

BIOSPECIMEN PROCESSING

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1. Background and rationale

At the 144-Month Clinic Visit (2016-2017) we will collect baseline 12 hour fasting blood and urine samples from new cohort study participants. Samples will be archived in a state-of-the art biospository for future analyses. Blood will be collected in three types of tubes for specialized processing of different blood components. After processing, biospecimens will be aliquoted into cryovials and shipped to the storage repository.

2. Equipment and supplies

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

- Centrifuge (4°C)
- -70 or -80° C freezer space is required
- -20° C freezer space (laboratory grade, not frost-free freezer)
- Refrigerator space
- Rainin pipette and tips (or similar pipette filler)
- Pipettes: disposable polyethylene transfer pipettes
- Preprinted specimen ID labels (see Appendix 3, provided by Coordinating Center)
- Cryovials, caps and cap inserts (provided by Coordinating Center)
- Cryovial storage boxes 9 x 9 grid (provided by Coordinating Center)
- PAXgene storage boxes (8 x 1 grid for UI; 3 x 1 grid for UAB)
- Urine-based pregnancy test kits
- Lab coat and gloves
- Biohazardous waste disposal container
- Aliquot rack
- Lab mat
- 10% bleach solution
- Barcode scanner (provided by Coordinating Center)
- Rubber bands
- Black sharpie permanent marker
- Styrofoam shipping containers
- Absorbent pads
- Dry ice (for shipping)
- FedEx shipping labels
- TO and FROM labels

2.1 Specimen ID labels

Specimen ID labels for each participant have a unique 5-digit specimen ID number (starting 144-month specimen ID number is 80001 for U-Iowa and 30001 for UAB). Labels with barcodes have a three-digit extension (e.g., B01) after the 5-digit specimen ID number that serves as a unique identifier for each storage cryovial or PAXgene tube. Barcodes will be used to inventory, track and manage samples at the Fisher BioServices repository.

Pre-printed labels are on large rolls with two labels (side by side) per row. There are 54 labels (27 rows) for each participant (see Appendix 3). Use of the labels is described in section 4.1 Preparation for processing.

Nonbarcode labels (8 labels)

- Row 1: Tube #1 (Lavender Top) and Tube #2 (Red Top)
- Row 2: Tube #3 Lavender Top) and Tube #4 (Red Top)
- Row 3: Tube #5 (Red Top) and Extra Tube (back-up for phlebotomists)
- Row 4: Urine Cup and Lab Form (Laboratory Processing form)

Barcode labels (46 labels)

- Row 5: one for PAX RNA (Red Top Tube) and one for PAX RNA grid form
- Rows 6 thru 11: Six for plasma vials (Blue 01-06) and six for plasma grid form
- Row 12: One for buffy coat vial (Yellow 07) and one for buffy coat grid form
- Rows 13 thru 24: Twelve for serum vials (Red 08-19) and twelve for serum grid form
- Row 25 thru 27: Three for urine vials (Clear 20-22) and three for urine grid form

2.1.1 Labels for use during laboratory processing

Laboratory staff will receive a set of 23 labels for use during laboratory processing (see Appendix 3, right-hand side labels in rows 4 thru 27).

- MOST grid Red Top Tube PAX RNA 144m (1 label)
- MOST grid Blue 01 (thru 06) 1.0mL plasma 144m (6 labels)
- MOST grid Yellow 07 buffy coat 144m (1 label)
- MOST grid Red 08 (thru 19) 1.0mL serum 144m (12 labels)
- MOST grid Clear 20 (thru 22) 1.5mL urine 144m (3 labels)

Check to make sure that the Lab Form label is already placed on the right upper right hand corner of the Laboratory Processing Form under the words “Bar Code Label” (see Section 6. Laboratory Processing Form) and that the 5-digit specimen ID# is clearly written in the boxes under the label (under the words “Enter ID from Bar Code 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. ***The process of matching the participant-specific MOST Participant ID# to the specimen-specific ID is crucial to being able to use data collected from laboratory tests.***

Laboratory staff will affix the 23 “MOST grid” labels to the MOST Lab Storage Box Grid form as detailed in section 5.1.7 MOST Lab Storage Box form.

2.1.2 Barcode labels

The PAXgene red top tube (9.5mL) for PAX RNA, and the 22 cryovials for aliquoting samples will already have a specimen ID barcode label affixed prior to sample collection (see MOST Biospecimen Collection Operation Manual). This should be done the day before the clinic visit so the laboratory staff can focus attention on specimen processing. The PAXgene tube and cryovial labels have a 3-digit extension (i.e., PAX, B01-B06, Y07, R08-R19, C20-C22) that serves as a unique identifier for each PAXgene tube and cryovial type within a specimen ID. Barcode labels allow the biorepository to inventory and track MOST samples.

- Lab staff will place “MOST grid” labels on a MOST Lab Storage Box form showing the exact sample location in the storage box.
- After each storage box is filled and the Excel logsheet called MOST Barcode Grid logsheet created using the barcode scanner (see Appendix 7A), the paper Lab Storage Box Grid form will be filed in a folder or binder at the clinic. The SF Coordinating Center will request a copy of the Lab Storage Box Grid form if needed to resolve inventory discrepancies.

Barcode labels for the PAXgene tube (Figures 1 and 2): The first line of the label has barcode information. The second line has an amalgamation of identifiers: the site ("B" or "T"), the 5-digit specimen ID# (Birmingham “B” 144-month specimen ID#s will start with **30001** and Iowa “T” 144-month specimen ID#s will start with **80001**), and PAXgene tube identifier (PAX). The third line has the either “MOST vial” (for the PAXgene tube) or “MOST grid” (for paper grid form). The fourth line has Red Top Tube (PAXgene vacutainer tube has a red stopper). The fifth line describes the sample (PAX RNA), and the study time point (144m).

Each PAX label has an identical twin label with the same amalgamation of identifiers (see Figures 1 and 2). The only difference is the word “vial” (Figure 1) is replaced with the word “grid” (Figure 2) in second line of text. The “grid” label will be placed on the MOST Lab Storage Box Grid form. The paper grid form will show PAXgene box contents. See Appendix 6B for Lab Storage Box Grid diagram for the PAXgene tube.

Figure 1 (PAXgene vial barcode label)

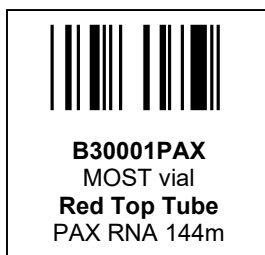
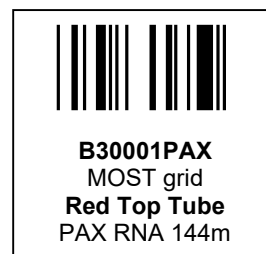


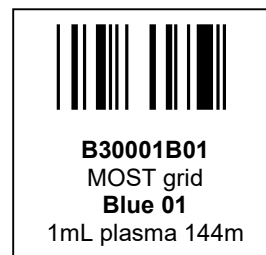
Figure 2 (PAXgene grid barcode label)



Barcode labels for cryovials (Figures 3 and 4): The first line of the label has barcode information. The second line has an amalgamation of identifiers: the site ("B" or "T"), the 5-digit specimen ID# (Birmingham “B” 144-month specimen ID#s will start with **30001** and Iowa “T” 144-month specimen ID#s will start with **80001**), cryovial cap color identifier (B, R, Y, C), and cryovial number (01-22). The third line has the either “MOST vial” (for vial) or “MOST grid” (for paper

grid form). The fourth line has the cap color (Blue, Red, Yellow, Clear) and the cryovial number. The fifth line describes the sample volume (1.0mL or 1.5mL, the sample type (plasma, buffy coat, serum or urine), and the study time point (144m)).

Each cryovial label has an identical twin label with the same amalgamation of identifiers (see Figures 3 and 4). The only difference is the word “vial” (Figure 3) is replaced with the word “grid” (Figure 4) in second line of text. The “grid” label will be placed on the MOST Lab Storage Box Grid form. The paper grid form will show cryovial box contents. Specimens will be boxed according to specimen type. See Appendix 6B for Lab Storage Box Grid diagram for each sample type.

Figure 3 (Cryovial vial barcode label)Figure 4 (Cryovial grid barcode label)

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 Biospecimen Collection Operations Manual, Appendix 2, Specimen Label (Placement on Cryovial)) and arranged in the appropriate working order (Biospecimen Collection Operations Manual, section 2.2.2 Blood collection).

There are a total of 54 labels per participant.

The following 31 labels will be affixed or discarded prior to the specimens arriving to the lab:

- five labels for the vacutainer tubes
- one label for the extra vacutainer tube
- one label for the urine cup
- one label for the PAXgene tube
- 22 labels for cryovials
- One label for the Lab Processing Form affixed to form by biospecimen collection staff
- 23 “MOST grid” labels for the MOST Lab Storage Box Grid forms will be clipped to the aliquot rack.

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: 1.0mL for plasma and serum, and 1.5mL for urine samples. Do not aliquot samples with less than 0.75ml of plasma or serum, or less than 1.0mL for urine.

The 144-month specimen processing and aliquoting schematic is shown in Appendix 2. It is helpful to post the appendix in the laboratory for quick reference.

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

5.1.2 Description of blood collection tubes

To aid in handling, each vacutainer draw tube is color coded: EDTA plasma 7.0mL tubes have lavender stoppers; serum 10.0mL tubes have red stoppers; and the PAXgene 9.5mL tube also has a red stopper.

Lavender top draw tube

Draw tubes #1 and #3 are 7.0mL lavender top vacutainers containing 15% EDTA as the anticoagulant. These tubes are used to collect plasma. It is very important that the tubes are completely filled. If the tubes are less than 2/3 full, discard the tube. After drawing the blood, the tubes will have been gently inverted 10 to 15 times minimum, protected from light contamination, and placed in a “wet ice” bath. Processing is detailed in section 5.1.3.1.

Red top draw tube

Draw tubes #2, #4, and #5 are 10.0mL red top vacutainers used to collect serum. These tubes contain no anticoagulant, so the blood will clot to form serum. After drawing the blood, protect the tubes 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). Processing is detailed in section 5.1.3.2.

PAXgene tube

The PAXgene tube is a 9.5mL PAXgene blood RNA vacutainer collection tube with a red stopper. This tube does not need to be processed. The sample will be used to isolate expressed RNA. The tube holds 9.5mL total volume but only 2.5mL of blood is collected in this tube as the tube already contains approximately 6.9mL of a chemical additive that stabilizes the RNA in the collected blood. After drawing, the tube should be inverted 10 times and placed on rack at room temperature for at least 2 hours. The PAXgene tube should undergo a room-temperature incubation period of at least 2 hours before the tubes are placed in the -20°C freezer. If this is not possible or the PAXgene tubes are inadvertently left overnight, the maximum amount of time that the tube should incubate is 36 hours. Avoid leaving the PAXgene tube to incubate over the entire weekend. Processing is detailed in section 5.1.3.3.

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 tubes #1 and #3. The rack should contain tubes #2, #4, #5 and #6. 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 tubes #1 and #3 should take place **immediately** (maximum time of 15 minutes after collection). Plasma and buffy coat aliquots will be made from the lavender top draw tubes. The plasma will be aliquoted into cryovials with blue caps. The plasma (blue cap cryovials 01 to 06) will be archived for future analysis. After the plasma is aliquoted, the buffy coat will be removed from the top layer of the red cells. The buffy coat (yellow cap cryovial 07) will be used for DNA analysis.

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 tubes are to be spun down in a refrigerated horizontal centrifuge at 4°C for 15 minutes at 2500 rpm.
- After centrifuging, immediately pipette and transfer 1.0mL of the plasma from the lavender top tubes into each of the 6 pre-labeled plasma cryovials 01 to 06 (blue caps).
- Cap the cryovials with blue caps, place in plasma cryovial box, and place in -70°C or -80°C freezer.

Note on Laboratory Processing form whether or not the participant consented to DNA/genetic testing (if the participant did not consent: do not aliquot buffy coat sample, discard the tubes with the buffy coat and clot remaining and note ending time of the EDTA plasma aliquoting on the Laboratory Processing Form).

- If the participant did consent: suction off the white cell layers remaining in the two EDTA lavender top tubes after the plasma is removed using a Pasteur pipette and transfer into the pre-labeled cryovial #07 (yellow cap). It is okay to include red cells in the cryovial. The main focus should be to obtain a large enough volume of white cells. The volume will vary.
- 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 tubes #2, #4, and #5 (10.0mL red top) must be protected from light exposure. The sample 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 tubes should display a clot by this time. The serum (red cap cryovials 08 thru 19) will be stored for future analysis.

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

- The red top tubes are to be spun down in a refrigerated centrifuge at 4°C for 15 minutes at 2500 rpm.
- After centrifuging, immediately pipette 1.0mL of serum into each of the pre-labeled red cap cryovials 08 thru 19. 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 box, and place in -70°C or -80°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 PAXgene processing

Note on Laboratory Processing form whether or not the participant consented to RNA/genetic testing (if the participant did not consent, you will properly discard the PAXgene tube).

Proceed with lab procedures if the participant consented to RNA/genetic testing. The PAXgene draw tube needs no further processing. After inverting the sample 10 times, set it upright in a rack at room temperature to incubate for at least 2 hours. The following protocol should be used: after drawing and inverting the PAXgene tube, set it upright on the lab bench until the end of the day. As more PAXgene tubes are drawn, continue adding them to the rack, making sure that the last one incubates at least 2 hours.

Document the time at start of incubation of PAXgene in the space provided in the appropriate section of the Laboratory Processing form.

At the end of the day, place the entire rack in the -20°C **degree freezer** (at that time, the previous day's rack will have been frozen at -20°C degrees, and these tubes should be transferred to the -70 degree freezer). Freeze the tube upright in a wire rack in a regular freezer (-20°C). Do not freeze tubes in a Styrofoam tray as this may cause the tubes to crack. Tube can be stored at -20°C and below. If tube is to be frozen at temperatures below -20°C , freeze the tubes first at -20°C for 24 hours, then transfer from rack to storage box and place them in -70°C or -80°C freezer.

- ***Note the ending time of incubation of PAXgene on the Laboratory Processing form.***
- ***Note PAXgene tube stored: Yes? or No? on the Laboratory Processing form.***

5.1.3.4 Urine sample

Please follow site-specific procedures for urine-pregnancy screening. Because the study includes imaging exams, female participants who are pregnant or trying to get pregnant were excluded from the study (New Cohort Telephone Screening Interview and Screen Visit). During the Telephone Screen Interview females are asked “When was your last natural menstrual period? Do not include bleeding due to taking female hormone pills or patches.” If the person had a natural period in the past 12 months or responds “Don’t know” and is between 45-55 years old, they will be required to have a urine pregnancy test before any imaging exams are done during that visit.

The Data from Prior Visits Report (DPVR) will inform you as to whether or not a urine-pregnancy test is required.

- Screening Visit DPVR Question #3:
“Is pregnancy test required (preliminary estimation based on the age)?”

- Clinic Visit DPVR Question #2a:
“Did participant require a pregnancy test at the Screening Visit?”

Imaging will also be done at the 168-month follow-up clinic visit. The clinical center x-ray and/or MRI technologists may find during their screening that a female participant must have a urine pregnancy test administered and results confirmed prior to imaging.

For all new cohort participants, urine specimens will be archived for future analysis. If the urine specimen cannot be immediately aliquoted into the three pre-labeled cryovials #20 to 22, place the filled urine cup in the refrigerator until the processing can begin (maximum time four hours). Do not allow the urine to be overly exposed to a light environment (remove light bulbs from refrigerators). The urine specimen is mixed by inverting the urine cup three to five times and then aliquoting 1.5mL into each of the three cryovials.

- Pipette 1.5mL of urine into the clear capped cryovials #20 to 22. 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.

Plasma (7.0mL EDTA tubes #1 and #3)

Aliquot	Cryovial(s)	Cap color	Volume
Plasma	6 (#01 to #06)	Blue	1.0mL

Buffy coat (7.0mL EDTA tubes #1 and #3)

Aliquot	Cryovial(s)	Cap color	Volume
Buffy coat	1 (#07)	Yellow	varies

Serum (10.0mL serum tubes #2, #4, and #5)

Aliquot	Cryovial(s)	Cap color	Volume
Serum	12 (#08 to #19)	Red	1.0mL

Urine (urine specimen cup)

Aliquot	Cryovial(s)	Cap color	Volume
Urine	3 (#20 to #22)	Clear	1.5mL

The cryovials will be placed into one of four boxes (**by sample type**) with the 9 x 9 grid. The front and top of the box must be marked and the boxes must be numbered sequentially by clinic. Write legibly with a Sharpie permanent marker or use printed labels with ink that won't bleed.

- **B0001** [*Plasma, Buffy Coat, Serum, or Urine*] (UAB) (*continue sequential numbering*)
or
- **T0001** [*Plasma, Buffy Coat, Serum, or Urine*] (UI) (*continue sequential numbering*)

Example for Numbering Shipment Boxes:

B0001 Buffy Coat
B0002 Urine
B0003 Urine
B0004 Plasma
B0005 Plasma
B0006 Plasma
B0007 Plasma

Note: Every box shipped from the clinic should be assigned a unique storage box number regardless of the specimen type.

Within each box the cryovials will be arranged sequentially by the participant's aliquot vial number. 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 box and all boxes will have specimens from more than one participant.

PAXgene tubes

Only 3 (UAB) or 8 (U-Iowa) PAXgene tubes will fit in each storage box. The grid forms for the PAXgene tubes are configured to match the number of tubes. See Appendix 6A, MOST Lab Storage Box Grid form.

5.1.5 Freezing specimens

Plasma, buffy coat, serum and urine samples must be frozen within 15 minutes (maximum) after aliquoting.

After aliquoting is complete, the rack containing the cryovials is removed from the ice bath and placed upright in the freezer at -70°C or -80°C for at least half an hour (preferably until the end of the day). Make sure the aliquots are not wet when placed in the freezer. If a freezer is not immediately available, place the rack of samples on dry ice. When the samples are thoroughly frozen, they should be added to the labeled 9 x 9 cryovial boxes being filled for shipment to Fisher BioServices. **Note: PAXgene tubes are not aliquoted; please refer to section 5.1.3.3 for PAXgene tube freezing procedures.**

5.1.6 Completed forms

The completed specimen collection packets can be set aside in a daily work folder. The Laboratory Processing forms are scanned into the data system (see Data Management Operations Manual) and filed in the participant's chart.

5.1.7 MOST Lab Storage Box Grid form

The Lab Storage Box Grid form will be filled with “grid” labels according to specimen type, left to right and top to bottom (see specimen layout on Appendix 6B). After the Lab Storage Box Grid form is completely filled, lab staff will use a barcode scanner to create a Barcode Grid Excel logsheet for each Lab Storage Box Grid form. Instructions to install and use the barcode scanner to create the Barcode Grid Excel logsheets are detailed in Appendix 7A. For inventory purposes, Barcode Grid Excel logsheets will be emailed to Fisher BioServices and the SF Coordinating Center when samples are shipped.

5.1.8 Completing forms

On the Laboratory Processing form, the MOST ID#, Acrostic, and Date Form Completed should be prefilled. If these are not prefilled, please contact the onsite study coordinator. **Fill in your staff ID#.** Check to make sure that the Bar Code Label is affixed to the form, that the 5-digit number is entered in the “Enter ID from Bar code label” boxes, and that the numbers are legible. 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.

Make every attempt possible to fill vials with 1.0mL (*at least 0.7mL*) for plasma and serum, and 1.5mL (*at least 1.0mL*) for urine.

- Mark “**Filled: Yes**” if the cryovial is filled and stored with the minimum amount specified previously.
- Mark “**Not filled**” if the cryovial is not filled because the volume is less than the minimum required for storage.
- Mark “H” if the specimen is **hemolyzed**. 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.

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 7.0mL tube **immediately** to 15 minutes
2. Buffy coat prior to discarding the EDTA plasma draw tubes
3. Serum 10.0mL tube **30 minutes** to 60 minutes maximum

4. PAXgene Tube **2 hours room temperature incubation** (36 hours max)
5. 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 specimens

5.4.1 General

Frozen blood and urine specimens are shipped about two times a month to Fisher BioServices by Federal Express (or other express delivery service) **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 Fisher are charged to your local Federal Express (or other express delivery service) 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 5) to prepare for shipping specimens. A sample of the Federal Express Airbill for Dry Ice Shipment is in Appendix 8 and the Dry Ice Outer Box Labeling Diagram is in Appendix 9.

5.4.2 Methods for shipping frozen specimens

Samples should be prepared for shipping to Fisher BioServices as follows:

- Wrap each freezer box in paper towels to absorb possible leakage. Put a rubber band around the towel-wrapped box or bag.
- Put the individual freezer boxes containing the samples into a leakproof zip-lock plastic bag. Seal the zip-lock bags.
- Line the styrofoam mailer with absorbent material (e.g., paper towels).
- Place approximately one third of the dry ice on the bottom of the mailer.
- Carefully place the freezer boxes into the styrofoam mailer. Place no more than a total of 4.0L of sample into the styrofoam shipping container. Use two or more styrofoam mailers when necessary. (In this case, label the mailers "1 of 2" and "2 of 2").
- Band sets of PAXgenes tubes together with a rubber band, wrap in paper towels and place in a ziplock storage bag and seal the bag and place in the shipping container. PAXgene tube should be frozen at -70°C degrees for at least 24 hours before shipping.

- Pack the shipping boxes with dry ice according to the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions for shipping frozen specimens. Place the remaining dry ice on top and around the samples to fill the styrofoam container.
- Seal the top of the styrofoam container with tape.
- Enclose the styrofoam container in the outer cardboard sleeve.
- **Attach** the following paper copies in the styrofoam shipment mailer:
 - Barcode Grid Excel logsheets
- Client Notification of Shipment form (see Appendix 7B) **E-mail** Project Manager at Fisher BioServices the day before you send a shipment, and send an email letting them know:
 - Date of shipment
 - Expected arrival
 - Number of Styrofoam mailers shipped
 - FedEx airbill number
 - **Note: Include electronic copies of the Barcode Grid Excel logsheets and Client Notification of Shipment form on the email**
 - Copy MOST Coordinating Center on the email


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 Fisher BioServices and the FROM: label from the clinical center.

The Fisher Bioservices mailing address is:

Fisher BioServices
14665 Rothgeb Drive
Rockville, MD 20850
Tel: (301) 315-8507
Mobile: (240) 506-9025

6. Laboratory Processing Form

Laboratory Processing

 57930	MOST ID # <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Acrostic <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Date Form Completed <input type="text"/> / <input type="text"/> / 20 <input type="text"/> <input type="text"/> <small>Month Day Year</small>	Staff ID# <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
--	--	---	--	--

Time at start of EDTA processing: <input type="text"/> : <input type="text"/> <input type="radio"/> am <input type="radio"/> pm <small>Hours Minutes</small>					
Collection Tubes	Cryo #	Vol.	Type	Filled	Problem
#1 and #3 EDTA Plasma Tubes (14 mL total)					
Plasma	01	1.0	B	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	02	1.0	B	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	03	1.0	B	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	04	1.0	B	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	05	1.0	B	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	06	1.0	B	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
Buffy coat	07	var	Y	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
Ending time of EDTA aliquoting: <input type="text"/> : <input type="text"/> <input type="radio"/> am <input type="radio"/> pm <small>Hours Minutes</small>					
#2, #4, and #5 Serum Tubes (30 mL total)					
Time at start of serum processing: <input type="text"/> : <input type="text"/> <input type="radio"/> am <input type="radio"/> pm <small>Hours Minutes</small>					
Serum	08	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	09	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	10	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	11	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	12	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	13	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	14	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	15	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	16	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	17	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
18	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled	
19	1.0	R	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled	
Ending time of serum aliquoting: <input type="text"/> : <input type="text"/> <input type="radio"/> am <input type="radio"/> pm <small>Hours Minutes</small>					
Urine	20	1.5	C	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	21	1.5	C	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
	22	1.5	C	<input type="radio"/> Yes	<input type="radio"/> H <input type="radio"/> Not filled
PAXgene	PAXgene tube stored? <input type="radio"/> Yes <input type="radio"/> No		Time at start of incubation: <input type="text"/> : <input type="text"/> <input type="radio"/> am <input type="radio"/> pm <small>Hours Minutes</small>		
			Time at end of incubation: <input type="text"/> : <input type="text"/> <input type="radio"/> am <input type="radio"/> pm <small>Hours Minutes</small>		

Bar Code Label

Enter ID from Bar Code label:

Did participant consent to DNA/genetic testing?

Yes No

Do NOT collect buffy coat sample.

H=Hemolyzed
Y=Yellow
B=Blue
W=White
R=Red
C=Clear

Did participant consent to RNA/genetic testing?

Yes No

Do NOT store PAXgene tube.

MOST Floating Form
Laboratory Processing
M19

57930



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
- Certification by the Department of Transportation or other organization for packaging and shipment of biological specimens (information on training courses can be found at <http://hazmat.dot.gov/training.htm#classes> or <http://www.fedex.com/us/services/options/seminars.html>)

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
- PAXgene tubes at room temperature
- Cryovials correctly labeled
- Cryovial and PAXgene tube storage boxes labeled correctly
- Hepatitis B vaccination given or offered to all personnel handling blood
- Non-permeable lab coats, gloves, and face shields used

Processing EDTA plasma, buffy coat and urine

- Time checked to ensure that tubes #1 and #3 are processed within 15 minutes of completion of phlebotomy
- Urine specimen refrigerated until processed
- Note start of EDTA processing on the form
- EDTA plasma tubes #1 and #3 at least 2/3 full, otherwise discarded
- Tubes #1 and #3 centrifuged at 4°C for 15 min at 2500 rpm
- Confirm that participant consent to DNA/genetic before aliquoting buffy coat
- Buffy coat correctly aliquoted
- Aliquots immediately placed upright 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 tubes #2, #4 and #5 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 tubes # 2, #4 and #5 were processed within 60 minutes
- Tubes #2, #4 and #5 centrifuged for 15 minutes at 2500 rpm
- Centrifuge correctly balanced with water tube(s)
- Serum correctly aliquoted
- Note ending time of serum processing

Processing PAXgene tube

- Confirm that participant consent to RNA/genetic before processing PAXgene tube
- Note start of PAXgene tube incubation on the form
- PAXgene tube inverted 10 times
- PAXgene tube placed upright at room temperature to incubate for at least 2 hours (36 hours max incubation)
- Note end of PAXgene incubation
- Mark “Yes” to PAXgene tube stored on the form

Cryovial freezing

- Aliquots checked to ensure they are not wet
- Cryovial rack placed upright in freezer or samples placed on dry ice immediately after aliquoting for at least 30 minutes before transferred to storage box
- Aliquots placed in separate boxes for shipment to Fisher (boxed by sample type) cryovial storage boxes and immediately frozen in -70°C freezer

PAXgene freezing

- PAXgene tub placed in conventional freezer -20°C (not frost-free)
- If tube to be frozen at temperatures below -20°C , tubes frozen at -20°C for 24 hours, then transferred to -70°C or -80°C

End of day procedure

- Laboratory Processing form reviewed for thoroughness and placed in daily work folder (faxed into data system)
- Freezer boxes correctly labeled
- MOST Lab Storage Box Grid form checked for completeness
- Scan grid form labels following procedures detailed in Appendix 7A to create MOST Barcode Grid Excel logsheet
- MOST Barcode Grid Excel logsheet saved on the computer using standardized naming conventions

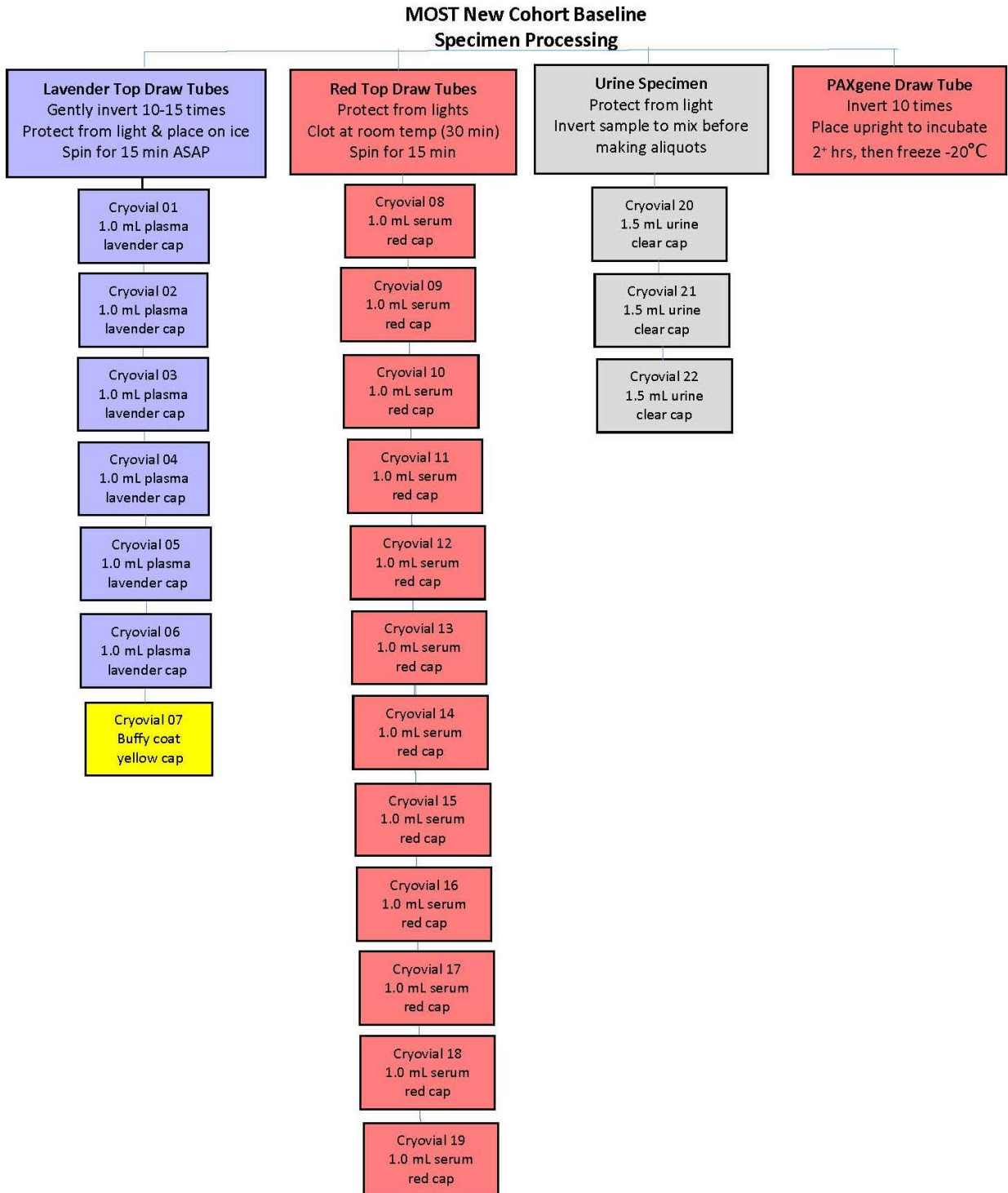
Shipment procedures – dry ice

- Shipment person certified by Department of Transportation
- 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
- Print and attach paper copies of the Barcode Grid Excel logsheets for each box included in the styrofoam shipment mailer
- 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
- Fisher BioServices notified by e-mail the day before shipment with FedEx airbill tracking number information
- Email the MOST Barcode Grid Excel logsheets and a Client Notification Shipment form to Ayana Robinson (Fisher BioServices)
- MOST Coordinating Center copied on the email to Fisher BioServices

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 boxes (9 x 9) for freezing specimens
- PAXgene tube storage boxes (U-Iowa: 8 tubes per box and UAB: 3 tubes per box)
- Lab Storage Box Grid forms
- Barcode scanner
- Rubber bands
- Black Sharpie marking pen

Appendix 2 MOST Specimen Processing Schematic



Appendix 3 Labels

Row 9	T80401B04 MOST vial Blue 04 1mL plasma 144m	T80401B04 MOST grid Blue 04 1mL plasma 144m	Row 18	T80401R13 MOST vial Red 13 1mL serum 144m	T80401R13 MOST grid Red 13 1mL serum 144m	Row 27	T80401C22 MOST vial Clear 22 1.5mL urine 144m	T80401C22 MOST grid Clear 22 1.5mL urine 144m
Row 8	T80401B03 MOST vial Blue 03 1mL plasma 144m	T80401B03 MOST grid Blue 03 1mL plasma 144m	Row 17	T80401R12 MOST vial Red 12 1mL serum 144m	T80401R12 MOST grid Red 12 1mL serum 144m	Row 26	T80401C21 MOST vial Clear 21 1.5mL urine 144m	T80401C21 MOST grid Clear 21 1.5mL urine 144m
Row 7	T80401B02 MOST vial Blue 02 1mL plasma 144m	T80401B02 MOST grid Blue 02 1mL plasma 144m	Row 16	T80401R11 MOST vial Red 11 1mL serum 144m	T80401R11 MOST grid Red 11 1mL serum 144m	Row 25	T80401C20 MOST vial Clear 20 1.5mL urine 144m	T80401C20 MOST grid Clear 20 1.5mL urine 144m
Row 6	T80401B01 MOST vial Blue 01 1mL plasma 144m	T80401B01 MOST grid Blue 01 1mL plasma 144m	Row 15	T80401R10 MOST vial Red 10 1mL serum 144m	T80401R10 MOST grid Red 10 1mL serum 144m	Row 24	T80401R19 MOST vial Red 19 1mL serum 144m	T80401R19 MOST grid Red 19 1mL serum 144m
Row 5	T80401PAX MOST vial Red Top Tube PAX RNA 144m	T80401PAX MOST grid Red Top Tube PAX RNA 144m	Row 14	T80401R09 MOST vial Red 09 1mL serum 144m	T80401R09 MOST grid Red 09 1mL serum 144m	Row 23	T80401R18 MOST vial Red 18 1mL serum 144m	T80401R18 MOST grid Red 18 1mL serum 144m
Row 4	T80401 Urine Cup	T80401 Lab Form	Row 13	T80401R08 MOST vial Red 08 1mL serum 144m	T80401R08 MOST grid Red 08 1mL serum 144m	Row 22	T80401R17 MOST vial Red 17 1mL serum 144m	T80401R17 MOST grid Red 17 1mL serum 144m
Row 3	T80401 Red Top Tube #5	T80401 Extra Tube	Row 12	T80401Y07 MOST vial Yellow 07 buffy coat 144m	T80401Y07 MOST grid Yellow 07 buffy coat 144m	Row 21	T80401R16 MOST vial Red 16 1mL serum 144m	T80401R16 MOST grid Red 16 1mL serum 144m
Row 2	T80401 Lavender Top Tube #3	T80401 Red Top Tube #4	Row 11	T80401B06 MOST vial Blue 06 1mL plasma 144m	T80401B06 MOST grid Blue 06 1mL plasma 144m	Row 20	T80401R15 MOST vial Red 15 1mL serum 144m	T80401R15 MOST grid Red 15 1mL serum 144m
Row 1	T80401 Lavender Top Tube #1	T80401 Red Top Tube #2	Row 10	T80401B05 MOST vial Blue 05 1mL plasma 144m	T80401B05 MOST grid Blue 05 1mL plasma 144m	Row 19	T80401R14 MOST vial Red 14 1mL serum 144m	T80401R14 MOST grid Red 14 1mL serum 144m

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.	4/participant if used for plasma, buffy coat, serum, and urine aliquots	plasma, buffy coat, 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, buffy coat, 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, buffy coat, serum, urine	RT-L1000
Barcode Scanner (Code Reader 900FD)	1/site		CDW-G: 3158370 (provided by SFCC)
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 BRL.	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
- Barcode scanner

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) – Cryovials
- Freezer boxes with 8 x 1 grids (rubber bands around box) – U-Iowa PAXgene tubes
- Freezer boxes with 3 x 1 grids (rubber bands around box) – UAB PAXgene tubes
- Leakproof Zip-lock bags
- Packaging tape
- Dry ice (approximately 20 lbs. per box)
- FedEx Labels (provided by carrier)
- Email Fisher BioServices (attach Barcode Grid Excel logsheet document for each Lab Storage Box being shipped) – copy MOST Coordinating Center on the email

Appendix 6A MOST Lab Storage Box Grid Form

Print form on 11" x 17" paper



MOST Lab Storage Box Grid – 144-Month Visit Specimens

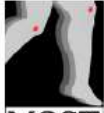
SPECIMEN TYPE (MARK ONE): Plasma Buffy Coat Serum Urine

MOST BOX #: _____ DATE SHIPPED: _____

NOTE: AFFIX MOST BIOSPECIMEN LABELS TO THE GRID EXACTLY MATCHING THE SLOT LOCATION OF EACH CRYOVIAL. FILL EACH ROW OF THE BOX SEQUENTIALLY FROM LEFT-TO-RIGHT AND TOP-TO-BOTTOM. Use a separate grid form for each cryovial box for each type of biological specimen – plasma, serum, urine, and buffy coat. Number the boxes sequentially with a site identifier ("B" or "T") and a four digit number starting from one (i.e., B0001, B0002, etc. for Birmingham and T0001, T0002, etc. for Iowa City) followed by specimen type (e.g., B0001 PLASMA, B0002 URINE, etc.).

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	81	
				BOTTOM					END

Print form on 8.5" x 11" paper



MOST **MOST Lab Storage Box Grid – 144-Month Visit Specimens**

SPECIMEN TYPE: PAXgene (UAB site only)

MOST BOX #: _____ DATE SHIPPED: _____

NOTE: AFFIX MOST BIOSPECIMEN LABELS TO THE GRID EXACTLY MATCHING THE SLOT LOCATION OF EACH TUBE SPACE. FILL EACH ROW OF THE BOX SEQUENTIALLY FROM LEFT-TO-RIGHT. Use a separate grid for each PAXgene storage box.

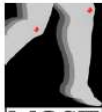
The front and top of the box must be numbered sequentially with a site identifier ("B") and a four digit number starting from 0001 followed by specimen type (e.g., B0001 PAX, B0002 PAX, etc.).

START

1	2	3
---	---	---

END

Print form on 8.5" x 11" paper



MOST **MOST Lab Storage Box Grid – 144-Month Visit Specimens**

SPECIMEN TYPE: PAXgene (UI site only)

MOST BOX #: _____ DATE SHIPPED: _____

NOTE: AFFIX MOST BIOSPECIMEN LABELS TO THE GRID EXACTLY MATCHING THE SLOT LOCATION OF EACH TUBE SPACE. FILL EACH ROW OF THE BOX SEQUENTIALLY FROM LEFT-TO-RIGHT. Use a separate grid for each PAXgene storage box.

The front and top of the box must be numbered sequentially with a site identifier ("T") and a four digit number starting from 0001 followed by specimen type (e.g., T0001 PAX, T0002 PAX, etc.).

START

1	2	3	4	5	6	7	8
---	---	---	---	---	---	---	---

END

Appendix 6B Lab Storage Box Grid Form Example

Plasma Example (page 1 of 5 examples)
(6 vials per participant)

Start #1			Top								
Ppt #1 01	02	03	04	05	06	Ppt #2 01	02	03			
04	05	06	Ppt #3 01	02	03	04	05	06			
Ppt #4 01	02	03	04	05	06	Ppt #5 01	02	03			
04	05	06	Ppt #6 01	02	03	04	05	06			
Ppt #7 01	02	03	04	05	06	Ppt #8 01	02	03			
04	05	06	Ppt #9 01	02	03	04	05	06			
Ppt #10 01	02	03	04	05	06	Ppt #11 01	02	03			
04	05	06	Ppt #12 01	02	03	04	05	06			
Ppt #13 01	02	03	04	05	06	Ppt #14 01	02	03			
Bottom						End					

Continue to next box.

Label top and front side of box: **Bnnnn Plasma** for UAB and **Tnnnn Plasma** for UI (nnnn is sequential numbering)

Buffy Coat Example (page 2 of 5 examples)
(1 vial per participant)

Start			Top						
Ppt #1 01	Ppt #2 01	Ppt #3 01	Ppt #4 01	Ppt #5 01	Ppt #6 01	Ppt #7 01	Ppt #8 01	Ppt #9 01	
Ppt #10 01	Ppt #11 01	Ppt #12 01	Ppt #13 01	Ppt #14 01	Ppt #15 01	Ppt #16 01	Ppt #17 01	Ppt #18 01	
Ppt #19 07	Ppt #20 07	Ppt #21 07	Ppt #22 07	Ppt #23 07	Ppt #24 07	Ppt #25 07	Ppt #26 07	Ppt #27 07	
Ppt #28 07	Ppt #29 07	Ppt #30 07	Ppt #31 07	Ppt #32 07	Ppt #33 07	Ppt #34 07	Ppt #35 07	Ppt #36 07	
Ppt #37 07	Ppt #38 07	Ppt #39 07	Ppt #40 07	Ppt #41 07	Ppt #42 07	Ppt #43 07	Ppt #44 07	Ppt #45 07	
Ppt #46 07	Ppt #47 07	Ppt #48 07	Ppt #49 07	Ppt #50 07	Ppt #51 07	Ppt #52 07	Ppt #53 07	Ppt #54 07	
Ppt #55 07	Ppt #56 07	Ppt #57 07	Ppt #58 07	Ppt #59 07	Ppt #60 07	Ppt #61 07	Ppt #62 07	Ppt #63 07	
Ppt #64 07	Ppt #65 07	Ppt #66 07	Ppt #67 07	Ppt #68 07	Ppt #69 07	Ppt #70 07	Ppt #71 07	Ppt #72 07	
Ppt #73 07	Ppt #74 07	Ppt #75 07	Ppt #76 07	Ppt #77 07	Ppt #78 07	Ppt #79 07	Ppt #80 07	Ppt #81 07	

Bottom

End

Continue to next box.

Label outside of box: **Bnnnn Buffy Coat** for UAB and **Tnnnn Buffy Coat** for UI (nnnn is sequential numbering)

Serum Example (page 3 of 5 examples)
(12 vials per participant)

Start	Top							
Ppt #1 08	09	10	11	12	13	14	15	16
17	18	19	Ppt #2 08	09	10	11	12	13
14	15	16	17	18	19	Ppt #3 08	09	10
11	12	13	14	15	16	17	18	19
Ppt #4 08	09	10	11	12	13	14	15	16
17	18	19	Ppt #5 08	09	10	11	12	13
14	15	16	17	18	19	Ppt #6 08	09	10
11	12	13	14	15	16	17	18	19
Ppt #7 08	09	10	11	12	13	14	15	16

Bottom

End

Continue to next box.

Label top and front side of box: **Bnnnn Serum** for UAB and **Tnnnn Serum** for UI (nnnn is sequential numbering)

Urine Example (page 4 of 5 examples)
(3 vials per participant)

Start			Top					
Ppt #1 20	21	23	Ppt #2 20	21	22	Ppt #3 20	21	22
Ppt #4 20	21	23	Ppt #5 20	21	22	Ppt #6 20	21	22
Ppt #7 20	21	23	Ppt #8 20	21	22	Ppt #9 20	21	22
Ppt #10 20	21	23	Ppt #11 20	21	22	Ppt #12 20	21	22
Ppt #13 20	21	23	Ppt #14 20	21	22	Ppt #15 20	21	22
Ppt #16 20	21	23	Ppt #17 20	21	22	Ppt #18 20	21	22
Ppt #19 20	21	23	Ppt #20 20	21	22	Ppt #21 20	21	22
Ppt #22 20	21	23	Ppt #23 20	21	22	Ppt #24 20	21	22
Ppt #25 20	21	23	Ppt #26 20	21	22	Ppt #27 20	21	22

Bottom

End

Continue to next box.

Label top and front side of box: **Bnnnn Urine** for UAB and **Tnnnn Urine** for UI (nnnn is sequential numbering)

PAXgene tube Example (page 5 of 5 examples)
(1 vial per participant)

U-Iowa Clinic:

Start		Top					
Ppt #1	Ppt #2	Ppt #3	Ppt #4	Ppt #5	Ppt #6	Ppt #7	Ppt #8
Bottom						End	

Continue to next box.

UAB Clinic:

Start		Top
Ppt #1	Ppt #2	Ppt #3
Bottom		End

Continue to next box.

Label outside of box: **Bnnnn PAX** for UAB and **Tnnnn PAX** for UI (nnnn is sequential numbering)

Appendix 7A Installation and use of barcode scanner

Each clinic will be provided with a set of 54 labels for each participant, 46 of the labels have barcodes. The provided barcode labels come in sets of 2 (like identical twins). These labels are side-by-side on the roll. “Vial” labels on the left are to be placed on the PAXgene tube and cryovials, and “grid” labels on the right are to be placed on the Lab Storage Box Grid form (see Appendix 6A). You will use a barcode scanner to scan each “grid” label placed on the form to create the electronic Excel logsheet called MOST Barcode Grid Excel logsheet.

1. First time set-up of barcode scanner

- Equipment: Barcode scanner, scanner stand, and USB cable.



- Insert the USB connector into the computer. A beeping sound should prompt you that the scanner has been successfully connected to your computer. Please wait for the beeping sound which takes about 10 seconds.

2. Preparing the logsheet

2.1 Open the zip files labeled [UAB or UI] ‘CRYOVIAL’ or ‘PAX’ MOST Barcode Grid Excel logsheet template document posted on the study website under Documents & Forms \ Study Documents \ Biospecimens.

- Row 1: manually enter the MOST Box # (**B**nnnn for UAB; **T**nnnn for U-Iowa), starting with 0001 for each specimen type.
- Row 2: manually enter the specimen type in the drop-down menu (*not applicable for the PAXgene logsheet*).
- Column F: manually enter comments (optional)

- Rows 4 thru 84: automated data entry via the barcode scanner (see section 3. Data entry with scanner). Note: PAXgene templates will have rows 4 thru 7 or rows 4 thru 12 depending on the PAXgene storage box size.

2.2 Data populated via the barcode scanner will also appear in the Grid Layout Table located on the right (starting in column F). Do not manually enter data on the Grid Layout Table except for in the “Comments” field. The layout of this table should correspond with the layout of the vials in each storage box.

Box Layout

- Cryovials (for both UAB and U-Iowa): **9 x 9** grid (81 vials maximum)
- PAXgene (for U-Iowa only): **8 x 1** grid (8 vials maximum)
- PAXgene (for UAB only): **3 x 1** grid (3 vials maximum)

2.3 Naming convention for Excel file:

- clinic box number (Bnnnn or Tnnnn)
- specimen type
- date file created

Naming convention example (change prefix “B” to “T” for Iowa):

- **BnnnnPlasmaYYYYMMDD.xlsx**
- **BnnnnBuffyYYYYMMDD.xlsx**
- **BnnnnSerumYYYYMMDD.xlsx**
- **BnnnnUrineYYYYMMDD.xlsx**
- **BnnnnPAXYYYYMMDD.xlsx**

2.4 Save the Excel file in your local hard drive “144m Biospecimens” folder.

2.5 When samples are shipped to Fisher BioServices

- **Electornic copies:** send notification email (see OM section 5.4 Shipping specimens) and attach all of the MOST Barcode Grid logsheets contained in the shipping box. Copy the MOST Coordinating Center on this email
- **Paper copies:** print paper copies of the Barcode Grid logsheets (the document is set up to print only the Grid Layout Table section starting on column F) and Client Notification Shipment form (see Appendix 7B) in the styrofoam mailer

3. Data entry with scanner

Open the specified Barcode Grid Excel logsheet. Place the mouse cursor over the field in Column C, Row 4 of the logsheet where you will begin data entry. Data should only be entered on Column C. Hover scanner over the barcode and pull the trigger button once. A 9-digit (alpha/numeric digit) will immediately appear in the field (cell C4) and move automatically to the next field below (cell C5) in preparation for the next data entry. Repeat until you’ve finished scanning all barcode labels on the form.

As data is entered on Column C, the Grid Layout Table (starting in Column F) will automatically be filled with the same number corresponding to the layout of the labels on the Lab Storage Grid form.

IMPORTANT:

1. Scan labels on the Lab Storage Grid form from **Left-to-Right and Top-to-Bottom**.
2. Remember to save the Excel file in your local hard drive when you are finished. You will email the Excel file to Fisher BioServices when specimens are shipped (Coordinating Center to be copied on the email to Fisher).

4. Quality assurance

- Storage boxes should be marked consecutively by specimen type.
- Check that each storage box contains the same biospecimen type and same cap insert color.
- Confirm that the labels in the Lab Storage Box Grid form (paper) match the position in the Barcode Grid Excel logsheet (electronic) (starting in Column F).
- Make use of the “Comments” field below the Grid Layout Table to write important comments.
- Save the MOST Barcode Grid ‘CRYO’ or ‘PAX’ Excel logsheet on your local hard drive in the “144m Biospecimens folder”.

Example of Barcode Grid Excel Logsheet

MOST Box #	5555	
Specimen type:	Plasma	
Row	Column	Barcode
1	1	T80401B01
1	2	T80401B02
1	3	T80401B03
1	4	T80401B04
1	5	T80401B05
1	6	T80401B06
1	7	T80402B01
1	8	T80402B02
1	9	T80402B03
2	1	T80402B04
2	2	T80402B05
2	3	T80402B06
2	4	T80403B01
2	5	T80403B02
2	6	T80403B03
2	7	T80403B04
2	8	T80403B05
2	9	T80403B06
3	1	T80404B01
3	2	T80404B02
3	3	T80404B03
3	4	T80404B04
3	5	T80404B05
3	6	T80404B06

MOST Grid Layout Table									
	1 column	2 column	3 column	4 column	5 column	6 column	7 column	8 column	9 column
1 row	T80401B01	T80401B02	T80401B03	T80401B04	T80401B05	T80401B06	T80402B01	T80402B02	T80402B03
2 row	T80402B04	T80402B05	T80402B06	T80403B01	T80403B02	T80403B03	T80403B04	T80403B05	T80403B06
3 row	T80404B01	T80404B02	T80404B03	T80404B04	T80404B05	T80404B06	T80405B01	T80405B02	T80405B03
4 row	T80405B04	T80405B05	T80405B06	T80406B01	T80406B02	T80406B03	T80406B04	T80406B05	T80406B06
5 row	T80407B01	T80407B02	T80407B03	T80407B04	T80407B05	T80407B06	T80408B01	T80408B02	T80408B03
6 row	T80408B04	T80408B05	T80408B06	T80409B01	T80409B02	T80409B03	T80409B04	T80409B05	T80409B06
7 row	T80410B01	T80410B02	T80410B03	T80410B04	T80410B05	T80410B06	T80410B07	T80411B01	T80411B02
8 row	T80411B03	T80411B04	T80411B05	T80411B06	T80412B01	T80412B02	T80412B03	T80412B04	T80412B05
9 row	T80412B06	T80413B01	T80413B02	T80413B03	T80413B04	T80413B05	T80413B06	T80414B01	T80414B02

Comments:

Appendix 7B Client Notification of Shipment Form



Fisher BioServices

PAGE NUMBER: 1 of 1	FORM NUMBER: 49005-2
EFFECTIVE DATE: 02 NOV 2015	REVISION LEVEL: 00

SUBJECT: CLIENT NOTIFICATION OF SHIPMENT – INDIVIDUAL ITEM

Step 1: When will your items arrive? Note: weekend shipments are not accepted unless specified in your contract.

Anticipated Date of Arrival: _____ (Date format: DD-MMM-YYYY)

Step 2: Please select the Fisher BioServices facility you are shipping to (REQUIRED):

- TO:
- 1001 Aldridge Road, Vacaville CA 95688
 - 10 Forge Park, Franklin MA 02038
 - 685 Lofstrand Lane, Rockville MD 20850
 - 12401 Washington Ave, Rockville MD 20852
 - 14665 Rothgeb Drive, Rockville MD 20850

If selecting the Rothgeb facility, please specify the departmental destination (if known)

- Repository Pharmacy
- Laboratory Production and Distribution

Step 3: FROM:

Site Name (Sender): _____
 Sender's Name/ Phone: _____
 Address: _____
 City, State, Zip Code: _____
 Sender's Email: _____
 Project Account Number: 0974
 Client/Study Name: MOST Study
 Project Manager: Ayana Robinson

Step 4: Electronic manifests are greatly preferred. Please contact your Project Manager for formatting instructions, and submit via email along with this form. When submitting electronically, please enter "see attached file" under "Description".

TOTAL QUANTITY: _____ (include units: vials, bottles, boxes, etc.)

Description	Qty	Unit	Lot Number (if applicable)
Cryovials (Specimen type: xx)	xx	cryovial box	N/A
PAXgene tubes	xx	PAXgene box	N/A

Step 5: Please enter the airbill or tracking number as well as the carrier (REQUIRED):

Airbill #: _____ Carrier: _____

Step 6: Please select storage conditions upon arrival at FBS (REQUIRED):

- Temperature
- 15°C to 30°C
 - 2°C to 8°C
 - 30°C to -15°C
 - 90°C to -70°C
 - 195°C to -135°C Vapor Phase LN₂
 - Other*

*By selecting "other," you are choosing a non-standard temperature. Doing so may conflict with your contract or may incur additional costs. Please contact your Project Manager or Client Services for any questions.

Step 7: Category A Category B N/A

Type: Supplies/Consumables Drug Return Drug
 Other (ex: shippers, temperature recorders, etc.)
 Samples

Material is: Chemical Biological Other _____

Step 8: Comments:

Step 9: Send this form by pressing the "Submit" button and attach any additional documents. If you experience issues using this functionality please refer to the table below for manual submission to the appropriate facility. Please include a copy of this form with the physical shipment.

Destination	E-mail	Fax	Phone
California	FBS.Vacaville.Customer.Service@thermofisher.com	(707) 469-9799	(707) 469-6226
Massachusetts	FBS.FranklinClient.Req@thermofisher.com	(508) 553-8857	(508) 533-0414
Maryland	FBS.Customer.service@thermofisher.com	(301) 294-4024	(301) 315-8433

FORM APPROVALS:

AUTHOR:	DATE:	DEPARTMENT MANAGER:	DATE:	QUALITY ASSURANCE:	DATE:
Stephanie Arnold	24 SEP 15	Michael R Adams	24 SEP 15	[Signature]	25 Sep 15

COMPANY CONFIDENTIAL AND PROPRIETARY

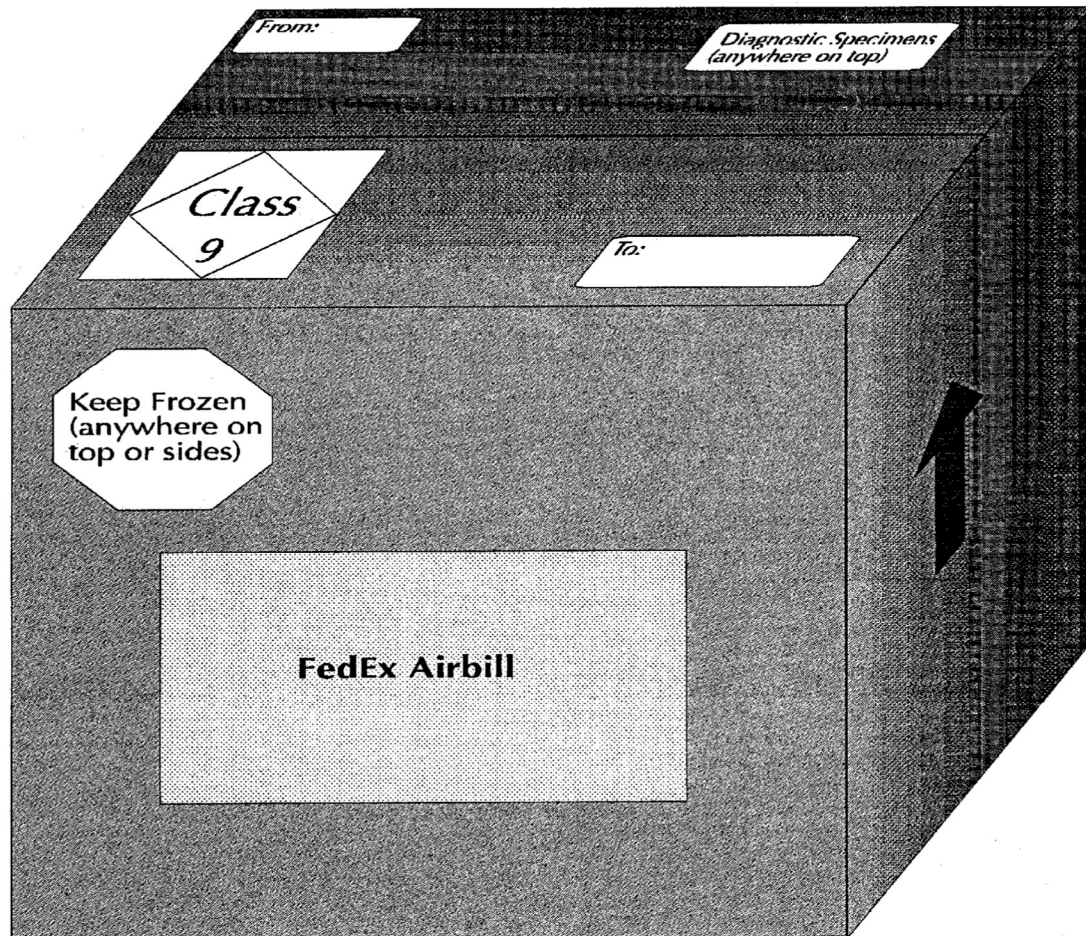
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Appendix 8 Federal Express Airbill for Dry Ice Shipment

		FedEx Tracking Number: 8059 5152 1159		Firm # No. 0200		Sender's Copy	
1 From Please print and press hard. Date _____ Sender's FedEx Account Number _____ <small>SENDER'S FEDEX ACCOUNT NUMBER ONLY</small> Sender's Name _____ Phone () _____ Company _____ Address _____ Dept./Floor/Suite/Room _____ City _____ State _____ ZIP _____				4 Express Package Service * To meet locations. NOTICE: Service order fees charged. Please select carefully. Packages up to 150 lbs. <small>For packages over 150 lbs., see the new FedEx Express Freight US Airbill.</small>			
2 Your Internal Billing Reference <small>First 24 characters will appear on invoice. OPTIONAL</small>				Next Business Day <input checked="" type="checkbox"/> FedEx First Overnight <small>Earliest next business morning delivery to select locations. Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.</small> <input type="checkbox"/> FedEx Priority Overnight <small>Next business morning. Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.</small> <input type="checkbox"/> FedEx Standard Overnight <small>Next business afternoon. Saturday Delivery NOT available.</small>		2 or 3 Business Days <input type="checkbox"/> FedEx 2Day A.M. <small>Second business morning. Saturday Delivery NOT available.</small> <input type="checkbox"/> FedEx 2Day <small>Second business afternoon. Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.</small> <input type="checkbox"/> FedEx Express Saver <small>Third business day. Saturday Delivery NOT available.</small>	
3 To Recipient's Name _____ Company FISHER BIOSERVICES Address 14665 ROTHGEB DRIVE <small>We cannot deliver to P.O. boxes or P.O. ZIP codes. Dept./Floor/Suite/Room _____</small> Address _____ <small>Use this line for the HOLD location address or for continuation of your shipping address.</small> City ROCKVILLE State MD ZIP 20850				5 Packaging * Declared value limit \$500. <input type="checkbox"/> FedEx Envelope* <input type="checkbox"/> FedEx Pak* <input type="checkbox"/> FedEx Box <input type="checkbox"/> FedEx Tube <input type="checkbox"/> Other			
6 Special Handling and Delivery Signature Options <input type="checkbox"/> SATURDAY Delivery <small>NOT available for FedEx Standard Overnight, FedEx 2Day A.M., or FedEx Express Saver.</small> <input type="checkbox"/> No Signature Required <small>Package will be left without obtaining a signature for delivery.</small>				<input type="checkbox"/> Direct Signature <small>Someone at recipient's address may sign for delivery. Fee applies.</small>		<input type="checkbox"/> Indirect Signature <small>If no one is available at recipient's address, someone at a neighboring address may sign for delivery. For residential deliveries only. Fee applies.</small>	
<input type="checkbox"/> HOLD Weekday <small>FedEx for any address. REQUIRED. NOT available for FedEx First Overnight.</small>				<input type="checkbox"/> HOLD Saturday <small>FedEx for any address. REQUIRED. AVAILABLE ONLY for FedEx Priority Overnight and FedEx 2Day to select locations.</small>			
7 Payment Bill to: Enter FedEx Acct. No. or Credit Card No. below. <input checked="" type="checkbox"/> Sender (Acct. No. in Section 7 will be billed.) <input type="checkbox"/> Recipient <input type="checkbox"/> Third Party <input type="checkbox"/> Credit Card <input type="checkbox"/> Cash/Check FedEx Acct. No. _____ Credit Card No. _____ Exp. Date _____				Does this shipment contain dangerous goods? <small>One box must be checked.</small> <input type="checkbox"/> No <input type="checkbox"/> Yes (As per attached Shipper's Declaration.) <input type="checkbox"/> Yes (Shipper's Declaration not required.) <input checked="" type="checkbox"/> Dry Ice (UN 1845) _____ kg _____ lb <small>Dangerous goods (including dry ice) cannot be shipped in FedEx packaging or placed in a FedEx Express Crisp Box. <input type="checkbox"/> Cargo Aircraft Only</small>			
8 Total Packages _____ Total Weight _____ Total Declared Value _____ <small>Do not include weight of container or packaging.</small>				Your liability is limited to USD\$100 unless you declare a higher value. See back for details. By using this Airbill you agree to the terms and conditions on the back of this Airbill and in the current FedEx Service Guide, including terms that limit our liability.			
				644			
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Appendix 9 Dry Ice and Labeling Diagram

Outer Box Labeling



NOTE: Labels must not overlap