

LAB SPECIMEN PROCESSING

Prepared by the Laboratory for Clinical Biochemistry Research (LCBR) at the University of Vermont in collaboration with the Coordinating Center

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1. Background and Rationale

During the year 2 clinic visit, one of the main aims of the blood collection will be to archive white blood cells and platelets for later studies of intracellular markers of aging and disease, for production of DNA for genetic analyses, and for analysis of mitochondrial enzymes and DNA.

The Health ABC study involves the collection of approximately 56 mL of blood from participants. The blood is collected in several types of tubes for specialized processing of different blood components. After processing, the specimens will be aliquoted into cryovials to be sent to LCBR for immediate analysis or to McKesson BioServices to store for later analyses.

2. Equipment and Supplies

A complete supply list with ordering information can be found in Appendix 1. Necessary supplies include:

- Refrigerated and non-refrigerated centrifuges capable of spinning at 30,000 g-minutes (A swinging bucket rotor is strongly recommended for the non-refrigerated centrifuge)
- -20° Freezer space is required
- -70° Freezer
- Dry Ice
- Pipets and tips: 50 µL and 0.5, 1.0, and 2.0 mL volumes
- Disposable pipets capable of measuring 20 mL of urine, or enough 20-25 mL graduated cylinders to allow washing and drying between participants
- Plastic transfer pipets
- Pasteur pipets
- Lab coat and gloves
- Biohazardous waste disposal container
- 10 and 15 mL tubes for pooling sample
- 15 mL graduated conical centrifuge tubes
- Balance tubes for the centrifuge
- Lab mat

- 10% bleach solution
- Freezer boxes with 9 x 9 cell grid (supplied by McKesson)
- 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 2. 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 35) that serves as a unique identifier for each cryovial within a sample ID. The labels for cryovials have bar codes to help McKesson and LCBR track the repository. To make it easy to differentiate cryovials that are to be sent to LCBR, their labels include the text “To LCBR”. See Appendix 2 for proper orientation of the barcode label.

Beneath the human-readable ID number, cryovial labels also have a line of text consisting 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 (W = white, B = blue, R = red, etc., 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 (“EDTA” for EDTA-treated plasma, “Buffy” for buffy coat, etc.). **The number refers to the cryovial volume (0.5 mL, 1.5 mL, or 2.0 mL for blood products, 2.0 mL for urine), not the volume aliquoted (e.g., 1 mL of plasma or serum is generally stored in a 1.5 mL cryovial).**

There are also 17 labels containing the ID number with no extension. Six are to be used for pre-labeling the 6 draw tubes, with 3 extras for backup vacutainers. There is a label for the urine specimen cup, three labels for the tubes you will use to pool the plasma and serum samples, and four labels for the conical centrifuge tubes used in the cell separation. These labels have no barcode, and they have 1-3 lines of text indicating which specimen container they are intended for, including the stopper color and volume, if applicable.

There are also 2 barcoded labels with the ID number, one with the words “Phlebotomy Form,” which is placed on the Phlebotomy Form (see Blood and Urine Collection chapter), and the other with the words “Laboratory Processing Form,” which is placed on the Laboratory Processing Form (Appendix 3). *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.

Finally, there are 6 barcoded labels with the same ID number and the words “BDID Form.” These labels may or may not be used, depending on whether there is extra sample left after processing the participant’s blood. Use of these labels is detailed under “Making Blind Duplicate Aliquots” below.

3. Safety Issues and Exclusions

3.1 Precautions for Handling Blood Specimens

In accordance with the OSHA regulations on blood borne pathogens (see Appendix 4 for complete OSHA regulations), the LCBR 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. Subject and Exam Room Preparation

4.1 Preparation for Processing

All items on the Sample Processing Checklist (Appendix 5) should be on hand before beginning processing.

Each day, new one-day supplies of the following solutions should be measured out and held as noted in parentheses. Discard unused solutions at the end of the day; do not put them back in the stock containers:

- Buffy coat freezing buffer A (hold on ice)
- Buffy coat freezing buffer B (room temperature)
- Vitamin C reducing buffer (hold on ice)
- Phosphate Buffered Saline (PBS) (room temperature)
- Platelet freezing buffer (hold on ice)

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 (see Aliquot Rack Setup diagram in Appendix 6). After labeling draw tubes, urine cup, and cryovials, there will be 18 labels left: 3 “Backup Vacutainer” labels, 1 “Phlebotomy Form,” 1 “Laboratory Processing Form” label, 3 labels for the tubes used to pool plasma and serum, 4 labels for the conical centrifuge tubes, and 6 “BDID Form” labels. 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. The labels for the pooling tubes and the “BDID Form” labels should be clipped to the corresponding aliquot rack.

There should also be a blind duplicate aliquot rack ready at all times. This aliquot rack is set up exactly like the other aliquot racks, *using a set of labels designated for blind duplicate samples (see Section 7, Quality Assurance, below)*. The “Phlebotomy Form,” and “Laboratory Processing Form” labels (not the “BDID Form” labels) from the same sheet should be affixed to a Phlebotomy form and a Laboratory Processing form pre-labeled with a dummy participant ID and Acrostic (See Section 7, below). The “BDID Form” labels should be used to label the upper right corner of each sheet of the Blind Duplicate Identification Form. The dummy Phlebotomy and Laboratory Processing forms should be clipped to the aliquot rack until all aliquots for LCBR are filled. The Blind Duplicate Identification Form should be kept with the aliquot rack until all aliquots are filled.

5. Detailed Procedures

5.1 Processing

5.1.1 General

Processing of the EDTA tubes (#1 & 4) should be done immediately following venipuncture - within 15 minutes of collection of the last tube. Personal protective

equipment (non-permeable lab coats, double-gloves with at least one latex pair, splatter shields) MUST BE worn. A flow chart is included in Appendix 7 to diagram this process.

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, put an X in the box next to each cryovial that is filled, whether partially or totally. If the tube is only partially filled, also put an X in the box marked P.

5.1.2 Description of Blood Collection Tubes

Each draw tube is color coded to aid in handling.

Tubes 1 and 4 are 10 mL lavender stoppered tubes containing 15% EDTA as the anticoagulant. These tubes will be processed to obtain plasma for archiving and for total cholesterol determination. The plasma is aliquoted into white-capped cryovials. One 0.5 mL aliquot will be mixed with special reducing agents in a 2.0 mL cryovial (yellow cap) and frozen for later analysis of Vitamin C level. The buffy coats from the EDTA tubes will no longer be collected (more viable buffy coats will be obtained and stored from the CPT tubes).

Tube 2 and 3 are special 8 mL cell separation (CPT) tubes with blue/black stoppers. These tubes will be processed to obtain viable buffy coats for later studies of intracellular markers of aging and disease and for production of DNA for genetic analyses. Platelets will also be collected for analysis of mitochondrial enzymes and DNA. These tubes use citrate as the anticoagulant, so the plasma from these tubes is aliquoted into blue-capped cryovials.

Tubes 5 and 6 are 10 mL siliconized red stoppered tubes. These tubes contain no anticoagulant so that the blood clots to form serum. After drawing, the blood is allowed to clot at room temperature for 40-45 minutes (Maximum = 90 minutes). Cryovial caps are coded red. The serum is used for analysis of fasting insulin and TSH, and for archiving.

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 tubes #2, 3, 5 and 6. The ice bath should contain tubes #1 and 4. If processing is delayed, keep tubes #1 and 4 on ice. The aliquot rack containing cryovials with the matching sample ID labels should be placed in an ice bath.

Tubes #5 and 6 must remain at room temperature for a minimum of 40 minutes. Allowing the tubes to stand longer may increase the yield of serum. The maximum allowable time before centrifugation is 90 minutes.

Tubes #1 and 4 must be processed within 15 minutes of the completion of phlebotomy. Tubes 2 and 3 can be held at room temperature for up to 2 hours and processed in batches. These tubes should not be placed on ice. Note the time that EDTA plasma processing started in the space provided on the Laboratory Processing Form. You will also note the time cell separation began in the space below the left column (see section 5.1.8). Use standard 12-hour time, and include am/pm.

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).

EDTA: Tubes #1 and 4 are centrifuged and the plasma supernatant is pooled into one 15 mL tube before aliquoting.
The total number of aliquots is: 12
1 x 0.5 mL mixed with 1.5 mL of reducing buffer for Vitamin C
(Color code = yellow)
7 x 0.5 mL (Color code = white)
4 x 1.0 mL (Color code = white)

CPT: Tubes #2 and 3 are held at room temperature up to 2 hours before processing. Buffy coats, platelets, and citrated plasma are separated out in a four-step process detailed below.

Buffy coats: Buffy coats from each tube (#2 & 3) are placed into separate 2.0 mL cryovials, mixed with a 2-part freezing solution, and frozen in a special 3-step freezing protocol.
2 x 1.5 mL (Color code = clear).

Platelets: Platelets from each tube (#2 & 3) are suspended in a special freezing buffer and placed into separate 2.0 mL cryovials.
2 x 2.0 mL (Color code = orange)

Citrate: The citrated plasma from tubes #2 & 3 is pooled into one 10 mL tube before aliquoting.
The total number of aliquots is: 4 (Color code = blue)
4 x 0.5 mL

Serum: The serum from the two collection tubes (#5 and 6) is pooled into one 15 mL tube before aliquoting.
The total number of aliquots is: 10 (Color code = red)
6 x 0.5 mL
4 x 1.0 mL

Urine: The urine is aliquoted into cryovials and larger 30-mL containers. One cryovial and one larger container contain acetic acid.
The total number of aliquots is: 4
1 x 2.0 mL (Color code = violet) (Cryovial 32)
1 x 20 mL (Cryovial 33)
1 x 2.0 mL with added 50 µL acetic acid (Color code = green) (Cryovial 34)
1 x 20 mL with added 500 µL acetic acid (Cryovial 35)

(Please note: If there is a small urine sample (less than 40 mL) the order of priority on the above urine specimens is 1. Cryovial 33; 2. Cryovial 34; 3. Cryovial 35; and 4. Cryovial 32.

The total number of aliquots from blood per participant is 31. A detailed listing of aliquots can be found on the Laboratory Processing form

5.1.5 Centrifugation of EDTA Plasma Samples

Tubes #1 and 4 are centrifuged at 4° C for 10 minutes at 3000 G. (A total of 30,000 g-minutes)

While these tubes are spinning:

- Aliquot the urine
- Restock the blood collection tray with tube rack and blood collection tubes, ice, and forms for the next participant.

- Recheck labels on the aliquot racks to ensure that they match the sample ID# on the draw tubes.
- Perform any necessary clean up.

5.1.6 Making Urine Aliquots

LCBR will provide a stock supply of 1 M acetic acid to the sites. Before urine is added to the green-capped cryovial #34, pipet 50 μ L (0.05 mL) of acetic acid into the cryovial first. Similarly, add 500 μ L (0.5 mL) of acetic acid to the 30-mL bottle labeled "Acid/20 mL"

- Pipet 2.0 mL of urine into one violet-capped cryovial and place on ice.
- Pipet 2.0 mL of urine into green-capped cryovial containing 50 μ L acetic acid (1 M) and place on ice
- Measure 20 mL of urine into one 30-mL bottle, and place on ice.
- Measure another 20 mL of urine into a second 30-mL bottle containing 500 μ L acetic acid (1 M) and place on ice.
- Discard the remaining urine (unless blind duplicate sample is needed, see below)
- See section 5.1.4 above for aliquoting priority when urine sample is small.

5.1.7 Making EDTA Plasma Aliquots

Once centrifuged, the maximum time allowed before aliquoting the EDTA plasma tubes (#1 and 4) is 15 minutes. 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 tubes (#1 and 4) on ice until aliquoting can occur.

- Allow the centrifuge(s) to come to a complete stop. Remove tubes from the 4° C centrifuge, being careful not to shake the tubes, and put them on ice.
- Before aliquoting the EDTA plasma from both EDTA plasma tubes (#1 and 4) is pooled in a 15 mL tube. Place the pooled tube on ice before aliquoting.
- The first 0.5 mL of EDTA plasma is placed into a 2.0 mL cryovial already containing 1.5 mL of the reducing buffer provided by LCBR and mixed by pipetting up and down 2 or 3 times. This cryovial should then be capped (color code=yellow) and immediately placed in the -70° freezer or on dry ice.
- **Change pipet tips before aliquoting the rest of the plasma.**
- Follow the outline on the Laboratory Processing form for aliquoting the rest of the plasma samples. Put an X in the box next to each cryovial that is filled,

whether partially or totally. If the tube is only partially filled, also put an X in the box marked P.

- Pipet the plasma with the *proper volume pipet*. Do not use the cryovial to estimate volume.
- Recap aliquots after each sample tube has been pipetted.

5.1.7.1 Work in the following sequence making sure aliquots are filled in this order

Vitamin C (Pooled EDTA plasma tube): Color coded Yellow

Aliquot:	1 x 0.5mL plasma	use 2.0 mL cryovial	McKesson
		prefilled with reducing	
		buffer (1.5 mL)	

If the caps on the Vitamin C tubes are being forced off because of expansion, reduce the volume of plasma from 0.5 mL to 0.4 mL and reduce the volume of Vitamin C buffer from 1.5 mL to 1.2 mL.

EDTA (Pooled EDTA plasma tube): Color coded White

Aliquots:	1 x 0.5 mL EDTA plasma	use 0.5 mL cryovial	LCBR
	5 x 0.5 mL EDTA plasma	use 0.5 mL cryovials	McKesson
	4 x 1.0 mL EDTA plasma	use 1.5 mL cryovials	McKesson
	2 x 0.5 mL EDTA plasma	use 0.5 mL cryovials	McKesson

Put an X in the box next to each cryovial that is filled, whether partially or totally. If the tube is only partially filled, also put an X in the box marked P. If a sample is hemolyzed, put an X in the box marked H. To determine whether a sample is hemolyzed, compare its color to the chart provided by LCBR.

Once these samples are aliquoted (~10 Minutes), any remaining sample can be used for blind duplicates (see below). When you are finished, the original blood collection tubes should be discarded in a biohazard, puncture-proof sharps container.

EDTA aliquots can remain on ice in the rack for up to 30 minutes before freezing at -70° C (or colder). Vitamin C aliquots should be placed into the -70° freezer immediately after processing.

If any tubes are accidentally mixed during pipetting so that plasma is contaminated with red cells, they may be recentrifuged.

5.1.8 Separation of Cellular Components

Tubes #2 & 3 may be held at room temperature for up to 2 hours to allow batching of CPT tubes from several participants. If samples from several participants are run at once, it is very important to ensure that the samples do not get mixed up. Labels have been made for every tube used in the process, and the processor must check and double-check each time sample is transferred from one tube to another that both tubes have the same sample ID. In addition, it is absolutely imperative that pipet tips and transfer pipets be changed between samples from different participants. Failure to do so could completely invalidate DNA results.

Step 1: Centrifugation of CPT Tubes. When ready to begin cell separation, the CPT tubes should be centrifuged at 30,000 G-min at room temperature. 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. 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.

Step 1a: Transferring supernatant. Using a transfer pipet, transfer a little over half the supernatant from each tube into a single 10 mL pooling tube for each participant. Be very careful not to disturb the cell layer. There is plenty of plasma, and extra plasma will be separated from the cell layer during the next centrifuge step. Place the plasma pooling tube on ice until you are ready to aliquot it.

Step 2: Pelleting the Buffy Coat. Next completely remove the cell layer from each CPT tube using a transfer pipet, and transfer it to a separate 15-mL conical centrifuge tube labeled “15 mL conical centrifuge tube Step 2: Cells.” Fill the centrifuge tube up to the 14 mL mark with PBS. Mix gently and centrifuge at 4500 G-min at room temperature. (If you need help calculating the centrifuge speed and time appropriate for your centrifuge, contact Elaine Cornell at LCBR).

Step 2a: Aliquoting Pooled Plasma. While the cells are being centrifuged, aliquot the pooled plasma into four blue-capped centrifuge tubes (0.5 mL aliquots). Place the centrifuge tubes on ice until they can be transferred to the -70° freezer.

Step 3: Separation of Platelets from Buffy Coats. Remove the centrifuge tubes from the centrifuge. There will be a small pellet (which may vary in color from slightly pink to white) overlaid by a large supernatant. Using a clean transfer pipet, transfer the supernatant to a second set of 15 mL conical centrifuge tubes labeled “15 mL

conical centrifuge tube Step 3: Platelets.” Be very careful not to disturb the cell pellet. It is better to leave a small amount of supernatant in the tube than to damage the pellet. It is okay to use the same transfer pipet for both tubes from a single participant. Change pipets between participants. Place the new centrifuge tubes in the centrifuge and spin at 30,000 G-min.

Step 3a: Resuspension of Buffy Coat Pellets. Add 0.5 mL of buffy coat freezing buffer A to each tube containing the buffy coat pellet. Resuspend the pellet by tapping the tube and/or flushing the pipet tip repeatedly. This may take a little patience. Do not use the same pipet tip for mixing on different participants. When the pellet has been resuspended, add 0.5 mL of buffy coat freezing buffer B and mix until the pellet is completely dissolved. Using a fresh transfer pipet for each participant, transfer the resuspended buffy coats to separate cryovials (i.e., one cryovial per centrifuge tube). Be sure the cryovial label matches the centrifuge tube label. Cap with a clear cap and place the cryovials in a rack that can be placed inside a styrofoam container.

Put a lid on the styrofoam box containing the rack of buffy coat aliquots and place the entire container in a -20° freezer for two hours. Set a timer to remind yourself to move the container after two hours. It is important not to let the container sit too long (or too short) in the -20° freezer. After two hours, the entire box should be transferred to a -70° freezer for at least 4 hours or overnight. After that, the cryovials may be put in the -145° sample shipment box and held at -70° until they are shipped to McKesson.

Step 4: Resuspension of Platelet pellet. After the second centrifuge tubes are finished spinning, there will be another pellet at the bottom. This is the platelet sample. First, using a transfer pipet, remove and discard the supernatant without disturbing the platelet pellet. Add 1.5 mL of platelet buffer to the centrifuge tube. This pellet is much more tightly packed and will be more difficult to resuspend. You may need to dislodge it with a glass rod. Using a combination of tapping and flushing the transfer pipet, resuspend the platelet pellet. It is ok if there are still some clumps, as long as the entire pellet is dislodged.

When the pellet has been resuspended, transfer the entire contents of the tube to an orange capped cryovial. These cryovials should be placed in the -70° freezer or on dry ice immediately.

Step 5: Completing the Year 2 Laboratory Processing Form. Because of the possibility that two staff will be handling the laboratory processing for any

individual participant, one person who will handle the cell processing and the other who will handle the blood and urine processing, we need to collect the Staff ID # of the second processor. The person who does the majority of the processing should record their number in the usual space for the Staff ID # at the top of the Year 2 Laboratory Processing Form. The person who does the cell processing should put their ID # at the bottom of the form in the space under the first column of the form. If one person does both the cell processing and the remainder of the processing, they should put their Staff ID # both at the top of the form and in the space under the first column of the form.

In addition, please write down the time at which cell separation began, below the cell processor ID# in the space under the first column of the form.

5.1.9 Centrifugation of Serum Samples

Tubes #5 and 6 should be left at room temperature for at least 40-45 minutes (maximum 90 minutes) after they are drawn. They should be displaying a clot by this time. They are centrifuged at 4° C for 10 minutes at 3000 G. A room temperature centrifuge may be used to ease processing flow, only if the centrifuge does not heat up and warm the samples. Samples centrifuged at room temperature should always be monitored to ensure that they remain cool.

5.1.10 Making Serum Aliquots

Allow the centrifuge to come to a complete stop. Carefully remove the tubes from the centrifuge, being careful not to shake the tubes, and place them on ice.

Serum from both collection tubes is pooled prior to aliquoting. You may pour the serum from the tubes or use a pipet, depending on the retraction of the clot. Place the pooled tube on ice before aliquoting.

Serum (Tubes #5 and 6) Color coded Red

Pool serum from both tubes before aliquoting

Aliquots:	1 x 1.0 mL serum	use 1.5 mL cryovial	LCBR
	5 x 0.5 mL serum	use 0.5 mL cryovial	McKesson
	3 x 1.0 mL serum	use 1.5 mL cryovial	McKesson
	2 x 0.5 mL serum	use 0.5 mL cryovial	McKesson

Put an X in the box next to each cryovial that is filled, whether partially or totally. If the tube is only partially filled, also put an X in the box marked P. If a sample is hemolyzed, put an X in the box marked H. To determine whether a sample is hemolyzed, compare its color to the chart provided by LCBR.

Extra serum can be used for blind duplicates (see below). When you are finished, the original blood collection tubes should be discarded in a biohazard, puncture-proof sharps container.

5.1.11 Making Blind Duplicates (if applicable)

Be sure you have read Section 7, Quality Assurance, below and that you understand how the blind duplicate scheme works. Ask your supervisor if you have any questions.

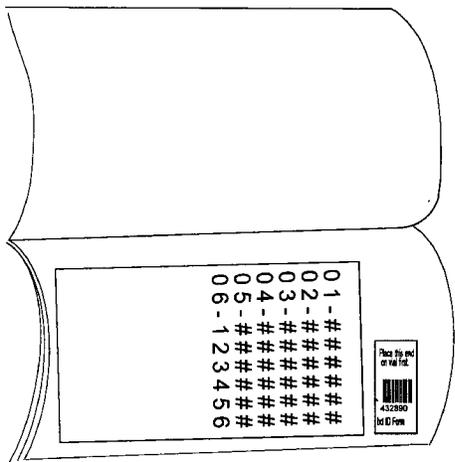
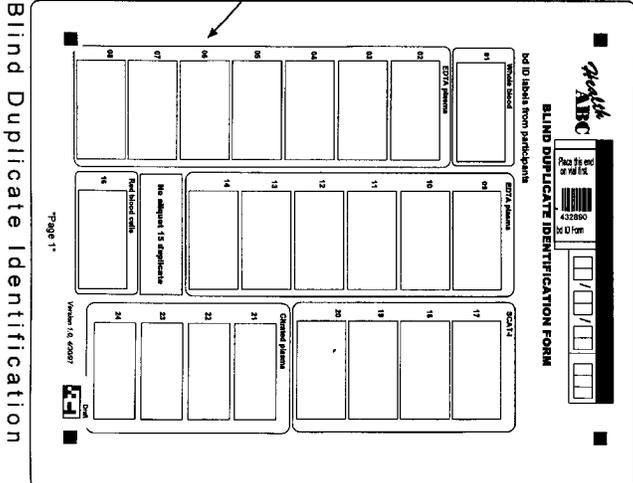
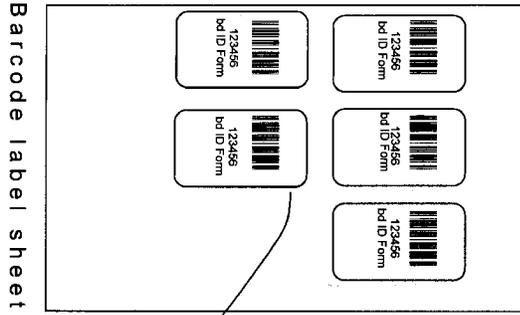
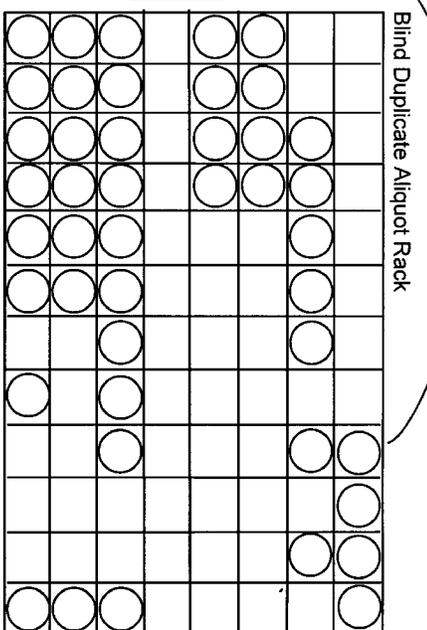
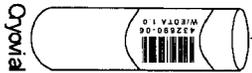
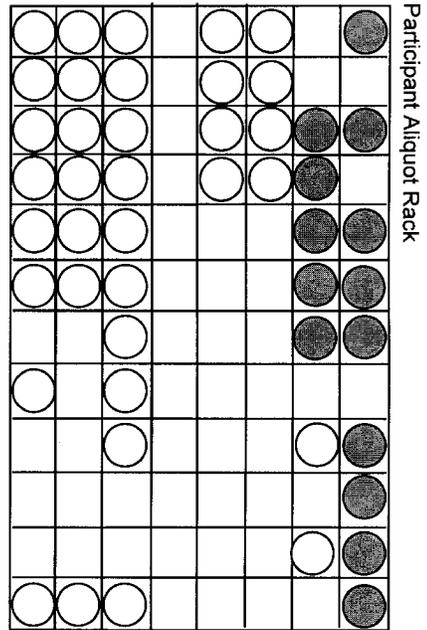
Each time sufficient sample exists to fill an extra cryovial, an empty cryovial of the correct type (e.g., vials 01 to 12 for EDTA-treated plasma) will be selected from the blind duplicate aliquot rack and filled with the appropriate quantity of sample (0.5 mL or 1.0 mL, according to the Laboratory Processing Form). The filled cryovial will be placed in the **participant's aliquot rack**, which is in the ice bath.

Before doing anything else, a "BDID Form" label must be removed from the **participant's** label sheet and affixed to the Blind Duplicate Identification Form in the spot corresponding to the aliquot number that was filled. You must also write the **participant's** sample ID number next to the aliquot number in your Blind Duplicate ID log book. This book is simply a notebook with each page devoted to a separate blind duplicate ID number (see Appendix 8 for example).

For example (see figure on page 16), suppose there is sufficient extra EDTA-treated plasma from sample ID #123456 to make a 1.0 mL aliquot. Cryovial 06 from the blind duplicate set ID #432890 has not yet been filled. You will pick up the pre-labeled cryovial 06 (#432890-07), put 1.0 mL of plasma into it, and place it in the

participant's aliquot rack (on ice). You will then remove a "BDID Form" label from the **participant's** ID label sheet (#123456) and place it in the spot marked aliquot 06 on the Blind Duplicate Identification Form. You will then write the **participant's** ID #123456 next to aliquot 06 in the log book on the page devoted to blind duplicate set #432890.

Note that there will never be extra buffy coat or platelets, as the entire sample is to be stored for each participant. Thus, you will not be preparing blind duplicates of aliquots 13, 14, 15, or 16. There will usually be plenty of extra urine for blind duplicates. Twenty mL urine blind duplicates may be partially filled if sufficient extra urine is hard to come by. Minimum volume is 15 mL.



5.1.12 Freezing

Upon completion of the processing steps, plasma and serum aliquots must be frozen at -70° or on dry ice within a maximum of 30 minutes. The platelet (#15 and 16) and vitamin C (#01) aliquots should be placed immediately into the -70° freezer or on dry ice. After aliquoting is complete the rack containing the remaining cryovials is removed from the ice bath and placed upright in the freezer at -70° C (or on dry ice or colder) 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. If there is not enough space in the -70° freezer, urine specimens only may be stored at -20° until shipped.

As described in section 5.1.8, the buffy coat aliquots (#13 and 14) should be placed in a rack inside a styrofoam box and put in the -20° freezer after aliquoting. Exactly two hours later (± 5-10 minutes) the buffy coat aliquots should be transferred to the -70° freezer still inside the styrofoam box. At least 4 hours later, or the next morning, the cryovials can be transferred to the freezer boxes in which they will be shipped to McKesson. **Note: the buffy coat cryovials are kept in a separate freezer box labeled “Freeze at -145 C” (see Appendix 9).**

5.1.13 Return Visit Aliquots

Occasionally, participants return to the clinic just to give a urine sample or have a blood draw. There are separate forms that must be filled out for return visits: the Return Visit Phlebotomy Form and the Return Visit Lab Processing Form (see Appendix 14). Use a new set of sample ID bar code labels. Place the Phlebotomy Form label in the Bar Code Label space on the Return Visit Phlebotomy Form. Place the Laboratory Processing Form label in the Bar Code Label space on the Return Visit Laboratory Processing Form. Use the rest of the labels in the same way as for the regular clinic visit. Be sure to fill out both forms with the header information including the Health ABC Enrollment ID #, Acrostic, Date Form Completed, and Staff ID #.

5.1.14 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 at each Field Center. The copies are enclosed with each

weekly shipment of samples to the LCBR and to McKesson Bioservices. Note: McKesson only needs a copy of the Laboratory Processing form; the Phlebotomy form is not needed in their sample shipment. Be sure the participant's Health ABC Enrollment ID# and acrostic, the sample ID, and the staff ID are legible on the copies (e.g., not cut off by the copier).

Completed Blind Duplicate Identification forms should also be scanned into the data system. Once a week, make copies of the current Blind Duplicate ID log page(s), the associated dummy Phlebotomy and Laboratory Processing Forms, and any Blind Duplicate Identification forms completed during the week, and *fax them to Emily Kenyon at the Coordinating Center*. This serves as a backup in case of catastrophic loss of these forms, which would render the blind duplicates unidentifiable and therefore useless.

5.2 End of the Day Procedures

- All urine cryovials are separated and placed in separate freezer boxes that will be sent to McKesson. The urine and blood sample freezer boxes to be sent to McKesson should be labeled with the name of the site and consecutive box numbers.
- The larger urine bottles should be placed in the larger urine bottle boxes (9 bottles per box) supplied by McKesson. Samples from one participant may overlap into two boxes. (See freezer box diagrams in Appendix 9.)
- Frozen cryovials in racks are packaged into freezer boxes by numeric order of cryovials per participant. Do not leave spaces in the boxes when the total number of cryovials is less than expected. Samples from one participant may overlap into two boxes. (See freezer box diagrams in Appendix 9)
- Filled blind duplicate cryovials should be temporarily stored in a separate freezer box until the full set has been completed. *The exception to this is that when both blind duplicate cryovials to be sent to LCBR (#02 and 21) have been filled, they should be placed in numerical ID# sequence if possible in the freezer box for LCBR shipment (see below).* When all cryovials in the blind duplicate set have been completed, place them in the next available freezer box for McKesson.
- Cryovials (#02 and 21) sent to LCBR in Vermont are separated out into other freezer boxes. *Note that the labels on these cryovials include the words "To LCBR" to make them easy to identify.* These freezer boxes should be numbered consecutively (1, 2, 3, etc.) and should also be labeled with the name of the site.
- If the buffy coat aliquots have been in the -70° freezer in their styrofoam container for at least 4 hours, they may be transferred to **the freezer box labeled "Freeze at -**

145 C.” Otherwise they should be left inside the styrofoam container in the -70° freezer until morning.

- 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.
- Wash graduated cylinders used for urine with laboratory detergent and rinse with distilled water. Drain upside down to dry.
- 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 10 mL	15 minutes
2.	CPT 8 mL	2 hours
3.	CPT 8 mL	2 hours
4.	EDTA 10 mL	15 minutes
5.	Serum 10 mL	90 minutes
6.	Serum 10 mL	90 minutes

Once centrifuged, maximum time before aliquoting: 15 Minutes

After aliquoting samples, freeze within 30 minutes. Freeze buffy coats, platelets (using special freezing protocol described above), and vitamin C immediately after processing.

5.4 Shipping the Blood Samples

5.4.1 General

Frozen blood samples are shipped **weekly** to the LCBR and McKesson Bioservices by Federal Express overnight delivery. The schedule will be as follows:

Mondays Memphis
Tuesdays Pittsburgh

This allows the laboratory and repository to stagger the arrival of samples on Tuesdays and Wednesdays for easier processing. When Monday is a holiday, the Monday shipment may be shipped on Tuesday.

Shipments to McKesson are charged to your local Federal Express account number. All shipments to LCBR are charged to the University of Vermont (recipient) Federal Express account.

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 10) should be kept in stock at all times.

5.4.2 Methods for shipping frozen samples

The frozen samples to be shipped are those from the previous week. There will be two separate shipments made: one to McKesson Bioservices and one to the University of Vermont.

The buffy coat cryovials (#13 and 14) are already packaged in a separate freezer box labelled "Freeze at -145°". Both these boxes and the boxes containing specimens to be stored at -70° may be shipped together to McKesson.

The frozen blood and urine cryovials are already packaged in pre-labeled freezer boxes and stored in the -70° C freezer by consecutive box number. Partial boxes should be sent.

Make complete copies (both pages) of corresponding Phlebotomy and Laboratory Processing forms for the LCBR shipment. Copies of the Laboratory Processing forms only are made for the McKesson shipment.

Samples should be prepared for shipping 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 and urine zip-locks into the styrofoam mailer. Place no more than a total of 4 L of sample into the styrofoam shipping container. Use two or more styrofoam mailers for the McKesson shipment when necessary. (In this case, label the mailers "1 of 2" and "2 of 2").

- 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 (LCBR) or Laboratory Processing form (McKesson) on top of the styrofoam container before closing up the outer sleeve with tape.

Fill out the FedEx Airbill as follows (Appendix 11):

- Type in your FedEx account number (for both McKesson and LCBR shipments)
- 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 box for the McKesson shipment
- Type an 'X' in the Bill Recipient box for the LCBR shipment. Fill in the University of Vermont account number (1531-6949-7) and internal reference number (5-26713) below the account number
- 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 12).

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 12):

- 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: placed on top in upper right hand corner.
(UN1845, see Appendix 12)
- 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 in the space provided and filled in on the FedEx airbill.

The LCBR mailing address at the University of Vermont is:

Elaine Cornell
University of Vermont-Pathology
55 A South Park Drive
Colchester, VT 05446
(802) 656-8963

The McKesson Bioservices mailing address is:

Steve Lindenfeser
McKesson BioServices
685 Lofstrand Lane
Rockville, MD 20850
(301) 340-1620

FAX the following information to McKesson Bioservices at (301) 340-3275 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 returned to the lab as soon as possible after the home visit, preferably within 2 hours. 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 Blind duplicate aliquots

7.1.1 Rationale:

In order to monitor reproducibility of the assays being carried out by LCBR, it is necessary for them to assay 5% of the samples twice, *without knowing which samples are duplicates*. In order for this process to work, the duplicate blood must appear to be blood from another participant. A further complication is that almost the full yield of plasma and serum collected from each participant will be aliquoted for immediate assay or storage. The process of producing these blind duplicates is therefore difficult to explain, but easy to carry out once the process is understood.

7.1.2 Blind duplicate sample IDs:

Before the sample ID labels sheets are sent to the clinics, the coordinating center will identify a random sample of 5% of the IDs. These sample IDs, *indistinguishable from regular sample IDs*, will become blind duplicate sample IDs. The labels for sample collection should be removed from these sheets so they cannot be confused with participant sample labels.

To create a “blind duplicate participant” the blood processing staff will need to aliquot into extra cryovials any extra serum or plasma left after completing the set of cryovials for any particular participant. The cryovials to be used for this process will be set up in an aliquot rack exactly like those for a participant except that there will be no cryovials 13-16. This blind duplicate aliquot rack must be kept handy during the processing of all participant samples.

A sheet of blind duplicate sample ID labels will be used to label the cryovials in the blind duplicate aliquot rack exactly as is done for regular cryovials. A “BDID Form” label from the sheet of **blind duplicate sample ID labels** will be placed **in the appropriate box at the top of each page** of the “Blind Duplicate Identification Form” (see Appendix 13), *and this form must be kept with the corresponding aliquot rack until all the cryovials are filled*. Since each aliquot labeled with the blind duplicate ID number may be filled with sample from a different participant, it is vital that the **participants’ sample IDs** be associated with the correct aliquots in the data system. Therefore, a “BDID Form” label from the **participant’s** label sheet will be placed in the bubble corresponding to the aliquot filled with that participant’s sample.

7.1.3 Paperwork:

In addition, dummy Phlebotomy and Laboratory Processing forms must be made for each blind duplicate ID number used. The Laboratory Processing and Phlebotomy forms should

be pulled out from a standard set of forms and the remaining pages discarded. This should be done on a case-by-case basis, not in advance, so the page link numbers are not out of sync with those of real participants. The dummy Phlebotomy and Laboratory Processing forms should be filled out with the same dummy Health ABC Enrollment ID#s and Acrostics used for the first visit. The Coordinating Center will provide a list of Health ABC Enrollment ID#s and Acrostics used. The dummy forms should be used in numerical order from lowest pagelink number to highest (i.e., low blind duplicate aliquot numbers should be associated with low page link numbers and low dummy Health ABC Enrollment IDs).

Keep the dummy forms with the blind duplicate aliquot rack until both cryovials to be sent to LCBR are filled. Record any comments pertaining to the aliquoting of LCBR samples (cryovials 02 and 21), then check all the rest of the cryovials as filled -- this includes cryovials 13-16, even though they will not be filled. Fill in the Phlebotomy form with dummy answers, making sure the "Time at Start of Venipuncture" and "Time at Start of Processing" are in reasonable agreement. In order to avoid tipping off LCBR that these are dummy forms, be sure to vary your answers from one set of dummy forms to another. Place the dummy forms with the other Phlebotomy and Laboratory Processing forms in the daily work folder. Once copies of the Phlebotomy and Laboratory Processing forms are sent to the Core Laboratory and McKesson, selected fields from the original Laboratory Processing form need to be entered into the data system. Only the Health ABC Enrollment ID#, acrostic, date that the form was completed, staff ID #, and the bar code will need to be entered. A separate screen has been created for the entry of this form (for data entry purposes, the form is called the "Laboratory Processing for Blind Duplicate Form"). These few fields will have to be keyboard entered (including the bar code number), not scanned.

A central file should be created for all of the Phlebotomy and Laboratory Processing Forms for the blind duplicates. Once the Laboratory Processing Form has been entered into the data system, both the Phlebotomy Form and the Laboratory Processing Form for the blind duplicates should be filed in this central file.

Completed Blind Duplicate Identification Forms should be scanned into the data system. Note that this is *in addition* to the weekly faxing of current forms to the Coordinating Center. Once the Blind Duplicate Identification form has been scanned it should be filed with its associated dummy Phlebotomy and Laboratory Processing forms.

7.1.4 Keeping the blind duplicates "in sync" with the normal samples:

Blind duplicate cryovials should be filled in the same order as regular cryovials *except when there is at least 0.5 mL but not 1.0 mL of extra sample available, in which case it is permissible to jump over 1.0 mL samples to get to the next 0.5 mL sample. Do not make partial blood aliquots for blind duplicates (partial 20 mL urine samples are acceptable; minimum volume is 15 mL).* If there is not enough sample left to fill a blind duplicate aliquot to the intended level, discard the remaining sample. If all aliquots of one type of sample are filled before the other sample types, you may stop saving extra samples of the completed type until you have completed the entire blind duplicate set for that blind duplicate ID. *If you are falling behind on blind duplicates (see below), you should start a second concurrent blind duplicate set when you have filled out one type of aliquots in the first set.*

Note that you should be completing a blind duplicate set for approximately every 20 participants. You should not get far ahead or fall far behind. If you fill up a blind duplicate set quickly, wait until the next blind duplicate ID is in the series of regular IDs that you are currently using on participants. If you fall behind, adjustments will have to be made.

If your blind duplicates are falling behind, the first thing to do is determine whether it is one sample type alone that is preventing the blind duplicate set from being filled out. If so, start a second concurrent blind duplicate set and keep filling the more plentiful sample types. If you are unable to catch up (i.e., there is a chronic shortage of, for example, serum), you should notify your supervisor and they should contact the Health ABC Coordinating Unit. It may be necessary to increase the volume of the collection tube for the sample that is in short supply.

If it is determined that all of the samples are in short supply, you should examine your pipetting technique. Variations in pipetting, especially the depth to which the pipet is dipped in the sample, can have large effects on sample loss during aliquoting. If this is not the problem and you find that you are unable to fill up blind duplicate sets as planned, notify your supervisor. This should be done as soon as the problem is detected, as alternative plans will have to be made.

7.2 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 administration by experienced examiner)
- Discuss problems and questions with local expert or QC officer

7.3 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
- Submit sample of processed blood to LCBR for evaluation (only for staff members who are processing the special CPT tubes to get platelets, buffy coats, and plasma)

7.4 Quality Assurance Checklist

Preparation

- Aliquot racks correctly set up
- Blind duplicate rack correctly set up
- Cryovials correctly labeled
- Vitamin C reducing buffer pre-measured into cryovial 01 just before aliquoting (cryovial 01 kept in rack on ice)
- Hepatitis B vaccination given or offered to all personnel handling blood
- Non-permeable lab coats, gloves, and face shields used
- One-day supply of freezing buffers and PBS measured out and kept on ice (except buffy coat freezing buffer B and PBS, which should be at room temperature)

Processing EDTA plasma and urine

- Time checked to ensure that tubes 1 and 4 processed within 15 minutes of completion of phlebotomy
- Tubes #1 and 4 centrifuged at 4° C for 10 min at 3000 G
- EDTA plasma from tubes #1 and 4 pooled and placed on ice before aliquoting
- First EDTA plasma aliquot (0.5 mL) added to vitamin C buffer in cryovial 01
- New pipet tips used for rest of EDTA plasma
- Plasma correctly aliquoted in correct order

- Vitamin C aliquots immediately placed in -70° freezer
- Urine correctly aliquoted

Cell Separation

Step 1

- CPT held at room temperature for no more than two hours before processing
- CPT tubes centrifuged for 30,000 G-min at room temperature

Step 1a

- Plasma pipetted from each tube without disturbing the cell layer and pooled in a single tube for each participant
- Pooling tube checked for matching sample ID
- New transfer pipet used for each participant's samples
- Pooling tube placed on ice until aliquoting

Step 2

- Entire cell layer transferred to a new conical centrifuge tube
- Centrifuge tube checked for matching sample ID
- New transfer pipet used for each participant's samples
- Fluid level brought up to 14 mL with PBS and gently mixed
- Tubes centrifuged at 4500 G-min at room temperature

Step 2a

- Pooled plasma mixed before aliquoting
- Plasma aliquoted into 4 blue-capped cryovials
- Cryovials checked for matching sample ID
- New pipet tip used for each participant's samples
- Cryovials held on ice until frozen

Step 3

- Supernatant transferred to a new conical centrifuge tube
- Centrifuge tube checked for matching sample ID
- New transfer pipet used for each participant's samples
- Pellet not disturbed during transfer of supernatant (a small amount may be left in the original tube)
- Tubes centrifuged at 30,000 G-min at room temperature

Step 3a

- 0.5 mL of buffy coat freezing buffer A added to each tube
- Tube tapped and/or pipet tip flushed repeatedly to resuspend pellet
- New pipet tip used for mixing each participant's samples
- 0.5 mL of buffy coat freezing buffer B added to each tube
- Resuspended sample mixed gently before transferring to cryovial
- Entire resuspended buffy coat sample transferred to clear-capped cryovial
- Cryovials placed in rack in styrofoam container with lid.
- Styrofoam container placed in -20° freezer
- Timer set for 2 hours to transfer to -70° freezer

Step 4

- Supernatant removed from each tube and discarded
- New transfer pipet used for supernatant from each participant's samples
- 1.5 mL of platelet buffer added to each tube
- Tube tapped and/or pipet tip flushed repeatedly to resuspend pellet
- Entire resuspended pellet transferred to orange-capped cryovial
- New transfer pipet used for each participant's samples
- Cryovials placed immediately in -70° freezer

Processing serum tubes

- Time checked to ensure that tubes 5 & 6 have stood at room temperature for at least 40 minutes, maximum 90 minutes
- Tubes 5 & 6 centrifuged for 10 minutes at 3000 G. If room temperature centrifuge used, temperature of serum after spinning is monitored by feel.
- Centrifuge correctly balanced with water tube(s)
- Serum from tubes 5 & 6 pooled before aliquoting
- Serum correctly aliquoted

Blind duplicates

- Extra sample stored as blind duplicates, if available
- All remaining sample discarded in hazardous waste container

Freezing

- Platelet and vitamin C cryovials placed immediately in -70° freezer after filling
- Special freezing protocol followed for buffy coats
- Remaining aliquots checked to ensure they are not wet
- Rack placed upright in -70° C freezer or samples placed on dry ice

End of day 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
- Aliquots 02 and 21 stored in LCBR box
- Aliquots 13 & 14 stored in a separate box for -145° freezing
- Aliquots 01, 03-12, 15-20, and 22-31 placed in blood freezer boxes for McKesson
- Aliquots 32 and 33 placed in urine freezer boxes for McKesson
- Aliquots 34 and 35 (large urine aliquots) placed in freezer boxes provided by McKesson.

Shipment procedures -- dry ice

- Freezer boxes correctly wrapped -- absorbent material, rubber band, and zip-lock bag
- Styrofoam mailers correctly packed -- absorbent material, dry ice, top sealed with tape
- Styrofoam mailer sealed in cardboard sleeve
- FedEx airbill correctly filled out
- Labels correctly affixed

**APPENDIX 1 Health ABC Cryovials and Processing
(3 pages)**

Vendor: VWR Scientific (800) 932-5000

Note: Prices are from the catalog. Educational discounts should apply.

Cryovials/Caps	#per participant	Sample Type	VWR Catalog Number	Price per pk (500)	Price per cs (5000)
0.5 mL with Skirt	18	multiple	20170-209	\$27.03	\$270.27
1.5 mL with Skirt	16	buffy, platelet	20170-213	\$27.03	\$270.27
2.0 mL with Skirt	3	multiple	20170-205	\$27.03	\$270.27
Cap- Natural	2	buffy coat	20170-241	\$48.00	\$480.00
Cap - White	11	EDTA	20170-274	\$48.27	\$482.74
Cap- Red	11	Serum	20170-247	\$48.30	\$482.74
Cap- Blue	4	Citrate	20170-251	\$48.30	\$483.00
Cap- Yellow	1	Vitamin C	20170-243	\$48.30	\$482.74
Cap-Green	1	Urine (acid.)	20170-253	\$48.30	\$483.00
Cap- Violet	1	Urine	20170-249	\$48.30	\$483.00
Cryovial Racks	1 (~10 total)	all	30128-346	\$45.96/5	-----
Cover for Cryo Rack	1 (helpful but not required)	all	30128-350	\$29.99/5	-----
Other	#per participant	Sample Type	Fisher Cat. No.	Price per pack	Price per case
30 mL LDPE bottles	2	Urine	02-896-1A	\$10.82/12	\$61.70/72
15 mL graduated conical polypropylene centrifuge tubes	4	Cell separation	14-959-70C OR 12-565-282F OR 07-200-178		\$144.20/500 \$162.90/500 \$175/500
Transfer pipets (3.2 mL is fine)	6		Fisher # 13-711-7		\$265/5000 \$32/500

Miscellaneous:

Pipets	Capable of pipeting 0.5, 1.0, and 2.0 mL volumes. For removal of buffy coat (white cell) layer For aspiration of platelets Disposable for urine, capable of pipetting 20 mL volumes (plus bulb), unless graduated cylinders will be used
Graduated cylinders	Capable of measuring 20 mL (if not using disposable pipets) Need enough for one day's worth of participants
Test tubes	For pooling serum and EDTA plasma before aliquoting. Plastic (polypropylene) is preferred. Disposable is okay 10 and 15 mL capacity
Urine Cups	info pending

Health ABC Materials for Blood Collection

BD = Becton Dickinson brand which is available through VWR, Baxter, and Fisher

Vacutainer blood collection sets, 21G 3/4", consists of 12" tubing with Luer adapter

VWR# VT7251

\$184.57/200

BD # 7250

Vacutainer needle holders

BD # 4893

\$112.64/720

Collection Tubes - Vacutainer brand

Tube#	Type	Volume	Size	Description	BD#	Price (100)
1,4	EDTA	10 mL	16x100	Liquid EDTA (K3) 15%	6457	\$25.00
5, 6	Serum	10 mL	16x100	No additive, silicon coated	6430	\$23.40

To be supplied by LCBR:

Buffy coat storage buffer A&B

Platelet storage buffer

Vitamin C reducing buffer

PBS or PBS tablets

CPT tubes

Acetic Acid (1 M)

Health ABC Materials for Sample Shipment

The following items will be provided by McKesson Bioservices/LCBR:

Freezer boxes (2")	VWR# 55705-424 Revco # 5954 or Krackeler Scientific* #114-5144-F12	~\$23.00/12 \$23.00/12
Box dividers (9x9)	VWR# 55701-762 Revco # 6212 or Krackeler Scientific* #114-5144-F29	~\$21.00/12 \$16.00/12

Styrofoam Shipping Containers:

Vendor: Polyfoam Packers 800-323-7442
Note: VWR carries Polyfoam Packers products

Polyfoam Packers #430

These boxes can be recycled and used many times. Four of each per site is required to start

The following items should be purchased at each site:

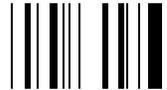
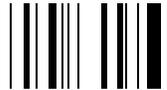
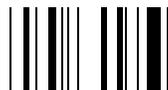
Zip Lock Bags (12x12") VWR#11217-128

Each site will use approximately 23 per week (based on 35 participants per week).

Ice Packs VWR#15715-105 U-TEK Reusable Refrigerant Packs

*Alternate vendor: Krackeler Scientific
 (800)334-7725 (Albany, NY)
 (800)221-6921 (Durham, NC)

**APPENDIX 2 Sample Label Sheet (Bar Codes) and Label Orientation on Cryovial
(1 of 3 pages)**

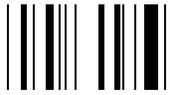
##### Draw Tube 1 Purple top 10 mL	##### Draw Tube 2 Black/blue top 8 mL	##### Draw Tube 3 Black/blue top 8 mL	##### EDTA Plasma Pool 15 mL	##### Serum Pool 15 mL	##### Citrate Plasma Pool 10 mL
##### Draw Tube 4 Purple top 10 mL	##### Draw Tube 5 Red top 10 mL	##### Draw Tube 6 Red top 10 mL	##### 15 mL conical centrifuge tube Step 2: Cells	##### 15 mL conical centrifuge tube Step 2: Cells	##### 15 mL conical centrifuge tube Step 3: Platelets
##### Backup Vacutainer	##### Backup Vacutainer	##### Backup Vacutainer	##### 15 mL conical centrifuge tube Step 3: Platelets	 ##### BDID Form	 ##### BDID Form
##### Urine cup	 ##### Phlebotomy Form	 ##### Laboratory Processing Form	 ##### BDID Form	 ##### BDID Form	 ##### BDID Form

 ##### BDID Form	Place this end on vial first  #####-01 Y/Vit C 2.0	Place this end on vial first  #####-02 W/EDTA 0.5 To LCBR
Place this end on vial first  #####-03 W/EDTA 0.5	Place this end on vial first  #####-04 W/EDTA 0.5	Place this end on vial first  #####-05 W/EDTA 0.5

APPENDIX 2
Sample Label Sheet (Bar Codes)
(page 2 of 3)

Place this end on vial first  #####-06 W/EDTA 0.5	Place this end on vial first  #####-07 W/EDTA 1.5	Place this end on vial first  #####-08 W/EDTA 1.5	Place this end on vial first  #####-18 B/Citrate 0.5	Place this end on vial first  #####-19 B/Citrate 0.5	Place this end on vial first  #####-20 B/Citrate 0.5
Place this end on vial first  #####-09 W/EDTA 1.5	Place this end on vial first  #####-10 W/EDTA 1.5	Place this end on vial first  #####-11 W/EDTA 0.5	Place this end on vial first  #####-21 R/Serum 1.5 To LCBR	Place this end on vial first  #####-22 R/Serum 0.5	Place this end on vial first  #####-23 R/Serum 0.5
Place this end on vial first  #####-12 W/EDTA 0.5	Place this end on vial first  #####-13 C/Buffy 2.0 Use special freezing protocol	Place this end on vial first  #####-14 C/Buffy 2.0 Use special freezing protocol	Place this end on vial first  #####-24 R/Serum 0.5	Place this end on vial first  #####-25 R/Serum 0.5	Place this end on vial first  #####-26 R/Serum 0.5
Place this end on vial first  #####-15 O/platelets 2.0	Place this end on vial first  #####-16 O/platelets 2.0	Place this end on vial first  #####-17 B/Citrate 0.5	Place this end on vial first  #####-27 R/Serum 1.5	Place this end on vial first  #####-28 R/Serum 1.5	Place this end on vial first  #####-29 R/Serum 1.5

Place this end
on vial first



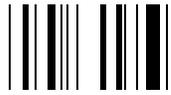
#####-30
R/Serum 0.5

Place this end
on vial first



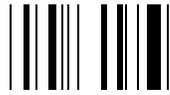
#####-31
R/Serum 0.5

Place this end
on vial first



#####-32
V/Urine 2.0

Place this end
on vial first



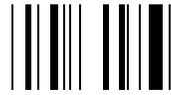
#####-33
V/Urine 20
30 mL Bottle

Place this end
on vial first



#####-34
G/Acid 2.0

Place this end
on vial first

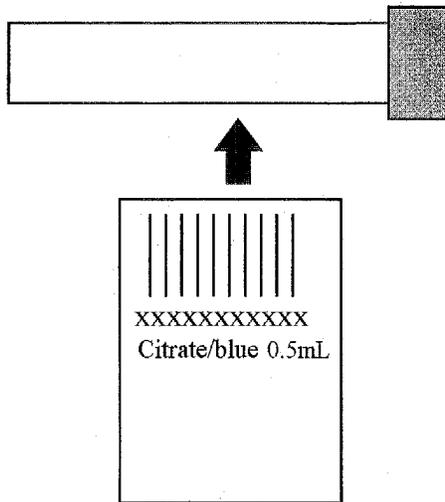


#####-35
G/Acid 20
30 mL Bottle

APPENDIX 2
Sample Label Sheet (Bar Codes)
(page 3 of 3)

HEALTH ABC STUDY

Label Orientation on Cryovial



APPENDIX 3 Laboratory Processing Form



HABC Enrollment ID #	Acrostic	Date Form Completed	Staff ID #
H		/ /	
		Month / Day / Year	

LABORATORY PROCESSING

Bar Code Label

Time at start of processing: | | : | | am
 pm

Collection Tubes	Cryo #	Vol.	Type	To	Check "X"	Problems	Collection Tubes	Cryo #	Vol.	Type	To	Check "X"	Problems
#1, 4 Vitamin C	01	0.5	Y/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	#2, 3 Citrate	17	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
#1, 4 EDTA	02	0.5	W/0.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		18	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	03	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		19	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	04	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		20	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	05	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	#5, 6 Serum	21	1.0	R/1.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	06	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		22	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	07	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		23	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	08	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		24	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	09	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		25	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
10	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	26		0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
11	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	27		1.0	R/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
12	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	28		1.0	R/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
#2, 3 Buffy Refused DNA collection	13	var	C/2.0	M*	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		29	1.0	R/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	14	var	C/2.0	M*	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		30	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
#2, 3 Platelets	15	var	O/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		31	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	16	var	O/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	URINE	32	2.0	V/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> P
						33		20	V/20	M	<input type="checkbox"/>	<input type="checkbox"/> P	
						(acidified)		34	2.0	G/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> P
							35	20	G/20	M	<input type="checkbox"/>	<input type="checkbox"/> P	

L=LCBR; M=McKesson; H=Hemolyzed; P=Partial; W=white; C=clear; Y=Vitamin C; B=blue; R=red; V=violet G=green, O=Orange

*Place in a styrofoam box at -20°C for 2 hours. Transfer to -80°C to hold for shipping.

Page Link #



APPENDIX 4 OSHA Guidelines
Enforcement Procedures for the Occupational Exposure to Bloodborne Pathogens
Standard, 29 CFR 1910.1030
(16 pages)

1910.1030 - Bloodborne pathogens.

- * Standard Number: 1910.1030
- * Standard Title: Bloodborne pathogens.
- * SubPart Number: Z
- * SubPart Title: Toxic and Hazardous Substances

Produced by USDOL OSHA - Directorate of Safety Standards &
Directorate of Health Standards
Maintained by USDOL OSHA - OCIS

(a) Scope and Application. This section applies to all occupational exposure to blood or other potentially infectious materials as defined by paragraph (b) of this section.

(b) Definitions. For purposes of this section, the following shall apply:

"Assistant Secretary" means the Assistant Secretary of Labor for Occupational Safety and Health, or designated representative.

"Blood" means human blood, human blood components, and products made from human blood.

"Bloodborne Pathogens" means pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

"Clinical Laboratory" means a workplace where diagnostic or other screening procedures are performed on blood or other potentially infectious materials.

"Contaminated" means the presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

"Contaminated Laundry" means laundry which has been soiled with blood or other potentially infectious materials or may contain sharps.

"Contaminated Sharps" means any contaminated object that can penetrate the skin including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed ends of dental wires.

"Decontamination" means the use of physical or chemical means to remove, inactivate, or destroy bloodborne pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.

"Director" means the Director of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, or designated representative.

"Engineering Controls" means controls (e.g., sharps disposal containers, self-sheathing needles) that isolate or remove the bloodborne pathogens hazard from the workplace.

"Exposure Incident" means a specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties.

"Handwashing Facilities" means a facility providing an adequate supply of running potable water, soap and single use towels or hot air drying machines.

"Licensed Healthcare Professional" is a person whose legally permitted scope of practice allows him or her to independently perform the activities required by paragraph (f) Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up.

"HBV" means hepatitis B virus.

"HIV" means human immunodeficiency virus.

"Occupational Exposure" means reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

"Other Potentially Infectious Materials" means (1) The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; (2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead); and (3) HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

"Parenteral" means piercing mucous membranes or the skin barrier

through such events as needlesticks, human bites, cuts, and abrasions.

"Personal Protective Equipment" is specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts or blouses) not intended to function as protection against a hazard are not considered to be personal protective equipment.

"Production Facility" means a facility engaged in industrial-scale, large-volume or high concentration production of HIV or HBV.

"Regulated Waste" means liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials.

"Research Laboratory" means a laboratory producing or using research-laboratory-scale amounts of HIV or HBV. Research laboratories may produce high concentrations of HIV or HBV but not in the volume found in production facilities.

"Source Individual" means any individual, living or dead, whose blood or other potentially infectious materials may be a source of occupational exposure to the employee. Examples include, but are not limited to, hospital and clinic patients; clients in institutions for the developmentally disabled; trauma victims; clients of drug and alcohol treatment facilities; residents of hospices and nursing homes; human remains; and individuals who donate or sell blood or blood components.

"Sterilize" means the use of a physical or chemical procedure to destroy all microbial life including highly resistant bacterial endospores.

"Universal Precautions" is an approach to infection control. According to the concept of Universal Precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

"Work Practice Controls" means controls that reduce the likelihood of exposure by altering the manner in which a task is performed (e.g., prohibiting recapping of needles by a two-handed technique).

..1910.1030(c) (c) Exposure Control. (1) Exposure Control Plan. (i) Each employer having an employee(s) with occupational exposure as defined by paragraph (b) of this section shall establish a written Exposure Control Plan designed to eliminate or minimize employee exposure. (ii) The Exposure Control Plan shall contain at least the following elements: (A) The exposure

determination required by paragraph (c)(2), (B) The schedule and method of implementation for paragraphs (d) Methods of Compliance, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, (g) Communication of Hazards to Employees, and (h) Recordkeeping, of this standard, and (C) The procedure for the evaluation of circumstances surrounding exposure incidents as required by paragraph (f)(3)(i) of this standard. (iii) Each employer shall ensure that a copy of the Exposure Control Plan is accessible to employees in accordance with 29 CFR 1910.1020(e). (iv) The Exposure Control Plan shall be reviewed and updated at least annually and whenever necessary to reflect new or modified tasks and procedures which affect occupational exposure and to reflect new or revised employee positions with occupational exposure.

..1910.1030(c)(1)(v) (v) The Exposure Control Plan shall be made available to the Assistant Secretary and the Director upon request for examination and copying. (2) Exposure Determination. (i) Each employer who has an employee(s) with occupational exposure as defined by paragraph (b) of this section shall prepare an exposure determination. This exposure determination shall contain the following: (A) A list of all job classifications in which all employees in those job classifications have occupational exposure; (B) A list of job classifications in which some employees have occupational exposure, and (C) A list of all tasks and procedures or groups of closely related task and procedures in which occupational exposure occurs and that are performed by employees in job classifications listed in accordance with the provisions of paragraph (c)(2)(i)(B) of this standard. (ii) This exposure determination shall be made without regard to the use of personal protective equipment. (d) Methods of Compliance. (1) General. Universal precautions shall be observed to prevent contact with blood or other potentially infectious materials. Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious materials.

..1910.1030(d)(2) (2) Engineering and Work Practice Controls. (i) Engineering and work practice controls shall be used to eliminate or minimize employee exposure. Where occupational exposure remains after institution of these controls, personal protective equipment shall also be used. (ii) Engineering controls shall be examined and maintained or replaced on a regular schedule to ensure their effectiveness. (iii) Employers shall provide handwashing facilities which are readily accessible to employees. (iv) When provision of handwashing facilities is not feasible, the employer shall provide either an appropriate antiseptic hand cleanser in conjunction with clean cloth/paper towels or antiseptic towelettes. When antiseptic hand cleansers or towelettes are used, hands shall be washed with soap and

running water as soon as feasible. (v) Employers shall ensure that employees wash their hands immediately or as soon as feasible after removal of gloves or other personal protective equipment. (vi) Employers shall ensure that employees wash hands and any other skin with soap and water, or flush mucous membranes with water immediately or as soon as feasible following contact of such body areas with blood or other potentially infectious materials.

..1910.1030(d)(2)(vii) (vii) Contaminated needles and other contaminated sharps shall not be bent, recapped, or removed except as noted in paragraphs (d)(2)(vii)(A) and (d)(2)(vii)(B) below. Shearing or breaking of contaminated needles is prohibited. (A) Contaminated needles and other contaminated sharps shall not be bent, recapped or removed unless the employer can demonstrate that no alternative is feasible or that such action is required by a specific medical or dental procedure. (B) Such bending, recapping or needle removal must be accomplished through the use of a mechanical device or a one-handed technique. (viii) Immediately or as soon as possible after use, contaminated reusable sharps shall be placed in appropriate containers until properly reprocessed. These containers shall be:

(A) puncture resistant;

(B) labeled or color-coded in accordance with this standard;

..1910.1030(d)(2)(viii)(C)

(C) leakproof on the sides and bottom; and

(D) in accordance with the requirements set forth in paragraph (d)(4)(ii)(E) for reusable sharps.

(ix) Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in work areas where there is a reasonable likelihood of occupational exposure. (x) Food and drink shall not be kept in refrigerators, freezers, shelves, cabinets or on countertops or benchtops where blood or other potentially infectious materials are present. (xi) All procedures involving blood or other potentially infectious materials shall be performed in such a manner as to minimize splashing, spraying, spattering, and generation of droplets of these substances. (xii) Mouth pipetting/suctioning of blood or other potentially infectious materials is prohibited. (xiii) Specimens of blood or other potentially infectious materials shall be placed in a container which prevents leakage during collection, handling, processing, storage, transport, or shipping.

..1910.1030(d)(2)(xiii)(A) (A) The container for storage, transport, or shipping shall be labeled or color-coded according to paragraph (g)(1)(i) and closed prior to being stored, transported, or shipped. When a facility utilizes Universal Precautions in the handling of all specimens, the labeling/color-coding of specimens is not necessary provided containers are recognizable as containing specimens. This exemption only applies while such specimens/containers remain within the facility. Labeling or color-coding in accordance with paragraph (g)(1)(i) is required when such specimens/containers leave the facility. (B) If outside contamination of the primary container occurs, the primary container shall be placed within a second container which prevents leakage during handling, processing, storage, transport, or shipping and is labeled or color-coded according to the requirements of this standard. (C) If the specimen could puncture the primary container, the primary container shall be placed within a secondary container which is puncture-resistant in addition to the above characteristics. (xiv) Equipment which may become contaminated with blood or other potentially infectious materials shall be examined prior to servicing or shipping and shall be decontaminated as necessary, unless the employer can demonstrate that decontamination of such equipment or portions of such equipment is not feasible. ..1910.1030(d)(2)(xiv)(A) (A) A readily observable label in accordance with paragraph (g)(1)(i)(H) shall be attached to the equipment stating which portions remain contaminated. (B) The employer shall ensure that this information is conveyed to all affected employees, the servicing representative, and/or the manufacturer, as appropriate, prior to handling, servicing, or shipping so that appropriate precautions will be taken. (3) Personal Protective Equipment. (i) Provision. When there is occupational exposure, the employer shall provide, at no cost to the employee, appropriate personal protective equipment such as, but not limited to, gloves, gowns, laboratory coats, face shields or masks and eye protection, and mouthpieces, resuscitation bags, pocket masks, or other ventilation devices. Personal protective equipment will be considered "appropriate" only if it does not permit blood or other potentially infectious materials to pass through to or reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used. ..1910.1030(d)(3)(ii) (ii) Use. The employer shall ensure that the employee uses appropriate personal protective equipment unless the employer shows that the employee temporarily and briefly declined to use personal protective equipment when, under rare and extraordinary circumstances, it was the employee's professional judgment that in the specific instance its use would have prevented the delivery of

health care or public safety services or would have posed an increased hazard to the safety of the worker or co-worker. When the employee makes this judgment, the circumstances shall be investigated and documented in order to determine whether changes can be instituted to prevent such occurrences in the future. (iii) Accessibility. The employer shall ensure that appropriate personal protective equipment in the appropriate sizes is readily accessible at the worksite or is issued to employees. Hypoallergenic gloves, glove liners, powderless gloves, or other similar alternatives shall be readily accessible to those employees who are allergic to the gloves normally provided. (iv) Cleaning, Laundering, and Disposal. The employer shall clean, launder, and dispose of personal protective equipment required by paragraphs (d) and (e) of this standard, at no cost to the employee. ..1910.1030(d)(3)(v) (v) Repair and Replacement. The employer shall repair or replace personal protective equipment as needed to maintain its effectiveness, at no cost to the employee. (vi) If a garment(s) is penetrated by blood or other potentially infectious materials, the garment(s) shall be removed immediately or as soon as feasible. (vii) All personal protective equipment shall be removed prior to leaving the work area. (viii) When personal protective equipment is removed it shall be placed in an appropriately designated area or container for storage, washing, decontamination or disposal. (ix) Gloves. Gloves shall be worn when it can be reasonably anticipated that the employee may have hand contact with blood, other potentially infectious materials, mucous membranes, and non-intact skin; when performing vascular access procedures except as specified in paragraph (d)(3)(ix)(D); and when handling or touching contaminated items or surfaces. (A) Disposable (single use) gloves such as surgical or examination gloves, shall be replaced as soon as practical when contaminated or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised. ..1910.1030(d)(3)(ix)(B) (B) Disposable (single use) gloves shall not be washed or decontaminated for re-use. (C) Utility gloves may be decontaminated for re-use if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, torn, punctured, or exhibit other signs of deterioration or when their ability to function as a barrier is compromised. (D) If an employer in a volunteer blood donation center judges that routine gloving for all phlebotomies is not necessary then the employer shall: {1} Periodically reevaluate this policy; {2} Make gloves available to all employees who wish to use them for phlebotomy; {3} Not discourage the use of gloves for phlebotomy; and {4} Require that gloves be used for phlebotomy in the following circumstances: [i] When the employee has cuts, scratches, or other breaks in his or her

skin; [ii] When the employee judges that hand contamination with blood may occur, for example, when performing phlebotomy on an uncooperative source individual; and [iii] When the employee is receiving training in phlebotomy. ..1910.1030(d)(3)(x) (x) Masks, Eye Protection, and Face Shields. Masks in combination with eye protection devices, such as goggles or glasses with solid side shields, or chin-length face shields, shall be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious materials may be generated and eye, nose, or mouth contamination can be reasonably anticipated. (xi) Gowns, Aprons, and Other Protective Body Clothing. Appropriate protective clothing such as, but not limited to, gowns, aprons, lab coats, clinic jackets, or similar outer garments shall be worn in occupational exposure situations. The type and characteristics will depend upon the task and degree of exposure anticipated. (xii) Surgical caps or hoods and/or shoe covers or boots shall be worn in instances when gross contamination can reasonably be anticipated (e.g., autopsies, orthopaedic surgery). (4) Housekeeping. (i) General. Employers shall ensure that the worksite is maintained in a clean and sanitary condition. The employer shall determine and implement an appropriate written schedule for cleaning and method of decontamination based upon the location within the facility, type of surface to be cleaned, type of soil present, and tasks or procedures being performed in the area. ..1910.1030(d)(4)(ii) (ii) All equipment and environmental and working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials. (A) Contaminated work surfaces shall be decontaminated with an appropriate disinfectant after completion of procedures; immediately or as soon as feasible when surfaces are overtly contaminated or after any spill of blood or other potentially infectious materials; and at the end of the work shift if the surface may have become contaminated since the last cleaning. (B) Protective coverings, such as plastic wrap, aluminum foil, or imperviously-backed absorbent paper used to cover equipment and environmental surfaces, shall be removed and replaced as soon as feasible when they become overtly contaminated or at the end of the workshift if they may have become contaminated during the shift. (C) All bins, pails, cans, and similar receptacles intended for reuse which have a reasonable likelihood for becoming contaminated with blood or other potentially infectious materials shall be inspected and decontaminated on a regularly scheduled basis and cleaned and decontaminated immediately or as soon as feasible upon visible contamination. ..1910.1030(d)(4)(ii)(D) (D) Broken glassware which may be contaminated shall not be picked up directly with the hands. It shall be cleaned up using mechanical means, such as a brush and dust pan, tongs, or forceps. (E) Reusable sharps that are contaminated with

blood or other potentially infectious materials shall not be stored or processed in a manner that requires employees to reach by hand into the containers where these sharps have been placed. (iii) Regulated Waste. (A) Contaminated Sharps Discarding and Containment. {1} Contaminated sharps shall be discarded immediately or as soon as feasible in containers that are: [a] Closable; [b] Puncture resistant; [c] Leakproof on sides and bottom; and [d] Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard. {2} During use, containers for contaminated sharps shall be: [a] Easily accessible to personnel and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found (e.g., laundries); [b] Maintained upright throughout use; and [c] Replaced routinely and not be allowed to overfill.

..1910.1030(d)(4)(iii)(A){3} {3} When moving containers of contaminated sharps from the area of use, the containers shall be: [a] Closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping; [b] Placed in a secondary container if leakage is possible. The second container shall be: [i] Closable; [ii] Constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping; and [iii] Labeled or color-coded according to paragraph (g)(1)(i) of this standard. {4} Reusable containers shall not be opened, emptied, or cleaned manually or in any other manner which would expose employees to the risk of percutaneous injury. (B) Other Regulated Waste Containment. {1} Regulated waste shall be placed in containers which are: [a] Closable; [b] Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping; [c] Labeled or color-coded in accordance with paragraph (g)(1)(i) this standard; and [d] Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

..1910.1030(d)(4)(iii)(B){2} {2} If outside contamination of the regulated waste container occurs, it shall be placed in a second container. The second container shall be: [a] Closable; [b] Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping; [c] Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard; and [d] Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping. (C) Disposal of all regulated waste shall be in accordance with applicable regulations of the United States, States and Territories, and political subdivisions of States and Territories. (iv) Laundry. (A) Contaminated laundry shall be handled as little as possible with a minimum of agitation. {1} Contaminated laundry shall be bagged or containerized at the location where it was used and shall not be sorted or rinsed in the location of use.

..1910.1030(d)(4)(iv)(A){2} {2} Contaminated laundry shall be placed and transported in bags or containers labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard. When a facility utilizes Universal Precautions in the handling of all soiled laundry, alternative labeling or color-coding is sufficient if it permits all employees to recognize the containers as requiring compliance with Universal Precautions. {3} Whenever contaminated laundry is wet and presents a reasonable likelihood of soak-through of or leakage from the bag or container, the laundry shall be placed and transported in bags or containers which prevent soak-through and/or leakage of fluids to the exterior. (B) The employer shall ensure that employees who have contact with contaminated laundry wear protective gloves and other appropriate personal protective equipment. (C) When a facility ships contaminated laundry off-site to a second facility which does not utilize Universal Precautions in the handling of all laundry, the facility generating the contaminated laundry must place such laundry in bags or containers which are labeled or color-coded in accordance with paragraph (g)(1)(i). ..1910.1030(e) (e) HIV and HBV Research Laboratories and Production Facilities. (1) This paragraph applies to research laboratories and production facilities engaged in the culture, production, concentration, experimentation, and manipulation of HIV and HBV. It does not apply to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissues, or organs. These requirements apply in addition to the other requirements of the standard. (2) Research laboratories and production facilities shall meet the following criteria: (i) Standard Microbiological Practices. All regulated waste shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens. (ii) Special Practices (A) Laboratory doors shall be kept closed when work involving HIV or HBV is in progress. (B) Contaminated materials that are to be decontaminated at a site away from the work area shall be placed in a durable, leakproof, labeled or color-coded container that is closed before being removed from the work area. ..1910.1030(e)(2)(ii)(C) (C) Access to the work area shall be limited to authorized persons. Written policies and procedures shall be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit procedures shall be allowed to enter the work areas and animal rooms. (D) When other potentially infectious materials or infected animals are present in the work area or containment module, a hazard warning sign incorporating the universal biohazard symbol shall be posted on all access doors. The hazard warning sign shall comply with paragraph (g)(1)(ii) of this standard. (E) All activities involving other potentially infectious materials shall be

conducted in biological safety cabinets or other physical-containment devices within the containment module. No work with these other potentially infectious materials shall be conducted on the open bench. (F) Laboratory coats, gowns, smocks, uniforms, or other appropriate protective clothing shall be used in the work area and animal rooms. Protective clothing shall not be worn outside of the work area and shall be decontaminated before being laundered. ..1910.1030(e)(2)(ii)(G) (G) Special care shall be taken to avoid skin contact with other potentially infectious materials. Gloves shall be worn when handling infected animals and when making hand contact with other potentially infectious materials is unavoidable. (H) Before disposal all waste from work areas and from animal rooms shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens. (I) Vacuum lines shall be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency and which are checked routinely and maintained or replaced as necessary. (J) Hypodermic needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) shall be used for the injection or aspiration of other potentially infectious materials. Extreme caution shall be used when handling needles and syringes. A needle shall not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe shall be promptly placed in a puncture-resistant container and autoclaved or decontaminated before reuse or disposal. ..1910.1030(e)(2)(ii)(K) (K) All spills shall be immediately contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with potentially concentrated infectious materials. (L) A spill or accident that results in an exposure incident shall be immediately reported to the laboratory director or other responsible person. (M) A biosafety manual shall be prepared or adopted and periodically reviewed and updated at least annually or more often if necessary. Personnel shall be advised of potential hazards, shall be required to read instructions on practices and procedures, and shall be required to follow them. (iii) Containment Equipment. (A) Certified biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protection or physical containment devices, such as special protective clothing, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals, shall be used for all activities with other potentially infectious materials that pose a threat of exposure to droplets, splashes, spills, or aerosols. ..1910.1030(e)(2)(iii)(B) (B) Biological

safety cabinets shall be certified when installed, whenever they are moved and at least annually. (3) HIV and HBV research laboratories shall meet the following criteria: (i) Each laboratory shall contain a facility for hand washing and an eye wash facility which is readily available within the work area. (ii) An autoclave for decontamination of regulated waste shall be available. (4) HIV and HBV production facilities shall meet the following criteria: (i) The work areas shall be separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors shall be the basic requirement for entry into the work area from access corridors or other contiguous areas. Physical separation of the high-containment work area from access corridors or other areas or activities may also be provided by a double-doored clothes-change room (showers may be included), airlock, or other access facility that requires passing through two sets of doors before entering the work area.

..1910.1030(e)(4)(ii) (ii) The surfaces of doors, walls, floors and ceilings in the work area shall be water resistant so that they can be easily cleaned. Penetrations in these surfaces shall be sealed or capable of being sealed to facilitate decontamination. (iii) Each work area shall contain a sink for washing hands and a readily available eye wash facility. The sink shall be foot, elbow, or automatically operated and shall be located near the exit door of the work area. (iv) Access doors to the work area or containment module shall be self-closing. (v) An autoclave for decontamination of regulated waste shall be available within or as near as possible to the work area. (vi) A ducted exhaust-air ventilation system shall be provided. This system shall create directional airflow that draws air into the work area through the entry area. The exhaust air shall not be recirculated to any other area of the building, shall be discharged to the outside, and shall be dispersed away from occupied areas and air intakes. The proper direction of the airflow shall be verified (i.e., into the work area). ..1910.1030(e)(5) (5) Training Requirements. Additional training requirements for employees in HIV and HBV research laboratories and HIV and HBV production facilities are specified in paragraph (g)(2)(ix). (f) Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up. (1) General. (i) The employer shall make available the hepatitis B vaccine and vaccination series to all employees who have occupational exposure, and post-exposure evaluation and follow-up to all employees who have had an exposure incident. (ii) The employer shall ensure that all medical evaluations and procedures including the hepatitis B vaccine and vaccination series and post-exposure evaluation and follow-up, including prophylaxis, are: (A) Made available at no cost to the employee; (B) Made available to the employee at a reasonable time and place; (C) Performed by or under the

supervision of a licensed physician or by or under the supervision of another licensed healthcare professional; and (D) Provided according to recommendations of the U.S. Public Health Service current at the time these evaluations and procedures take place, except as specified by this paragraph (f).

..1910.1030(f)(1)(iii) (iii) The employer shall ensure that all laboratory tests are conducted by an accredited laboratory at no cost to the employee.

(2) Hepatitis B Vaccination. (i) Hepatitis B vaccination shall be made available after the employee has received the training required in paragraph (g)(2)(vii)(I) and within 10 working days of initial assignment to all employees who have occupational exposure unless the employee has previously received the complete hepatitis B vaccination series, antibody testing has revealed that the employee is immune, or the vaccine is contraindicated for medical reasons. (ii) The employer shall not make participation in a prescreening program a prerequisite for receiving hepatitis B vaccination. (iii) If the employee initially declines hepatitis B vaccination but at a later date while still covered under the standard decides to accept the vaccination, the employer shall make available hepatitis B vaccination at that time. (iv) The employer shall assure that employees who decline to accept hepatitis B vaccination offered by the employer sign the statement in Appendix A.

..1910.1030(f)(2)(v) (v) If a routine booster dose(s) of hepatitis B vaccine is recommended by the U.S. Public Health Service at a future date, such booster dose(s) shall be made available in accordance with section (f)(1)(ii).

(3) Post-exposure Evaluation and Follow-up. Following a report of an exposure incident, the employer shall make immediately available to the exposed employee a confidential medical evaluation and follow-up, including at least the following elements: (i) Documentation of the route(s) of exposure, and the circumstances under which the exposure incident occurred; (ii) Identification and documentation of the source individual, unless the employer can establish that identification is infeasible or prohibited by state or local law; (A) The source individual's blood shall be tested as soon as feasible and after consent is obtained in order to determine HBV and HIV infectivity. If consent is not obtained, the employer shall establish that legally required consent cannot be obtained. When the source individual's consent is not required by law, the source individual's blood, if available, shall be tested and the results documented.

..1910.1030(f)(3)(ii)(B) (B) When the source individual is already known to be infected with HBV or HIV, testing for the source individual's known HBV or HIV status need not be repeated. (C) Results of the source individual's testing shall be made available to the exposed employee, and the employee shall be informed of applicable laws and regulations concerning disclosure

of the identity and infectious status of the source individual. (iii) Collection and testing of blood for HBV and HIV serological status; (A) The exposed employee's blood shall be collected as soon as feasible and tested after consent is obtained. (B) If the employee consents to baseline blood collection, but does not give consent at that time for HIV serologic testing, the sample shall be preserved for at least 90 days. If, within 90 days of the exposure incident, the employee elects to have the baseline sample tested, such testing shall be done as soon as feasible. (iv) Post-exposure prophylaxis, when medically indicated, as recommended by the U.S. Public Health Service; ..1910.1030(f)(3)(v) (v) Counseling; and (vi) Evaluation of reported illnesses. (4) Information Provided to the Healthcare Professional. (i) The employer shall ensure that the healthcare professional responsible for the employee's Hepatitis B vaccination is provided a copy of this regulation. (ii) The employer shall ensure that the healthcare professional evaluating an employee after an exposure incident is provided the following information: (A) A copy of this regulation; (B) A description of the exposed employee's duties as they relate to the exposure incident; (C) Documentation of the route(s) of exposure and circumstances under which exposure occurred; (D) Results of the source individual's blood testing, if available; and (E) All medical records relevant to the appropriate treatment of the employee including vaccination status which are the employer's responsibility to maintain. (5) Healthcare Professional's Written Opinion. The employer shall obtain and provide the employee with a copy of the evaluating healthcare professional's written opinion within 15 days of the completion of the evaluation. ..1910.1030(f)(5)(i) (i) The healthcare professional's written opinion for Hepatitis B vaccination shall be limited to whether Hepatitis B vaccination is indicated for an employee, and if the employee has received such vaccination. (ii) The healthcare professional's written opinion for post-exposure evaluation and follow-up shall be limited to the following information: (A) That the employee has been informed of the results of the evaluation; and (B) That the employee has been told about any medical conditions resulting from exposure to blood or other potentially infectious materials which require further evaluation or treatment. (iii) All other findings or diagnoses shall remain confidential and shall not be included in the written report. (6) Medical Recordkeeping. Medical records required by this standard shall be maintained in accordance with paragraph (h)(1) of this section. (g) Communication of Hazards to Employees. (1) Labels and Signs. (i) Labels. (A) Warning labels shall be affixed to containers of regulated waste, refrigerators and freezers containing blood or other potentially infectious material; and other containers used to store, transport or ship blood or other potentially infectious materials,

except as provided in paragraph (g)(1)(i)(E), (F) and (G).

..1910.1030(g)(1)(i)(B) (B) Labels required by this section shall include the following legend:

BIOHAZARD

(C) These labels shall be fluorescent orange or orange-red or predominantly so, with lettering and symbols in a contrasting color. (D) Labels shall be affixed as close as feasible to the container by string, wire, adhesive, or other method that prevents their loss or unintentional removal. (E) Red bags or red containers may be substituted for labels. (F) Containers of blood, blood components, or blood products that are labeled as to their contents and have been released for transfusion or other clinical use are exempted from the labeling requirements of paragraph (g). ..1910.1030(g)(1)(i)(G) (G) Individual containers of blood or other potentially infectious materials that are placed in a labeled container during storage, transport, shipment or disposal are exempted from the labeling requirement. (H) Labels required for contaminated equipment shall be in accordance with this paragraph and shall also state which portions of the equipment remain contaminated. (I) Regulated waste that has been decontaminated need not be labeled or color-coded. (ii) Signs. (A) The employer shall post signs at the entrance to work areas specified in paragraph (e), HIV and HBV Research Laboratory and Production Facilities, which shall bear the following legend:

BIOHAZARD

(Name of the Infectious Agent)
(Special requirements for entering the area)
(Name, telephone number of the laboratory director or other responsible person.)

..1910.1030(g)(1)(ii)(B) (B) These signs shall be fluorescent orange-red or predominantly so, with lettering and symbols in a contrasting color. (2) Information and Training. (i) Employers shall ensure that all employees with occupational exposure participate in a training program which must be provided at no cost to the employee and during working hours. (ii) Training shall be provided as follows: (A) At the time of initial assignment to tasks where occupational exposure may take place; (B) Within 90 days after the effective date of the standard; and (C) At least annually thereafter. (iii) For employees who have received training on bloodborne pathogens in the year preceding the effective date of the standard, only training with respect to

the provisions of the standard which were not included need be provided. (iv) Annual training for all employees shall be provided within one year of their previous training. (v) Employers shall provide additional training when changes such as modification of tasks or procedures or institution of new tasks or procedures affect the employee's occupational exposure. The additional training may be limited to addressing the new exposures created. ..1910.1030(g)(2)(vi) (vi) Material appropriate in content and vocabulary to educational level, literacy, and language of employees shall be used. (vii) The training program shall contain at a minimum the following elements: (A) An accessible copy of the regulatory text of this standard and an explanation of its contents; (B) A general explanation of the epidemiology and symptoms of bloodborne diseases; (C) An explanation of the modes of transmission of bloodborne pathogens; (D) An explanation of the employer's exposure control plan and the means by which the employee can obtain a copy of the written plan; (E) An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials; (F) An explanation of the use and limitations of methods that will prevent or reduce exposure including appropriate engineering controls, work practices, and personal protective equipment; ..1910.1030(g)(2)(vii)(G) (G) Information on the types, proper use, location, removal, handling, decontamination and disposal of personal protective equipment; (H) An explanation of the basis for selection of personal protective equipment; (I) Information on the hepatitis B vaccine, including information on its efficacy, safety, method of administration, the benefits of being vaccinated, and that the vaccine and vaccination will be offered free of charge; (J) Information on the appropriate actions to take and persons to contact in an emergency involving blood or other potentially infectious materials; (K) An explanation of the procedure to follow if an exposure incident occurs, including the method of reporting the incident and the medical follow-up that will be made available; (L) Information on the post-exposure evaluation and follow-up that the employer is required to provide for the employee following an exposure incident; (M) An explanation of the signs and labels and/or color coding required by paragraph (g)(1); and ..1910.1030(g)(2)(vii)(N) (N) An opportunity for interactive questions and answers with the person conducting the training session. (viii) The person conducting the training shall be knowledgeable in the subject matter covered by the elements contained in the training program as it relates to the workplace that the training will address. (ix) Additional Initial Training for Employees in HIV and HBV Laboratories and Production Facilities. Employees in HIV or HBV research laboratories and HIV or HBV production facilities shall receive the following initial training in

addition to the above training requirements. (A) The employer shall assure that employees demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the facility before being allowed to work with HIV or HBV. (B) The employer shall assure that employees have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV. (C) The employer shall provide a training program to employees who have no prior experience in handling human pathogens. Initial work activities shall not include the handling of infectious agents. A progression of work activities shall be assigned as techniques are learned and proficiency is developed. The employer shall assure that employees participate in work activities involving infectious agents only after proficiency has been demonstrated.

..1910.1030(h) (h) Recordkeeping. (1) Medical Records. (i) The employer shall establish and maintain an accurate record for each employee with occupational exposure, in accordance with 29 CFR 1910.1020. (ii) This record shall include: (A) The name and social security number of the employee; (B) A copy of the employee's hepatitis B vaccination status including the dates of all the hepatitis B vaccinations and any medical records relative to the employee's ability to receive vaccination as required by paragraph (f)(2); (C) A copy of all results of examinations, medical testing, and follow-up procedures as required by paragraph (f)(3); (D) The employer's copy of the healthcare professional's written opinion as required by paragraph (f)(5); and (E) A copy of the information provided to the healthcare professional as required by paragraphs (f)(4)(ii)(B)(C) and (D). (iii) Confidentiality. The employer shall ensure that employee medical records required by paragraph (h)(1) are: ..1910.1030(h)(1)(iii)(A) (A) Kept confidential; and (B) Not disclosed or reported without the employee's express written consent to any person within or outside the workplace except as required by this section or as may be required by law. (iv) The employer shall maintain the records required by paragraph (h) for at least the duration of employment plus 30 years in accordance with 29 CFR 1910.1020. (2) Training Records. (i) Training records shall include the following information: (A) The dates of the training sessions; (B) The contents or a summary of the training sessions; (C) The names and qualifications of persons conducting the training; and (D) The names and job titles of all persons attending the training sessions. (ii) Training records shall be maintained for 3 years from the date on which the training occurred. (3) Availability. (i) The employer shall ensure that all records required to be maintained by this section shall be made available upon request to the Assistant Secretary and the Director for examination and copying. ..1910.1030(h)(3)(ii) (ii) Employee training records required by this paragraph shall be provided upon

request for examination and copying to employees, to employee representatives, to the Director, and to the Assistant Secretary. (iii) Employee medical records required by this paragraph shall be provided upon request for examination and copying to the subject employee, to anyone having written consent of the subject employee, to the Director, and to the Assistant Secretary in accordance with 29 CFR 1910.1020. (4) Transfer of Records. (i) The employer shall comply with the requirements involving transfer of records set forth in 29 CFR 1910.1020(h). (ii) If the employer ceases to do business and there is no successor employer to receive and retain the records for the prescribed period, the employer shall notify the Director, at least three months prior to their disposal and transmit them to the Director, if required by the Director to do so, within that three month period. (i) Dates. ..1910.1030(i)(1) (1) Effective Date. The standard shall become effective on March 6, 1992. (2) The Exposure Control Plan required by paragraph (c) of this section shall be completed on or before May 5, 1992. (3) Paragraph (g)(2) Information and Training and (h) Recordkeeping shall take effect on or before June 4, 1992. (4) Paragraphs (d)(2) Engineering and Work Practice Controls, (d)(3) Personal Protective Equipment, (d)(4) Housekeeping, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, and (g)(1) Labels and Signs, shall take effect July 6, 1992. [56 FR 64004, Dec. 06, 1991, as amended at 57 FR 12717, April 13, 1992; 57 FR 29206, July 1, 1992; 61 FR 5507, Feb. 13, 1996]

APPENDIX 5 Sample Processing Checklist

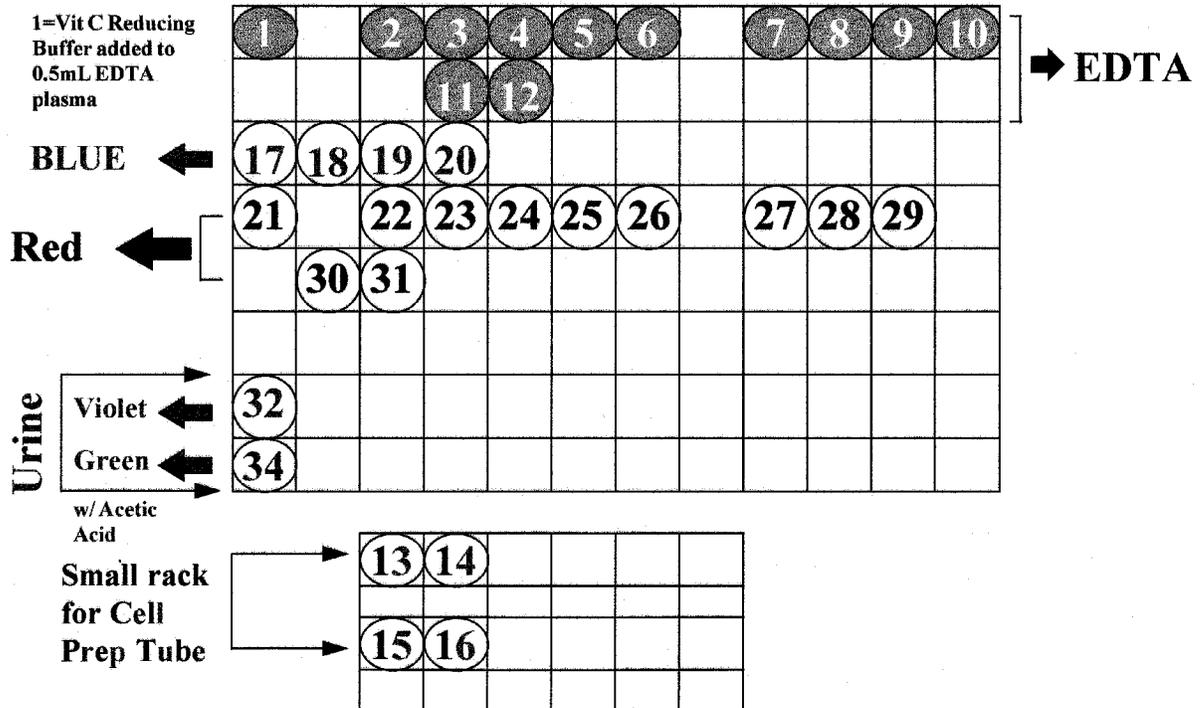
- Blind duplicate aliquot rack
- Crushed ice in ice bucket or plastic tub
- Pipets: 0.5, 1.0, and 2.0 volumes and pipet tips
- Disposable pipets or graduated cylinders capable of measuring 20 mL of urine
- Pasteur pipets
- Prepared reagents (PBS, Freezing Media A & B, Vit C Solution, Platelet Solution, 1 M Acetic Acid)
- Labeled cryovials in rack
- Lab coat and gloves
- Biohazardous waste disposal
- Refrigerated centrifuge capable of spinning at 30,000 g-minutes
- Room-temperature centrifuge
- 15 mL conical centrifuge tubes
- Tubes for pooling sample
- Balance tubes for the centrifuge
- 10% bleach solution
- Styrofoam container for freezing cell cryovials
- Freezer boxes with 9 x 9 grid
- Rubber bands

APPENDIX 6 Aliquot Rack Setup

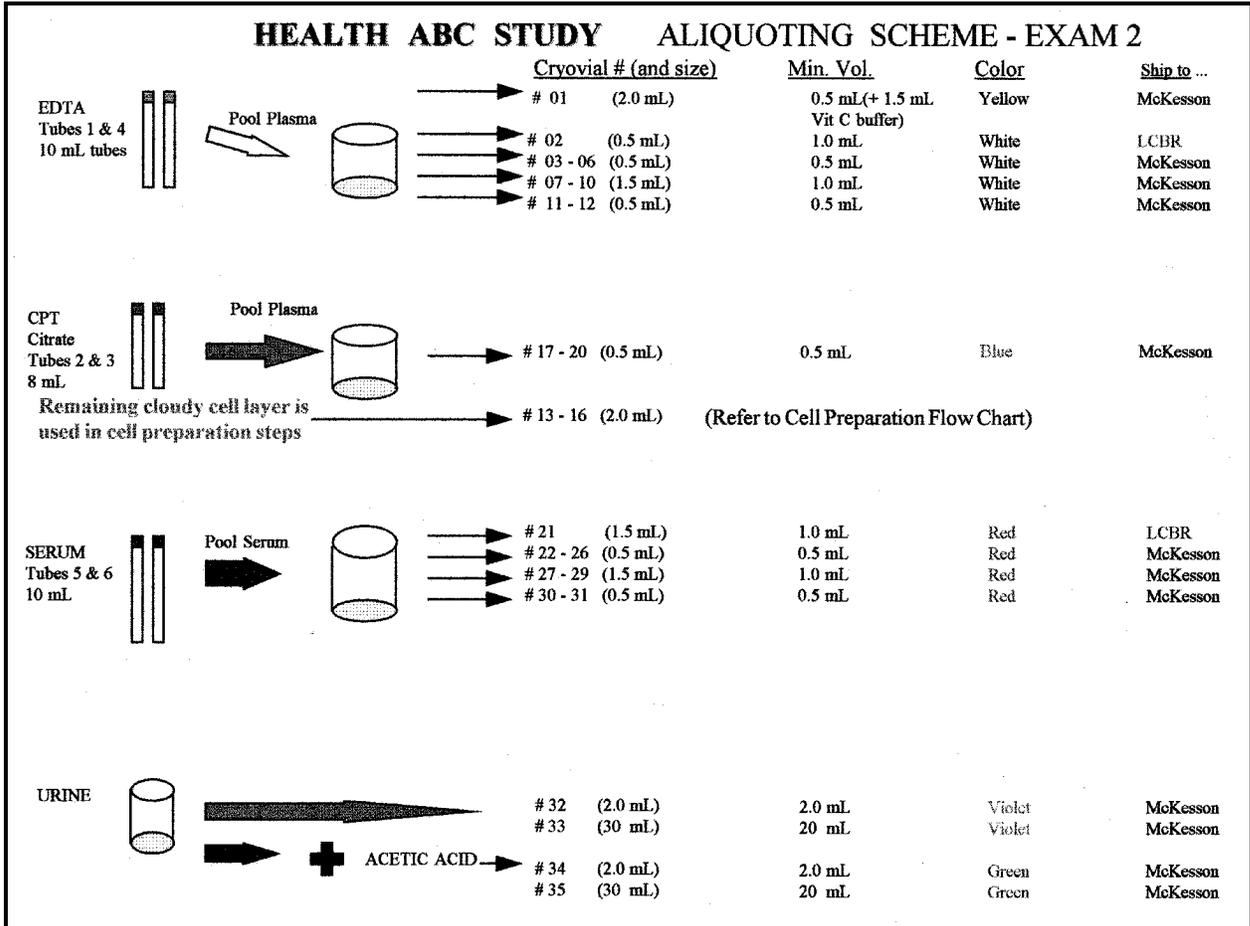
HEALTH ABC STUDY

Aliquot Rack Setup - EXAM 2

Rack should be kept on ice while aliquotting samples

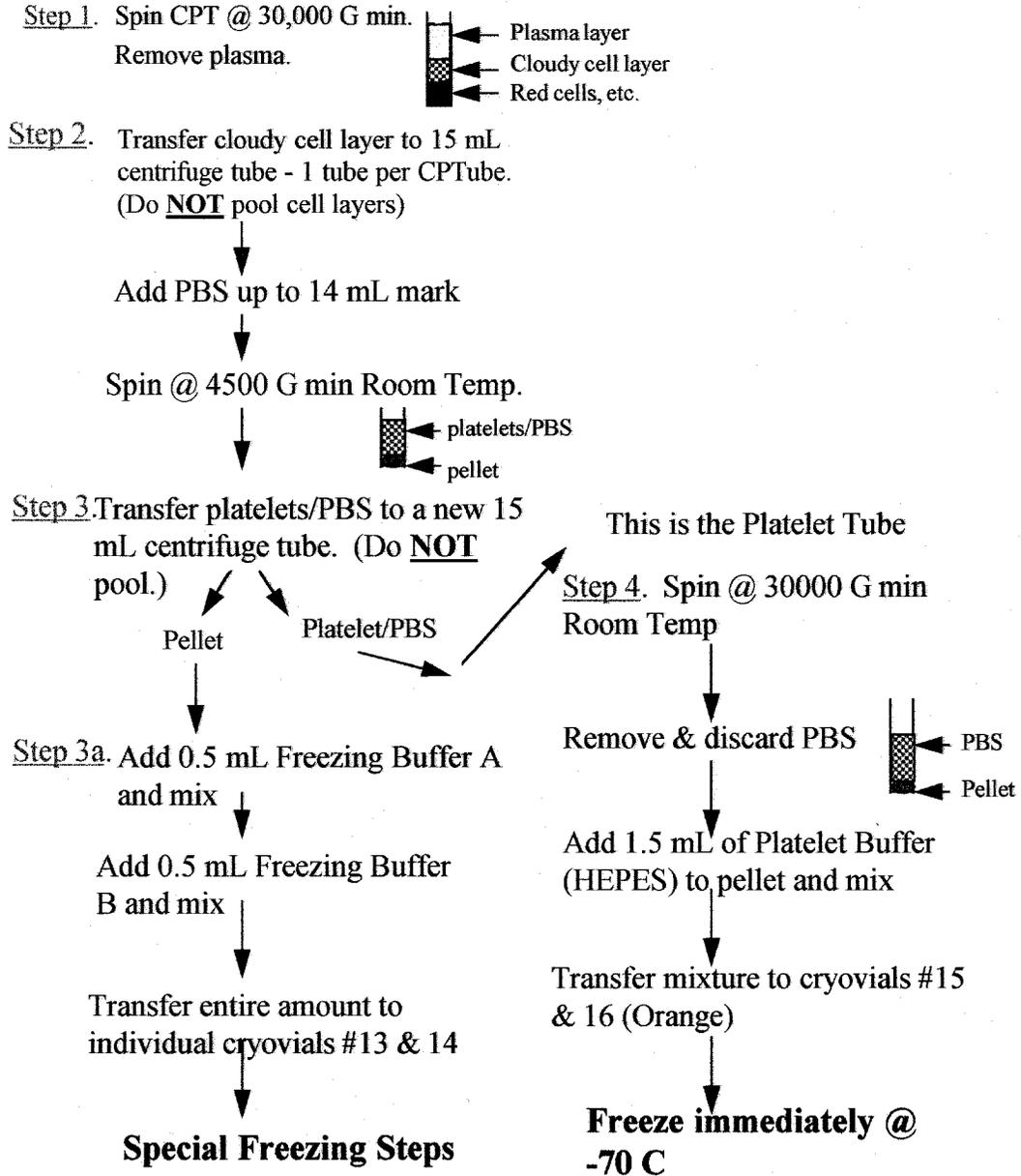


APPENDIX 7 Flow Chart/Aliquoting Scheme



Cell Preparation Flow Chart

HEALTH ABC STUDY - Exam 2
Cell Preparation from CPTubes (#2&3)



APPENDIX 8 Example of Blind Duplicate ID Log Page

bd ID Form
label from
blind duplicate
label set



	Sample ID Bar Code #		Sample ID Bar Code #
aliquot 1		aliquot 21	
aliquot 2		aliquot 22	
aliquot 3		aliquot 23	
aliquot 4		aliquot 24	
aliquot 5		aliquot 25	
aliquot 6		aliquot 26	
aliquot 7		aliquot 27	
aliquot 8		aliquot 28	
aliquot 9		aliquot 29	
aliquot 10		aliquot 30	
aliquot 11		aliquot 31	
aliquot 12		aliquot 32	
aliquot 17		aliquot 33	
aliquot 18		aliquot 34	
aliquot 19		aliquot 35	
aliquot 20			

APPENDIX 9 Freezer Box Diagrams
(page 1 of 5)

**Freezer Box Diagram for Shipping Plasma and Serum Samples
to McKesson Bioservices**

Numbers = cryovial #

Complete sets of cryovials available for these four participants. 29 total blood sample cryovials per participant / 25 are sent to McKesson Bioservices for -70° storage, 2 for -145° storage (separate box).

start #1

Top

Ppt #1 01	03	04	05	06	07	08	09	10
11	12	15	16	17	18	19	20	22
23	24	25	26	27	28	29	30	31
Ppt #2 01	03	04	05	06	07	08	09	10
11	12	15	16	17	18	19	20	22
23	24	25	26	27	28	29	30	31
Ppt #3 01	03	04	05	06	07	08	09	10
11	12	15	16	17	18	19	20	22
23	24	25	26	27	28	29	30	31

Bottom

End#81

Label outside of box: Health ABC Plasma/Serum Box #1 Date: ___ / ___ / ___

APPENDIX 9
Freezer Box Diagrams
(page 3 of 5)

Freezer Box Diagram for Shipping Larger Urine Bottles to McKesson

Numbers = bottle #

start #1 ^

Top

Ppt #1 33 → 35		Ppt #2 33
35	Ppt #3 33 → 35	35
Ppt #4 33 → 35		Ppt #5 33

Bottom

End#9

continue to
next box....

Label outside of box: Health ABC Urine Bottles #1 Date: ____ / ____ / ____

APPENDIX 9
Freezer Box Diagrams
(page 4 of 5)

Freezer Box Diagram for Shipping Buffy Coat Samples to McKesson

Note: this box must be clearly labeled “Freeze at -145°”

Numbers = cryovial #

start #1 ^

Top

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

Bottom

End#81
continue to
next box....

Label outside of box: Health ABC Buffy Coat Box #1 Date: ____ / ____ / ____
Freeze at -145°C.

APPENDIX 9
Freezer Box Diagrams
(page 5 of 5)

Freezer Box Diagram for Shipping Plasma/Serum Samples to LCBR/Vermont

Numbers = cryovial #

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

Bottom

End#81
continue to
next box....

Label outside of box: Health ABC Plasma/Serum Box #1 Date: ___ / ___ / ___
TO LCBR

APPENDIX 10 Sample Shipping Checklist

- Styrofoam Mailing Container (2 different sizes)
- with outer cardboard sleeve
- Polyfoam Packers # 430*
- Polyfoam Packers # 346*
- Absorbent material
- Freezer boxes with 9x9 grids (rubber bands around box)
- Leakproof Zip-lock bags
- Packaging tape
- Dry ice (approximately 20 lbs. per week)
- FedEx Labels (provided by carrier)
- Copies of Completed Phlebotomy/Processing Forms

Shipping materials can be purchased from:

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

Ice Packs:

VWR 1-800-234-5277

Cat. No., 14715-105 U-TEK Reusable Refrigerant Packs

FedEx airbills and airbill pouches:

Local FedEx office

Class 9 labels:

Local FedEx office

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

APPENDIX 11 Federal Express Airbill for Dry Ice Shipment

FedEx USA Airbill Tracking Number **9260216991**

1 From (please print)
Date _____ Sender's FedEx Account Number _____
Sender's Name _____ Phone (____) _____
Company _____ Dept./Floor/Suite/Room _____
Address _____
City _____ State _____ Zip _____

2 Your Internal Billing Reference Information
(Optional) (Max 26 characters will appear on invoice)

3 To (please print)
Recipient's Name **SUSAN GREENHUT** Phone **801 309-3667**
Company **MCKESSON BIOSERVICES**
Address **685 LOPSTRAND LANE**
(To "HOLD" at FedEx location, print FedEx address here) (We Cannot Deliver to P.O. Boxes or P.O. Zip Codes)
City **ROCKVILLE** State **MD** Zip **20850**

For HOLD at FedEx Location check here
 Hold Weekday (Not available with FedEx First Overnight or FedEx Standard Overnight)
 Hold Saturday (Not available at all locations) (Not available with FedEx First Overnight or FedEx Standard Overnight)

For Saturday Delivery check here
 (Extra Charge. Not available at all locations) (Not available with FedEx First Overnight or FedEx Standard Overnight)

Service Conditions, Declared Value, and Limit of Liability - By using this Airbill, you agree to the service conditions in our current Service Guide or U.S. Government Service Guide. Both are available on request. See back of Sender's Copy of this airbill for information and additional terms. We will not be responsible for any claim in excess of \$100 per package whether the result of loss, damage, or delay, non-delivery, misdelivery, or misrouting, unless you declare a higher value, pay an additional charge, and document your actual loss in a timely manner. Your right to recover from us for any loss includes intrinsic value of the package, loss of sales, interest, profit, attorney's fees, costs, and other forms of damage, whether direct, incidental, consequential, or special, and is limited to the greater of \$100 or the declared value but cannot exceed actual documented loss. The maximum declared value for any FedEx Letter and FedEx Pak is \$500. Federal Express may, upon your request, and with some limitations, refund all transportation charges paid. See the FedEx Service Guide for further details.

Questions?
Call 1-800-Go-FedEx (1-800-463-3339)

The World On Time

Sender's Copy

4 Service Delivery commitment may be later in some areas.
 FedEx Priority Overnight (Next business morning) FedEx Standard Overnight (Second business day)
 FedEx Govt. Overnight (Authorized user only)
 FedEx Overnight Freight FedEx 2Day Freight
(For packages over 150 pounds. Call for delivery schedule.)
 NEW FedEx First Overnight (FedEx first business morning delivery to select locations) (Higher rates apply) *FedEx Letter Rate not available. Minimum charge: One pound FedEx 2Day rate.

5 Packaging
 FedEx Letter* FedEx Pak* FedEx Box FedEx Tube Other Packaging
(Declared value limit \$500)

6 Special Handling
Does this shipment contain dangerous goods? Yes (See attached Shipper's Declaration) Yes (Shipper's Declaration not required)
 Dry Ice (Dry Ice, 9, UN 1845) (Shipper's Declaration not required) CA Cargo Aircraft Only

7 Payment
Bill to: Sender (Account no. in section 1 will be billed) Recipient Third Party Credit Card Cash/Check
(Enter FedEx account no. or Credit Card no. below)

FedEx Account No. _____ Exp. Date _____
Credit Card No. _____

Total Packages	Total Weight	Total Declared Value*	Total Charges*
		\$.00	\$.00

*When declaring a value higher than \$100 per package, you pay an additional charge. See SERVICE CONDITIONS, DECLARED VALUE AND LIMIT OF LIABILITY section for further information.

8 Release Signature
Your signature authorizes Federal Express to deliver this shipment without obtaining a signature and agrees to indemnify and hold harmless Federal Express from any resulting claims.
FORM ID NO. 0200
Rev. Date 10/95 • PART # 147382
©1994-95 FedEx • PRINTED IN U.S.A.
GBFE 1/06

FedEx USA Airbill Tracking Number **9260217002**

1 From (please print)
Date _____ Sender's FedEx Account Number _____
Sender's Name _____ Phone (____) _____
Company _____ Dept./Floor/Suite/Room _____
Address _____
City _____ State _____ Zip _____

2 Your Internal Billing Reference Information
(Optional) (Max 26 characters will appear on invoice)

3 To (please print)
Recipient's Name **ELAINE CORNELL** Phone **(802) 656-8963**
Company **UNIVERSITY OF VERMONT - PATHOLOGY**
Address **55A SOUTH PARK DR**
(To "HOLD" at FedEx location, print FedEx address here) (We Cannot Deliver to P.O. Boxes or P.O. Zip Codes)
City **COLCHESTER** State **VT** Zip **05446**

For HOLD at FedEx Location check here
 Hold Weekday (Not available with FedEx First Overnight or FedEx Standard Overnight)
 Hold Saturday (Not available at all locations) (Not available with FedEx First Overnight or FedEx Standard Overnight)

For Saturday Delivery check here
 (Extra Charge. Not available at all locations) (Not available with FedEx First Overnight or FedEx Standard Overnight)

Service Conditions, Declared Value, and Limit of Liability - By using this Airbill, you agree to the service conditions in our current Service Guide or U.S. Government Service Guide. Both are available on request. See back of Sender's Copy of this airbill for information and additional terms. We will not be responsible for any claim in excess of \$100 per package whether the result of loss, damage, or delay, non-delivery, misdelivery, or misrouting, unless you declare a higher value, pay an additional charge, and document your actual loss in a timely manner. Your right to recover from us for any loss includes intrinsic value of the package, loss of sales, interest, profit, attorney's fees, costs, and other forms of damage, whether direct, incidental, consequential, or special, and is limited to the greater of \$100 or the declared value but cannot exceed actual documented loss. The maximum declared value for any FedEx Letter and FedEx Pak is \$500. Federal Express may, upon your request, and with some limitations, refund all transportation charges paid. See the FedEx Service Guide for further details.

Questions?
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The World On Time

Sender's Copy

4 Service Delivery commitment may be later in some areas.
 FedEx Priority Overnight (Next business morning) FedEx Standard Overnight (Second business day)
 FedEx Govt. Overnight (Authorized user only)
 FedEx Overnight Freight FedEx 2Day Freight
(For packages over 150 pounds. Call for delivery schedule.)
 NEW FedEx First Overnight (FedEx first business morning delivery to select locations) (Higher rates apply) *FedEx Letter Rate not available. Minimum charge: One pound FedEx 2Day rate.

5 Packaging
 FedEx Letter* FedEx Pak* FedEx Box FedEx Tube Other Packaging
(Declared value limit \$500)

6 Special Handling
Does this shipment contain dangerous goods? Yes (See attached Shipper's Declaration) Yes (Shipper's Declaration not required)
 Dry Ice (Dry Ice, 9, UN 1845) (Shipper's Declaration not required) CA Cargo Aircraft Only

7 Payment
Bill to: Sender (Account no. in section 1 will be billed) Recipient Third Party Credit Card Cash/Check
(Enter FedEx account no. or Credit Card no. below)

FedEx Account No. **1531-6949-7** Exp. Date _____
Credit Card No. **(5-26713)**

Total Packages	Total Weight	Total Declared Value*	Total Charges*
		\$.00	\$.00

*When declaring a value higher than \$100 per package, you pay an additional charge. See SERVICE CONDITIONS, DECLARED VALUE AND LIMIT OF LIABILITY section for further information.

8 Release Signature
Your signature authorizes Federal Express to deliver this shipment without obtaining a signature and agrees to indemnify and hold harmless Federal Express from any resulting claims.
FORM ID NO. 0200
Rev. Date 10/95 • PART # 147382
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GBFE 1/06

APPENDIX 12 Dry Ice Label and Labeling Diagram
(page 1 of 2)

Shipper's Declaration not Required.

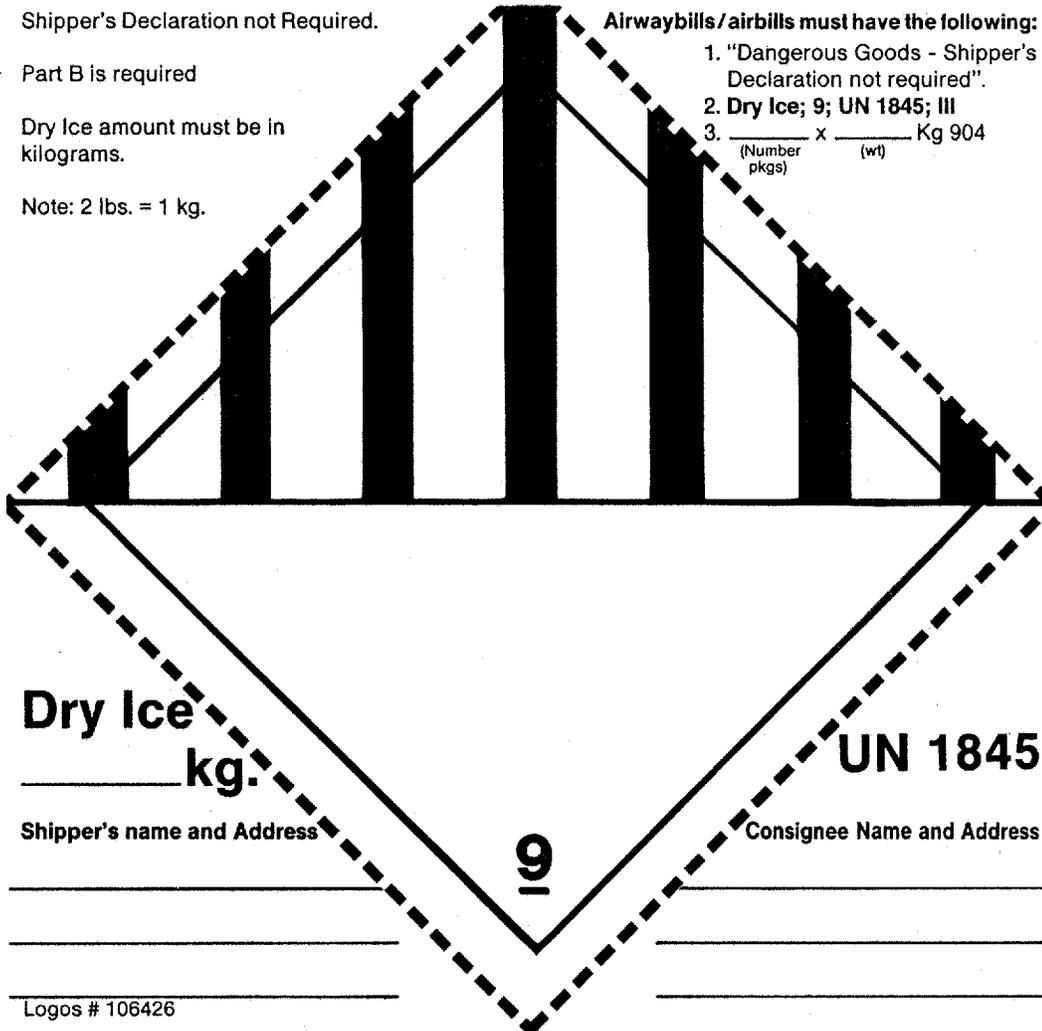
Part B is required

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills/airbills must have the following:

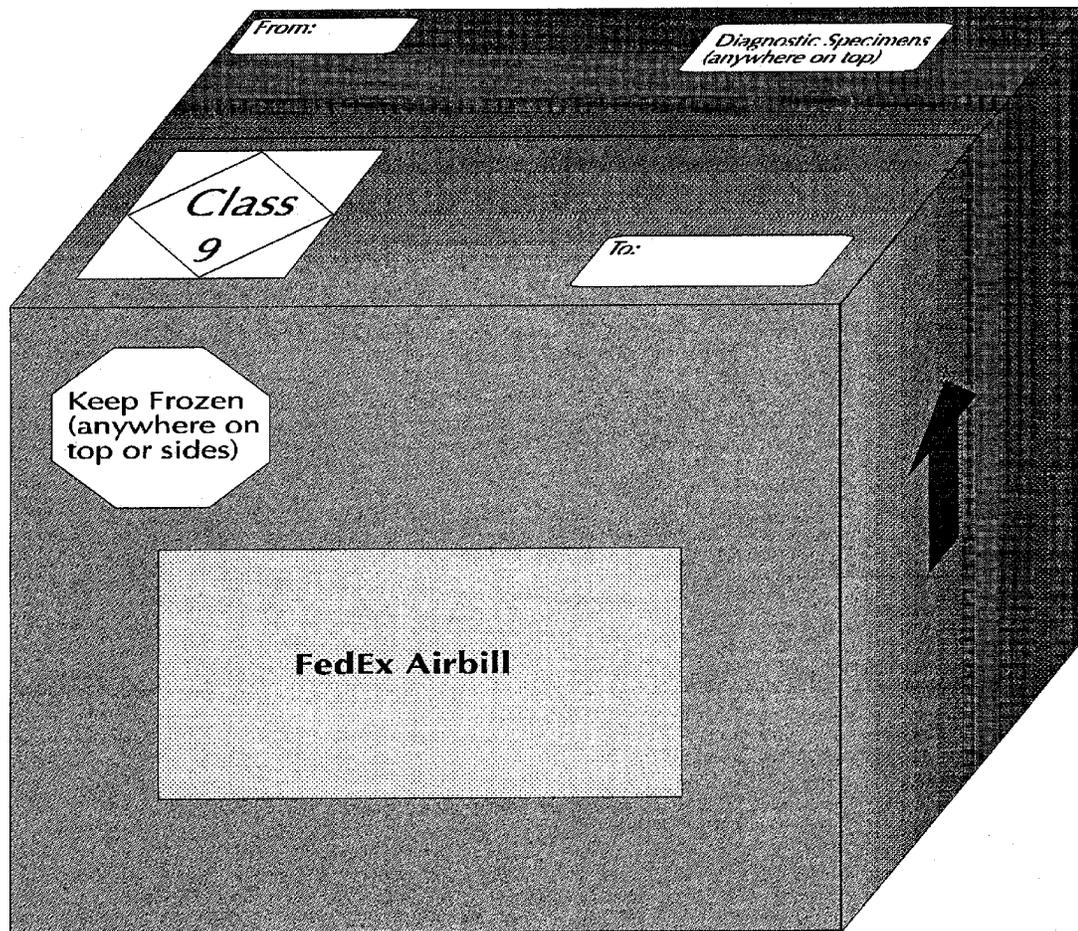
1. "Dangerous Goods - Shipper's Declaration not required".
2. Dry Ice; 9; UN 1845; III
3. $\frac{\text{Number}}{\text{pkgs}}$ x $\frac{\text{wt}}{\text{kg}}$ Kg 904



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

APPENDIX 12
Dry Ice and Labeling Diagram
(page 2 of 2)

Outer Box Labeling



NOTE: Labels must not overlap

APPENDIX 13 Blind Duplicate ID Form
(page 1 of 2)



Date Form Completed			Staff ID#	Attach BDID label from Blind Duplicate Label Sheet
<input type="text"/>				
Month	Day	Year		

**YEAR 2 BLIND DUPLICATE
IDENTIFICATION FORM**

Attach BDID labels from Participants' Label Sheets in appropriate spaces below.

Vitamin C	EDTA plasma	EDTA plasma	No aliquot 13-16 duplicates	Citratd plasma
01	05	09		17
EDTA plasma	06	10		18
02	07	11		19
03	08	12		20
04				

Page Link #

Draft



APPENDIX 13
Blind Duplicate ID Form
(page 2 of 2)



Date Form Completed			Staff ID#		Attach BDID label from Blind Duplicate Label Sheet
<input type="text"/>	/	<input type="text"/>	/	<input type="text"/>	<input type="text"/>
Month		Day		Year	

**YEAR 2 BLIND DUPLICATE
IDENTIFICATION FORM**

Attach BDID labels from Participants' Label Sheets in appropriate spaces below.

Serum	Serum	Serum	Urine
21	25	29	32
22	26	30	33
23	27	31	(acidified) 34
24	28		35

Page Link #

◆Page 2◆

Version 1.0, 6/24/98

Draft



APPENDIX 14 Return Visit Laboratory Forms
(page 1 of 2)



HABC Enrollment ID #	Acrostic	Date Form Completed	Staff ID #
H <input type="text"/>	<input type="text"/>	<input type="text"/> / <input type="text"/> / <input type="text"/> <small>Month Day Year</small>	<input type="text"/>

**YEAR 2 RETURN VISIT
PHLEBOTOMY**

Bar Code Label

1 Do you bleed or bruise easily?

Yes No Don't know

2 Have you ever experienced fainting spells while having blood drawn?

Yes No Don't know

3 Time at start of venipuncture?

: am pm
Hours Minutes

a. Was any blood drawn?

Yes No

↓

Please describe why not?

4 Time blood draw completed:

: am pm
Hours Minutes

5 Total tourniquet time:
(If tourniquet was reapplied, enter total time tourniquet was on. Note that 2 minutes is optimum.)

minutes

Comments on phlebotomy:

6 Quality of venipuncture:

Clean Traumatic

↓

<input type="checkbox"/> Vein collapse	<input type="checkbox"/> Excessive duration of draw
<input type="checkbox"/> Hematoma	<input type="checkbox"/> Leakage at venipuncture site
<input type="checkbox"/> Vein hard to get	<input type="checkbox"/> Other <i>(Please specify):</i>
<input type="checkbox"/> Multiple sticks	_____

7 Were tubes filled to specified capacity?
If not, comment why.

Blood Volume/Tube	Filled to Capacity?	Filled to Capacity?		Comment
		Yes	No	
1. EDTA 10 ml	<input type="checkbox"/>	<input type="checkbox"/>	→	_____
2. CPT 8 ml	<input type="checkbox"/>	<input type="checkbox"/>	→	_____
3. CPT 8 ml	<input type="checkbox"/>	<input type="checkbox"/>	→	_____
4. EDTA 10 ml	<input type="checkbox"/>	<input type="checkbox"/>	→	_____
5. Serum 10 ml	<input type="checkbox"/>	<input type="checkbox"/>	→	_____
6. Serum 10 ml	<input type="checkbox"/>	<input type="checkbox"/>	→	_____
Urine:				
1. Urine (50-60 ml)	<input type="checkbox"/>	<input type="checkbox"/>	→	_____

8 What is the date and time you last ate or drank anything except water?

a. Date of last meal:

/ /
Month Day Year

b. Time of last meal:

: am pm
Hours Minutes

c. How many hours has participant fasted?
(Question 4 minus Question 8b. Round to nearest hour)

hours

4047

LCBR Use only: Received Date: _____ Time: _____

Frozen? Yes No *Version 1.1, 7/30/98*

APPENDIX 14
Return Visit Laboratory Forms
(page 2 of 2)



HABC Enrollment ID #	Acrostic	Date Form Completed	Staff ID #
H <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> / <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
		Month Day Year	

**YEAR 2 RETURN VISIT
LABORATORY PROCESSING**

Bar Code Label

Time at start of processing: : am pm

Collection Tubes	Cryo #	Vol.	Type	To	Check "X"	Problems	Collection Tubes	Cryo #	Vol.	Type	To	Check "X"	Problems
#1, 4 Vitamin C	01	0.5	Y/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	#2, 3 Citrate	17	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
#1, 4 EDTA	02	0.5	W/0.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		18	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	03	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		19	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	04	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		20	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	05	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	#5, 6 Serum	21	1.0	R/1.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	06	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		22	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	07	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		23	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	08	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		24	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	09	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		25	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	10	1.0	W/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		26	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	11	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		27	1.0	R/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	12	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		28	1.0	R/1.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	#2, 3 Buffy Refused DNA collection	13	var	C/2.0	M*	<input type="checkbox"/>		<input type="checkbox"/> H <input type="checkbox"/> P	29	1.0	R/1.5	M	<input type="checkbox"/>
14		var	C/2.0	M*	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		30	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
#2, 3 Platelets	15	var	O/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		31	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	16	var	O/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	URINE	32	2.0	V/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> P
						33		20	V/20	M	<input type="checkbox"/>	<input type="checkbox"/> P	
						(acidified)		34	2.0	G/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> P
							35	20	G/20	M	<input type="checkbox"/>	<input type="checkbox"/> P	

L=LCBR; M=McKesson; H=Hemolyzed; P=Partial; W=white; C=clear; Y=Vitamin C; B=blue; R=red; V=violet G=green, O=Orange

*Place in a styrofoam box at -20°C for 2 hours. Transfer to -80°C to hold for shipping.

3881

Version 1.1, 7/30/98

