

LAB SPECIMEN PROCESSING**TABLE OF CONTENTS**

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1. Background and rationale

The Health ABC study 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, specimens will be sent to Dr. Bernd Meibohm's laboratory at the University of Tennessee, Memphis for storage and analysis. An additional tube will be drawn to be sent to a local clinical lab for lipid determinations.

2. Equipment and supplies

Necessary supplies include:

- Centrifuges capable of spinning at 3000 g-minutes
- -20° Freezer space is required
- -80° Freezer space is required
- Refrigerator space
- Dry Ice
- Pipets and tips: 1.0-mL volumes
- Lab coat and gloves
- Biohazardous waste disposal container
- Balance tubes for the centrifuge
- Lab mat
- 10% bleach solution
- Freezer racks
- Rubber bands

2.1 Sample ID Labels

You will be supplied with sheets of sample ID barcode labels to use for labeling forms, draw tubes, and cryovials. A sample sheet of barcode labels can be found in Appendix 1. All labels on each sheet have the same 6-digit sample ID number (the first digit identifies the clinic: Memphis = 1, Pittsburgh = 2).

Each cryovial label also has a 2-digit extension (01 to 02) that serves as a unique identifier for each cryovial within a sample ID. The labels for cryovials have bar codes to Dr. Meibohm keep track of the samples. See Appendix 1 for proper orientation of the barcode label.

Beneath the human-readable ID number, cryovial labels also have 1-3 lines of text. The first line consists of a letter, a word, and a number. This line of text is intended to increase accuracy in labeling and filling the cryovials. The letter refers to the color of the cryovial cap (R= red, W= white, C= clear, B=Blue, complete code can be found at the bottom of the Laboratory Processing form). The word corresponds to the type of sample to be stored in the cryovial (e.g. Citrate for citrate-treated plasma). **The number refers to the cryovial volume (4.0 mL), not the volume aliquoted.**

There are also 6 labels containing the ID number with no extension. Four are to be used for pre-labeling the 3 draw tubes, with 1 extra. Of these labels, only the Draw Tube #3 label has a barcode. They all have 1-3 lines of text indicating which specimen container they are intended for, including the stopper color and volume, if applicable.

There are 2 barcoded labels with the ID number, one with the words “Phlebotomy Form,” which is placed on the Phlebotomy Form (see Blood Collection chapter), and the other with the words “Laboratory Processing Form,” which is placed on the Laboratory Processing Form (Appendix 2). *This process of matching the participant-specific Health ABC Enrollment ID# (**already on the form brought to the lab by the participant**) to the sample-specific ID barcode is crucial to being able to use the data collected from laboratory tests.*

3. Safety issues and exclusions

3.1 Precautions for handling blood specimens

In accordance with the OSHA regulations on blood borne pathogens (see copy on file in laboratory), the study recommends the following laboratory safety protocol for the field 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 products.
- Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leakproof container. Never recap or break needles.
- Hepatitis B vaccine should be offered to all unvaccinated technicians handling blood and documentation of vaccination or technician's declining to be vaccinated should be kept.

4. Participant and exam room preparation

4.1 Preparation for processing

All items on the Sample Processing Checklist (Appendix 3) 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 sample ID bar codes (see Label Orientation diagram in Appendix 2) and arranged in appropriate working order. After labeling draw tubes and cryovials, there will be 3 labels left: 1 “Backup” label, 1 “Phlebotomy Form,”

and 1 “Laboratory Processing Form” label. These can be separated into 2 mini-sheets: The “Backup Vacutainer,” “Phlebotomy Form” and “Laboratory Processing Form” labels should be clipped to the corresponding blood collection tray. “

5. Detailed procedures

5.1 Processing

5.1.1 General

Tube #1 should be mixed (for about 30 seconds) and immediately placed on ice. The tube for local determinations should be handled according to the local lab’s specifications. Tube (#3) should be inverted 10 times and placed in a -20°C freezer. 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 phlebotomy. During processing, work in the order specified and make as many aliquots as possible while meeting the volume requirement of each cryovial. On the Laboratory Processing form, fill the bubble next to each cryovial that is filled, whether partially or totally. If the sample is hemolyzed, fill the bubble marked H. 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 (marked not filled).

5.1.2 Description of blood collection tubes

Each draw tube is color coded to aid in handling.

Tube #1 is a special 8-mL cell separation (CPT) tube with a blue/black stopper. This tube will be processed to obtain clean buffy coat for the RNA-later ancillary study. The plasma from this tube will also be used. This tube uses citrate as the anticoagulant, so the plasma from these tubes is aliquoted into a blue-capped cryovial.

Tube #2 is a tube to send to a local lab to get lipid determinations. This should be handled according to local laboratory directions.

Tube #3 is a special PAXgene blood RNA collection tube with a red stopper. This tube does not need to be processed and will be used to isolate messenger RNA in an ancillary study. Even though the tube is a 10 mL tube, only 2.5 mL of blood are collected in this tube. The tube contains already approximately 5 mL of a chemical additive that stabilizes the RNA in the collected blood.

5.1.3 Immediate processing

Upon reaching the blood processing station, remove the blood drawing rack and ice bath containing tubes from the blood collection tray. The rack should contain tube #3. The ice bath should contain tube #1.

5.1.4 Aliquots per sample type:

The following is a summary of the processing. Detailed instructions follow (volume indicates sample size, not cryovial size).

Citrated Plasma: Immediately after the draw, this tube is spun. The citrated plasma from tube #1 is aliquoted into a single 4.0-mL cryovial, which is sent to Dr. Meibohm. The total number of aliquots is 1 (Color code = B). Do not overfill the tube because the sample volume will expand on freezing.
1 cryovial, volume varies

Buffy coat: The buffy coat is removed from tube #1 and placed into one 4.0-mL cryovial prefilled with 2.0-mL of *RNAlater* (Color code = B). After storing this cryovial in the 4°C refrigerator for at least 12 hours, the tube is frozen and sent to Dr. Meibohm.
1 cryovial, volume varies

PAXgene: The 10 ml drawtube (#3) needs no further processing. After inverting the sample 10 times, the tube can be placed into a regular freezer (-20°C).

The total number of aliquots per participant is 2 plus one unaliquotted specimen collection tube. A detailed listing of aliquots can be found on the Laboratory Processing form (Appendix 3).

5.1.5 Centrifugation of CPT tubes

Tube #1 (CPT-tube) should be centrifuged as soon as possible. Until centrifugation, tube #1 should be stored upright **on ice**.

When ready to begin cell separation, the CPT tubes should be centrifuged for at least 20 minutes at 1500-1800 RCF (relative centrifugal force) in a horizontal (swing-out head), refrigerated centrifuge. To calculate the RCF of your centrifuge, please use the calculator at: <http://www.bd.com/vacutainer/products/molecular/citrate/procedure.asp>. Input the rotor radius and 1500-1800 RCF to see what speed to run the centrifuge at. **The RCF should not exceed 2000, beyond which tube breakage may occur.**

Note that these tubes are taller than other sample collection tubes, and a different rotor may be necessary. When centrifugation is complete, several layers will be evident. On top will be a clear plasma layer, under which will be a cloudy cell layer (buffy coat). Below these layers there is a gradient gel layer that acts as a barrier to prevent contamination by red cells, which are at the very bottom of the tube.

While the tubes are spinning, aliquot 2 mL of RNAlater™ solution into cryovial #2.

5.1.6 Making citrate plasma aliquots

Once centrifuged, the maximum time allowed before aliquoting the citrated plasma in the CPT tube (#1) is 15 min. If aliquoting is not immediate (within 15 minutes from removal of tubes from the centrifuge), please note the delay on the comment section of Laboratory Processing Form. Keep the collection tube (#1) on ice until aliquoting can occur.

Aliquots: 1 x 3.5-4 mL plasma use 4.0 mL cryovial Meibohm

- Allow the centrifuge(s) to come to a complete stop. Remove tube from the centrifuge, being careful not to shake the tubes, and put them on ice.
- Using a transfer pipet, transfer the supernatant from each tube into the respective cryovials. Be very careful not to disturb the cell layer. It is fine if some plasma is left on top of the cell layer. Put all of the plasma obtained into one 4.0 mL cryovial. (Do not overfill, because the tube may burst on freezing).
- Fill in the bubble next to cryovial #1 on the Laboratory Processing form and indicate whether the cryovial is filled partially or totally. If the tube is not filled at all, fill the bubble in the last column (“Not filled”). If the tube is only partially filled (e.g., less than 3 mL), also fill the bubble marked P. If a sample is hemolyzed, fill the bubble marked H. To determine whether a sample is hemolyzed, compare its color to the chart provided by LCBR in year 6. If the tube is both hemolyzed and partially filled, fill the bubble marked B (only one P, H, or B bubble should be filled for each cryovial, if applicable).

5.1.7 Making buffy coat aliquot for RNA preservation

Tube #1 remains on ice as long as it is not further processed after the centrifugation. The buffy coat aliquot is obtained from tube #1 after the plasma is removed. Use a Pasteur pipette to completely suction off the white cell layer and transfer it into one clear-coded 4-mL cryovial prefilled with 2 mL of RNAlater™ solution. It is okay to include some plasma in this cryovial. The main focus should be to obtain a large enough volume of white cells. The volume of buffy coat will vary (approximately 200-400 µL).

Close the labeled cryovial and invert it twice to ensure a homogenous cell suspension. Store the vial overnight (for at least 12 hours) at 4°C in the refrigerator. Record the time at which you place the cryovial in the refrigerator on the Laboratory Processing form (Appendix 2). If samples are processed on Fridays, they may be stored in the refrigerator until the following Monday.

After at least 12 hours of storage in the refrigerator, take the cryovial out of the refrigerator and store it in the -70°C freezer. Record the date and time at which you place the cryovial in the freezer on the Laboratory Processing Addendum. Be very careful to double check that the barcode on the tube matches the barcode number written on the Laboratory Processing Addendum.

5.1.8 Freezing

Upon completion of the processing steps, citrated plasma, must be frozen at -70° or on dry ice within a maximum of 30 minutes. **The buffy coat + RNA later will not be frozen until it has been refrigerated for at least 12 hours.**

After aliquoting is complete, the rack containing the citrated plasma cryovial is removed from the ice bath and placed upright in the freezer at -70°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.

5.1.9 Return visit aliquots

Occasionally, participants return to the clinic after their Year 8 clinic visit just to have a fasting blood draw or because they were unable to give a sample at the regular clinic visit. The same types of forms are used for the first sample collection and lab processing as for the second. The only difference is that for the first sample collection the “first sample collection” bubble on the first page is filled; for a repeat collection the “repeat sample collection” bubble is filled. Be sure to fill out all 3 forms with the header information including the Health ABC Enrollment ID #, Acrostic, Date Form Completed, and Staff ID #.

5.1.10 Completed forms

The completed Phlebotomy and Laboratory Processing forms can be set aside in a daily work folder. These forms are copied (one copy of the Phlebotomy Form and two of the Laboratory Processing Form), and then the originals are scanned into the data system and filed in the participants' charts. The copies are enclosed with each shipment of samples to Dr. Meibohm.

End of the Day Procedures

- Cryovials #1 and #2 should also be stored in freezer boxes, which should be included in a semi-monthly shipment to Dr. Meibohm (see Appendix 6).
- Drawtube #3 should be stored in a ziplock bag in the regular freezer (-20°C), which should also be included in the semi-monthly shipment to Dr. Meibohm.

- Re-stock blood collection trays with supplies.
- Label the next day's draw tubes and cryovials.
- Arrange draw tubes and aliquots in their proper racks.
- Wipe down all work areas with 10% Clorox solution.

5.2 Summary of processing time limitations

From end of venipuncture to start of processing:

1. CPT 8 mL 15 minutes

Once centrifuged, maximum time before aliquoting: 15 minutes. After aliquoting samples, freeze within 30 minutes.

5.3 Shipping the blood samples

5.3.1 General

All samples are shipped on Monday to Dr. Meibohm by Federal Express overnight delivery.

When Monday is a holiday, the Monday shipment may be shipped on Tuesday.

Pittsburgh will charge shipments to its Federal Express account. Dr. Meibohm's lab will collect the specimens directly from the Memphis clinic.

This shipping protocol follows the procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 904. All items from the shipping checklist (Appendix 5) should be kept in stock at all times.

5.3.2 Methods for shipping frozen samples

Make complete copies (all pages) of corresponding Phlebotomy and Laboratory Processing forms for the shipment. Samples should be prepared for shipping as follows **(Pittsburgh Only)**:

- 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 L of sample into the styrofoam shipping container.
- 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.
- Place the remaining dry ice (approximately 7 - 14 lbs total) 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.
- Place the copies of the Phlebotomy and Laboratory Processing forms on top of the styrofoam container before closing up the outer sleeve with tape.

Fill out the FedEx Airbill as follows: PITTSBURGH ONLY

- Type in your FedEx account number
- Type the date of the shipment
- Type the name of the person sending the shipment under Section one, where it says 'From'
- Type in your address and telephone number in Section one.
- Type the recipient's name, address, and telephone number in Section two. *The telephone number is mandatory.*
- Type an 'X' in the Bill Sender (Pittsburgh's budget has money to defray shipping costs)
- Type an 'X' in Priority Overnight under Section 4a
- Type an 'X' in the Other Packaging box in Section 5
- Type an 'X' in the Deliver Weekday box (Box 2).
- Place an 'X' in the "Dry Ice" box in Section five (Box 6). Enter the weight of the dry ice in kilograms as specified and the number of boxes shipped.
- In section 6, place an 'X' in the 'Yes (Shipper's declaration not required)' box

Affix the completed airbill to the front side of the package in the plastic pouch (see Appendix 6).

The following additional labels are to be attached to each shipping box. (A diagram showing the placement of these labels on the shipping container is shown in Appendix 6):

- | | |
|---|---|
| • Return Address Label: | placed on top in upper left corner. |
| • Consignee Address Label: | placed on top in bottom right corner. |
| • Black and White Class 9 Label:
(UN1845, see Appendix 10) | placed on top in upper right hand corner. |
| • Diagnostic Specimen Label: | placed on top under the return address label. |
| • Keep Frozen Label (optional): | placed on any side |

It is necessary to weigh the entire shipping container. The weight of the dry ice in kilograms is written on the Black and White Class 9 Label (Appendix 6) in the space provided and filled in on the FedEx airbill.

The mailing address is:

Dr. Bernd Meibohm
University of Tennessee Health Sciences Center
Dept. of Pharmaceutical Sciences
847 Union Ave, Suite 5p
Memphis, TN 38163
(901) 448-6990

FAX the following information to Dr. Meibohm at (901) 448-6940 , when a shipment is sent:

Date of shipment
Expected arrival date
Number of styrofoam mailers shipped
FedEx airbill number

6. Procedures for performing the measurements at home

This procedure is the same for home visits as for clinic visits. The samples will be placed on ice (except for serum) and returned to the lab as soon as possible after the home visit, preferably within 1 hour. Be sure to check the "time blood draw completed" field on the Phlebotomy form and begin processing within the time limits described in Section 5.3. This may not be possible if there was a delay in getting the samples back to the lab. It is therefore doubly important to record the time processing was started on the Lab Processing form.

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 Health ABC training session on techniques (or observe processing 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
- Explain blind duplicate aliquoting scheme
- Recite shipping schedule for applicable field center
- Process samples 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
- Hepatitis B vaccination given or offered to all personnel handling blood
- Remove buffy coat cryovials that have been stored for at least 12 hours at 4° C from the refrigerator and move to the freezer.

Processing CPT tubes

- Time checked to ensure that tube #1 is processed within 15 minutes of completion of phlebotomy
- Tube #1 centrifuged for 20 min at 1500-1800 RCF
- 2 mL of RNA_{later} added to cryovial #2 while tube is spinning
- Plasma correctly aliquoted into a 4.0-mL cryovial
- Buffy coat correctly aliquoted into a 4.0-mL cryovial
- Cryovial inverted twice to mix
- Time buffy coat cryovial is put into the refrigerator recorded on the Laboratory Processing Form
- Buffy coat cryovial stored in the refrigerator (4°C) for at least 12 hours.
- After at least 12 hours, buffy coat cryovial moved to freezer
- Time buffy coat cryovial moved to freezer recorded on the Laboratory Processing Addendum

Processing PAXgene Tube

- Invert tube #2 10 times
- Puts tube in conventional freezer -20° C

Semi-monthly procedure

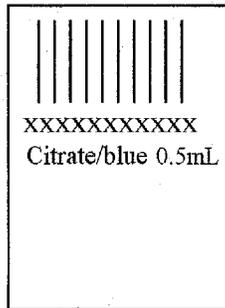
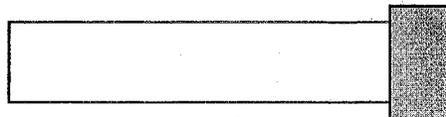
- Phlebotomy and Laboratory Processing forms placed in daily work folder
- Frozen aliquots removed from rack and placed in appropriate freezer boxes
- Freezer boxes correctly labeled
- Shipment person certified by DOT
- Freezer boxes correctly wrapped -- absorbent material, rubber band, and zip-lock bag
- Styrofoam mailers correctly packed -- absorbent material, dry ice, top sealed with tape
- Styrofoam mailer sealed in cardboard sleeve
- FedEx airbill correctly filled out
- Labels correctly affixed

Appendix 1 Sample Label Sheet (Bar Codes)

<p>##### Draw Tube 1 Blue/Black CPT 8 mL</p>	<p>##### Draw Tube 2 Local Tube Lipid Panel</p>	 <p>##### Draw Tube 3 Red top PAXgene 2.5 mL</p>
<p>Place this end on vial first</p>  <p>##### Back-up Vacutainer</p>	<p>Place this end on vial first</p>  <p>##### Back-up Vacutainer</p>	 <p>##### Phlebotomy Form</p>
 <p>##### Laboratory Processing Form</p>	<p>Place this end on vial first</p>  <p>#####-01 C/Citrate 4.0</p>	<p>Place this end on vial first</p>  <p>#####-02 C/Buffy 4.0 Buffy coat + RNA-later</p>

HEALTH ABC STUDY

Label Orientation on Cryovial



Appendix 2 Laboratory Processing

Health ABC

HABC Enrollment ID #	Acrostic	Type of Annual Contact	Staff ID#
H [] [] [] [] []	[] [] [] [] []	<input checked="" type="radio"/> Year 8 <input type="radio"/> Year 10	[] [] []

LABORATORY PROCESSING

First sample collection Second sample collection

Bar Code Label

1. Draw Tube # 1 (CPT)

Time at start of processing: [] [] : [] [] am
 pm

Collection Tubes	Cryo #	Vol.	Type	To	Fill in Bubble	Problems	Not Filled
Citrated plasma	1	var	b/4.0	M	<input type="radio"/>	<input type="radio"/> H <input type="radio"/> P <input type="radio"/> B	<input type="radio"/>
Buffy + RNA-later	2	var	b/4.0	M	<input type="radio"/>	<input type="radio"/> H <input type="radio"/> P <input type="radio"/> B	<input type="radio"/>

PAXgene

2. Time draw tube #3 placed in regular (-20°C) freezer :

[] [] : [] [] am pm
 Hours Minutes

RNA-later

3. Time buffy coat aliquoted into cryovial #2 containing RNA-later and placed in 4°C refrigerator:

[] [] : [] [] am pm
 Hours Minutes

4. Date cryovial #2 placed in -70°C freezer:

[] [] / [] [] / [] [] [] []
 Month Day Year

5. Time cryovial #2 placed in -70°C freezer (should be at least 12 hours after being placed in cryovial #2 containing RNA-later):

[] [] : [] [] am pm
 Hours Minutes

b=Blue; M=Meibohm lab; H=Hemolyzed; P=Partial; B=Both



Appendix 3 Sample Processing Checklist

- Crushed ice in ice bucket or plastic tub
- Pipets: 1.0 mL volumes
- Transfer pipets
- Labeled cryovials in rack
- Lab coat and gloves
- Biohazardous waste disposal
- Refrigerated centrifuge capable of spinning at 3000 g-minutes
- Room-temperature centrifuge
- Balance tubes for the centrifuge
- 10% bleach solution
- Styrofoam container for freezing cell cryovials
- Freezer boxes with 9 x 9 grid
- Rubber bands

Appendix 4 Freezer Box Diagrams

Freezer Box Diagram for Shipping Citrated Plasma and Buffy Coat with RNAlater to Dr. Meibohm

Numbers = cryovial #

start #1

Top

Ppt #1 01	02	Ppt #2 01	02	Ppt #3 01	02	Ppt #4 01	02	Ppt #5 01
Ppt #5 02	Ppt #6 01	02	Ppt #7 01	02	Ppt #8 01	02	Ppt #9 01	02
Ppt #10 01	02	Ppt #11 01	02	Ppt #12 01	02	Ppt #13 01	02	Ppt #14 01
Ppt #14 02	Ppt #15 01	02	Ppt #16 01	02	Ppt #17 01	02	Ppt #18 01	02
Ppt #19 01	02	Ppt #20 01	02	Ppt #21 01	02	Ppt #22 01	02	Ppt #23 01
Ppt #23 02	Ppt #24 01	02	Ppt #25 01	02	Ppt #26 01	02	Ppt #27 01	02
Ppt #28 01	02	Ppt #29 01	02	Ppt #30 01	02	Ppt #31 01	02	Ppt #32 01
Ppt #32 02	Ppt #33 01	02	Ppt #34 01	02	Ppt #35 01	02	Ppt #36 01	02
Ppt #37 01	02	Ppt #38 01	02	Ppt #39 01	02	Ppt #40 01	02	Ppt #41 01

Bottom

End#81

continue to
next box....

Label outside of box: PFT Ancillary Study Box #1 Date: ____ / ____ / ____

Appendix 5 Sample Shipping Checklist

- Styrofoam Mailing Container with outer cardboard sleeve
 - Polyfoam Packers # 430*
- Absorbent material
- 3" Freezer boxes with 9x9 grids (supplied by Dr. Kritchevsky)
- Leakproof Ziplock bags
- Packaging tape
- Dry ice (approximately 20 lbs. per shipment)
- FedEx Labels (provided by carrier)
- Copies of Completed Phlebotomy/Processing Forms
- Rubber bands for boxes

FedEx airbills, airbill pouches, and class 9 labels:
Local FedEx office

“Diagnostic Specimens” and “Keep Frozen” labels:
The sites can produce these labels.

Shipping boxes and freezer storage boxes will be supplied by Dr. Kritchevsky:

Insulated shipping boxes:

Polyfoam Packers 1-800-323-7442

Catalog No. 346 - for shipping up to twelve 2" freezer boxes

Catalog No. 430 - for shipping up to five 2" freezer boxes

Leakproof ziplock bags:

VWR 1-800-234-5227

Cat. No. 11217-128 - Bitran 12" x 12" zip-lock bag

Freezer storage boxes:

VWR 1-800-234-5227

Cat. No. 5954 - 2" freezer boxes for 2 mL cryovials

Cat. No. 6212 - 81-cell dividers for freezer boxes

Appendix 6 Dry Ice Label and Labeling Diagram
(page 1 of 2)

Shipper's Declaration not Required.

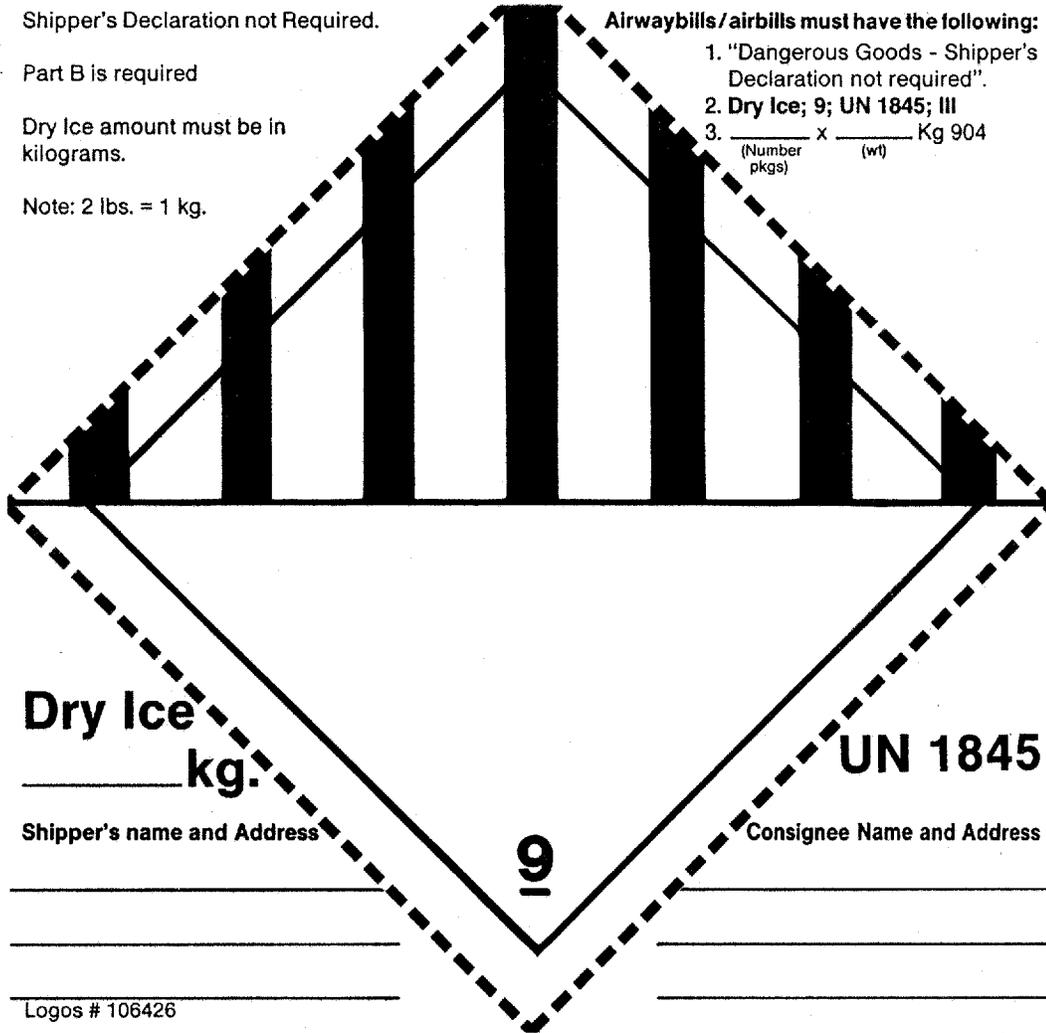
Part B is required

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills/airbills must have the following:

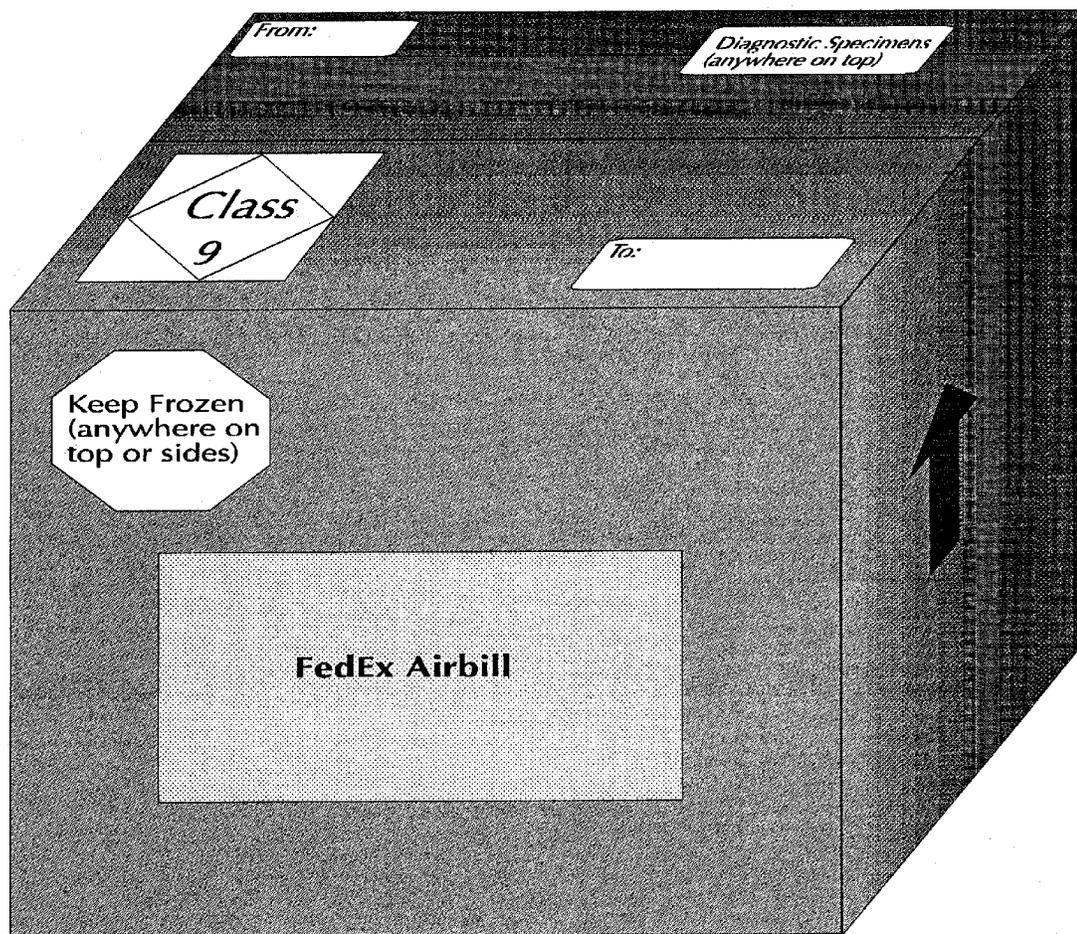
1. "Dangerous Goods - Shipper's Declaration not required".
2. Dry Ice; 9; UN 1845; III
3. $\frac{\text{Number}}{\text{(Number pks)}} \times \frac{\text{wt}}{\text{(wt)}} \text{ Kg 904}$



'DIAGNOSTIC SPECIMENS'
"PACKED IN COMPLIANCE WITH
IATA PACKING INSTRUCTION 650"

Appendix 6 Dry Ice and Labeling Diagram
(page 2 of 2)

Outer Box Labeling



NOTE: Labels must not overlap