ] Yes — Shipper's Declaration not required
- [X] Dry Ice — Dry Ice, 9, UN 1845 ____ x ____ kg
- [ ] Cargo Aircraft Only

Dangerous Goods (including Dry Ice) cannot be shipped in FedEx packaging.

7 Payment Bill to: — Enter FedEx Acct. No. or Credit Card No. below.
- [X] Sender — Acct. No. in Section 1 will be billed.
- [ ] Recipient
- [ ] Third Party
- [ ] Credit Card
- [ ] Cash/Check

FedEx Acct. No.
Credit Card No.
Exp. Date

Total Packages	Total Weight	Total Declared Value†
		$ .00

†Our liability is limited to $100 unless you declare a higher value. See back for details.
FedEx Use Only

8 Release Signature — Sign to authorize delivery without obtaining signature.
By signing you authorize us to deliver this shipment without obtaining a signature and agree to indemnify and hold us harmless from any resulting claims.

447

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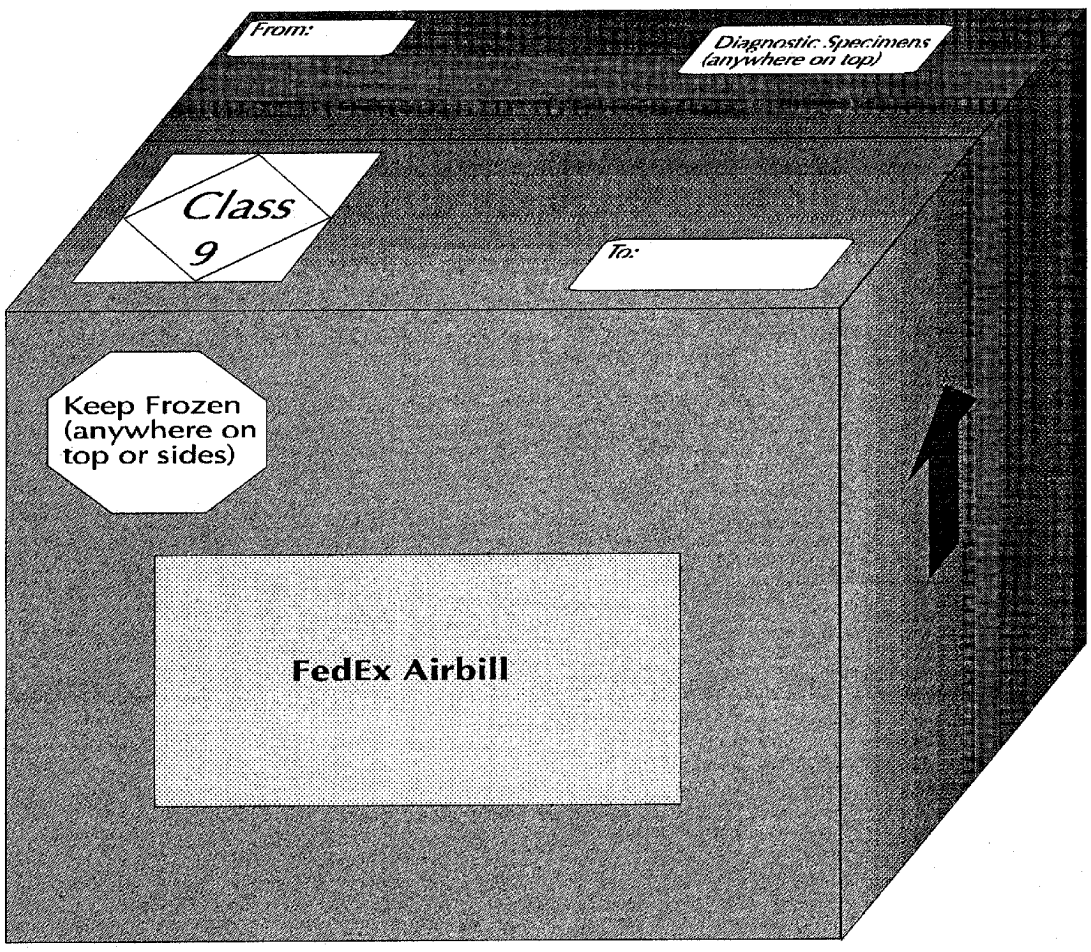
Appendix 9 Dry Ice and Labeling Diagram

Outer Box Labeling



NOTE: Labels must not overlap