

LAB SPECIMEN PROCESSING

Prepared by the Laboratory for Clinical Biochemistry Research (LCBR) at the University of Vermont

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1. Background and Rationale

The Health ABC study involves the collection of approximately 75 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 centrifuge capable of spinning at 30,000 g-minutes
- -70° Freezer
- Pipets and tips: 50 µL and 0.5, 1.0, and 2.0 mL volumes
- Pasteur pipets
- Lab coat and gloves
- Biohazardous waste disposal container
- 15 and 20 mL tubes for pooling sample
- 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 49) 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 volume to be aliquoted (0.5 mL or 1.0 mL for blood products, 2.0 mL for urine).

There are also 15 labels containing the ID number with no extension. Nine are to be used for pre-labeling the 9 draw tubes, with 3 extras for backup vacutainers. There is a label for the urine specimen cup and two labels for the tubes you will use to pool the EDTA-treated plasma samples and the serum samples. 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 called “Phlebotomy Form,” which is placed on the Phlebotomy Form (see Blood and Urine Collection chapter) , and the other called “Laboratory Processing Form,” which is placed on the Laboratory Processing Form (Appendix 3). *This process of matching the participant-specific 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.

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 13 labels left: 3 "Backup Vacutainer" labels, 1 "Phlebotomy Form," 1 "Laboratory Processing Form" label, 2 labels for the tubes used to pool plasma and serum, and 6 "BDID Form" labels. These can be separated into 2 mini-sheets: The "Backup Vacutainer" and "Phlebotomy 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 8, 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 8, 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 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 requirements. 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.

NOTE: Tubes #9 and #10 are used to collect extra blood as needed for the study. The amount taken can be 5 mL for tube #9 and #10, or 10 mL of just #9 or 10 mL of just #10, depending upon shortfalls of serum or plasma.

Tubes 1, 5, and 6 are 10 mL siliconized red stoppered tubes. Tube 9 is a 10 mL or a 5 mL siliconized red stoppered tube. 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 glucose, insulin, albumin, alkaline phosphatase, and creatinine.

Tubes 2 and 7 are 10 mL lavender stoppered tubes containing a 15% EDTA solution as the anticoagulant. Tube 10 is either a 10 mL or 5 mL lavender stoppered tube containing a 15% EDTA solution. The plasma is aliquoted into white-capped cryovials. The plasma will be used for cholesterol, and the buffy coat is collected for DNA analysis into a clear-capped 2.0 mL cryovial. Red cells (2.0 mL volume) are also collected in a white-capped cryovial.

Tube 3 is a 5 mL red stoppered "Special Coagulation" /SCAT-I tube provided by LCBR. It contains a special combination of anticoagulants that ensure long-term stability of the plasma sample. Specifically, this tube, when filled, will contain 4.5 NM EDTA, 150 KIU/mL aprotinin, and 20 μ M D-Phe-Pro-Arg-chloroketone. Aliquots for this tube are coded yellow. NOTE: This tube contains a white powdered anticoagulant, *and it must be stored refrigerated until used.* (It may be left out overnight after labeling, but should be re-refrigerated if not used the next day).

Tube 4 is a 4.5 mL blue stoppered tube containing 0.5 mL of 3.8% sodium citrate. Plasma from this tube is aliquoted into blue-capped cryovials and will be stored in repository.

Tube 8 is a 5 mL siliconized, red stoppered tube used for the 2-Hr OGTT collection. Aliquots are coded orange. The serum is used for 2-Hr glucose measurements. ***Since this tube will be separated from the rest of the draw tubes after the fasting phlebotomy, double check that you are aliquoting it into the cryovial with the matching sample ID.***

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 #1, 5, 6, (and 9). The ice bath should contain 4 (or 5) tubes: #2, 3, 4, 7 (and 10). If processing is delayed, keep tubes #2, 3, 4, 7 (and 10) on ice. The aliquot rack containing cryovials with the matching sample ID labels should be placed in an ice bath.

Tubes #1, 5, 6, (and 9) 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.

All other tubes (#2, 3, 4, 7 [and 10]) must be processed within 15 minutes of the completion of phlebotomy. Note the time that processing started on the Laboratory Processing Form.

5.1.4 Aliquots per Sample Type: The following is a summary of the processing. Detailed instructions follow.

Serum: The serum from the four collection tubes (#1, 5, 6 [& 9]) is pooled into one 20 mL tube before aliquoting.
The total number of aliquots is: 21 (Color code = red)
15 x 0.5 mL
6 x 1.0 mL

EDTA: One 0.5 mL aliquot of whole blood is removed from tube #2 before centrifuging. *This blood must not be frozen* and will be shipped separately to LCBR twice a week.
1 x 0.5 mL (Color code = white)

The rest of the blood in tubes #2, 7 (and 10) is centrifuged and the EDTA plasma is pooled into one 15 mL (or larger) tube before aliquoting
The total number of aliquots is: 13 (Color code = white)
8 x 0.5 mL
5 x 1.0 mL

Buffy coats:	Buffy coats are removed from both tubes (#2 & 7) and placed into one 2.0 mL cryovial 1 x 2.0 mL (Color code = clear).
Red cells:	Red cells are removed from one tube (#2 or 7) and placed into one 2.0 mL cryovial 1 x 2.0 mL (Color code = white)
SCAT-I:	The plasma from the SCAT-I tubes (tube #3) is aliquoted after centrifugation. The total number of aliquots is: 4 4 x 0.5 mL (Color code = yellow)
Citrate:	The citrated plasma is aliquoted from tube #4. The total number of aliquots is: 4 (Color code = blue) 4 x 0.5 mL
OGTT Serum:	The serum from the 2-Hr post-OGTT collection tube (#8) is aliquoted from the tube. The total number of aliquots is: 1 (Color code = orange) 1 x 0.5 mL
Urine:	The urine is aliquoted into cryovials, one of which contains acetic acid. The total number of aliquots is: 3 2 x 2.0 mL (Color code = violet) 1 x 2.0 mL with added 50 μ L acetic acid (Color code = green)

The total number of aliquots from blood per participant is 46. A detailed listing of aliquots can be found on the Laboratory Processing form.

5.1.5 Stage 1 First Run - Centrifugation of Plasma Samples

A 0.5 mL aliquot of whole blood is removed from tube #2 and placed in cryovial 01 (white cap). If you will be making a blind duplicate aliquot 01 (see Making Blind Duplicates, below), a second 0.5 mL of whole blood should also be reserved for that. The remaining blood in tubes #2, 3, 4, 7 (and 10) is centrifuged at 4° C for 10 minutes at 3000 G. (A total of 30,000 g-minutes)

Once centrifuged, the maximum time allowed before aliquoting 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 (#2, 3, 4, 7 [and 10]) on ice until aliquoting can occur.

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 Stage 1A Making Urine Aliquots

LCBR will provide a stock supply of 1 M acetic acid to the sites. Before urine is added to the green cryovial, pipet 50 μ L (0.05 mL) of acetic acid into the cryovial first.

- Pipet 2.0 mL of urine into two violet-capped cryovials 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
- Discard the remaining urine (unless blind duplicate sample is needed, see below)

5.1.7 Stage 2 Making Plasma Aliquots

NOTE: aliquot 26 should be omitted for diabetics taking insulin. If it is determined that the participant is taking insulin, the phlebotomist will put an X in the N/A box next to aliquot 26. Discard cryovial 26 without filling it.

- 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.
- Follow the outline on the Laboratory Processing form for aliquoting the samples. Note that all the EDTA plasma is pooled into one temporary tube before aliquoting. 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.
- Use a new pipet tip for each sample type.
- Be careful not to disturb the red or white cell layers. Avoid having red cells in the plasma/serum aliquots. If plasma volume is lower than expected, make fewer aliquots rather than disturb the red cells or buffy coat.
- Recap aliquots after each sample tube has been pipetted.

5.1.7.1 Buffy Coat Aliquot The buffy coat aliquot is obtained from tubes 2 and 7. After the plasma is removed, use a Pasteur pipet to suction off the white cell layer. It is okay to include red cells in this cryovial. The main focus should be to obtain enough volume

of white cells. The volume will vary (approximately 200-400 μ L). The white cells from both tubes are placed into one clear-coded 2.0 mL cryovial.

5.1.7.2 Red Cell Aliquot Remove approximately 2.0 mL of the packed red cells from either of the EDTA tubes after the buffy coat is removed. Place in a white color-coded 2.0 mL cryovial.

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

EDTA (Tubes 2, 7 [& 10]): Color coded White

Pool plasma from both tubes before aliquoting: Pipet plasma from tubes using a 2-5 mL pipet, *without disturbing the cell layer and without tilting the tubes.*

Aliquots:	1 x 1.0 mL plasma	use 2.0 mL cryovial	LCBR
	3 x 0.5 mL plasma	use 0.5 mL cryovial	McKesson
	4 x 1.0 mL plasma	use 2.0 mL cryovial	McKesson
	5 x 0.5 mL plasma	use 0.5 mL cryovial	McKesson

Buffy Coat: Color coded Clear

Combine cells from both EDTA tubes (Tubes 2 & 7) . Total volume will vary

Aliquot:	1 x buffy coat	use 2.0 mL cryovial	McKesson
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Red Cells: Color coded White

From either or both EDTA tubes (Tubes 2 & 7)

Aliquot:	1 x 2.0 mL cells	use 2.0 mL cryovial	McKesson
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SCAT-I (Tube 3): Color coded Yellow

Aliquots:	4 x 0.5 mL plasma	use 0.5 mL cryovial	McKesson
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Citrate (Tube 4): Color coded Blue

Aliquots:	4 x 0.5 mL plasma	use 0.5 mL cryovial	McKesson
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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.

Aliquots can remain on ice in the rack for up to 30 minutes before freezing at -70° C (or colder).

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

5.1.8 Stage 3 Second Run - Centrifugation of Serum Samples

Note: this procedure will also be used for tube #8.

Tubes #1, 5, 6, (and 9) should be displaying a clot by this time. They are centrifuged at 4° C for 10 minutes at 3000 G. A 10 mL balance tube filled with water is necessary.

5.1.9 Stage 4 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 all three 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.

Serum (Tubes #1, 5, 6, [and 9]) Color coded Red

Pool serum from all tubes before aliquoting

Aliquots:	2 x 0.5 mL serum	use 0.5 mL cryovial	LCBR
	7 x 0.5 mL serum	use 0.5 mL cryovial	McKesson
	6 x 1.0 mL serum	use 2.0 mL cryovial	McKesson
	6 x 0.5 mL serum	use 0.5 mL cryovial	McKesson

2-Hr OGTT Serum (Tube #8) Color coded Orange

Aliquot:	1 x 0.5 mL serum	use 0.5 mL cryovial	LCBR
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NOTE: The phlebotomist will put an X in the N/A box next to aliquot 46 if the participant was not administered the OGTT. A 2-hour draw will not be done. Discard cryovial 46 without filling it.

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.

5.1.10 Return Visit Aliquots

Occasionally, participants return to the clinic just to give a urine sample or have a fasting blood draw and oral glucose tolerance test. There are new forms that must be filled out for return visits: the Return Visit Phlebotomy Form, the Return Visit Oral Glucose Tolerance Form, and the Return Visit Lab Processing Form (see Appendix 15). 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 draw tube labels for Draw Tube 1, 2, and 8; and the urine specimen cup label for the urine specimen. Use the cryovial labels for blood (for cryovials 01, 02, 25, 26, and 46) and urine cryovials (47, 48, and 49) as usual. Be sure to fill out all three forms with the header information including the Health ABC ID #, Acrostic, Date Form Completed, and Staff ID #.

5.1.11 Stage 5 Making Blind Duplicates (if applicable)

Be sure you have read Section 8, 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 02 to 14 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 cryovial label). 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 *bound* (not loose-leaf) notebook with each page devoted to a separate blind duplicate ID number (see Appendix 8 for example).

For example (see figure on next page), 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 prelabeled cryovial 06 (#432890-06), 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, as the entire sample is to be stored for each participant. Thus, you will not be preparing blind duplicates of aliquot 15. There will, however, be plenty of red cells, so you can prepare a blind duplicate aliquot 16. There will also always be plenty of extra urine. You may prepare all three blind duplicate urine aliquots from one participant's urine if you wish, however it should be noted that there are only 6 BDID Form labels per participant.

To prepare a blind duplicate aliquot 01 (whole blood), you will have to use your judgment. If a participant appears to have plenty of volume in tubes 2 & 7 and you have not yet filled aliquot 01 from your blind duplicate set, aliquot two 0.5 mL samples before centrifuging. One will be the regular aliquot 01 for the participant, the other will be used to fill the blind duplicate aliquot 01. To avoid accidentally freezing the blind duplicate whole blood, it is recommended that you place the blind duplicate aliquot 01 right next to the participant's aliquot 01 in the participant's aliquot rack. When you remove the participant's aliquot 01 to the refrigerator, you are more likely to notice the extra aliquot 01 and remove it, too.

Take care to note whether removing two whole blood aliquots usually results in inability to fill out the aliquots of EDTA-treated plasma for the participant. If so, notify your supervisor. A change to a larger draw tube may be necessary when making future whole blood blind duplicates.

5.1.12 Stage 6 Freezing

Upon completion of the processing steps, aliquots must be frozen within a maximum of 30 minutes. *Do NOT freeze aliquot 01 containing whole blood.* Remove that cryovial from the rack and place it in a separate freezer box you will be keeping in your refrigerator (4° C). The rack containing the remaining cryovials is removed from the ice bath and placed upright in the freezer at -70° C (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.

5.1.13 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 using the small backup scanner only 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.

Completed Blind Duplicate Identification forms and completed OGTT forms should also be scanned into the data system using the small backup scanner only. Once a week, make copies of the current Blind Duplicate ID log page(s) and any Blind Duplicate Identification forms *worked on or started* 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

- You should already have put all whole blood samples (cryovials 01) into a separate box immediately after processing and have stored that box in the refrigerator (4° C). This box should be labeled with the clinic name and all whole blood samples collected until the next shipment placed in the same box (see below for shipping instructions).

- All urine cryovials are separated and placed in a separate freezer box 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.
- 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 the full set of blind duplicate cryovials to be sent to LCBR (#02, 25, 26, and 46) 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, 25, 26, and 46) 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.
- Re-stock blood collection trays with supplies.
- Label the next day's participants' draw tubes, aliquots, and forms.
- Arrange draw tubes and aliquots in their proper racks.
- 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.	Serum 10 mL	90 minutes
	2.	EDTA 10 mL	15 minutes
	3.	SCAT-I	15 minutes
	4.	Citrate 4.5 mL	15 minutes
	5.	Serum 10 mL	90 minutes
	6.	Serum 10 mL	90 minutes
	7.	EDTA 10 mL	15 minutes
	9.	Serum (extra)	90 minutes
	10.	EDTA (extra)	15 minutes
2-Hr	8.	Serum 5 mL	90 minutes

Once centrifuged, maximum time before aliquoting: 15 Minutes

After aliquoting samples, freeze within 30 minutes. Do NOT freeze aliquot #01); place it in the box you are storing in the 4° C refrigerator.

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.

Whole blood samples (cryovial #01) must be shipped separately twice weekly by Federal Express overnight delivery to LCBR. This must be done on Monday and Wednesday to allow LCBR to process the tubes within 7 days of collection. Again, when Monday is a holiday, that shipment may be delayed until 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 frozen blood and urine samples are already packaged in prelabeled 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 form 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.
- Put the individual freezer boxes containing the samples into a leakproof zip-lock plastic bag. Seal the zip-lock bags.
- Line the styrofoam mailer with absorbent material (e.g., paper towels).
- Place approximately one third of the dry ice on the bottom of the mailer.
- Carefully place the freezer boxes into the styrofoam mailer. Place no more than a total of 4 L of sample into the styrofoam shipping container. (This is a shipping regulation. You will never come close to this much sample.) Use two 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 freezer boxes 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).
- For dry ice shipments, 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 Lindenfesler
McKesson BioServices
685 Lofstrand Lane
Rockville, MD 20850
(301) 309-3667

FAX the following information to McKesson Bioservices at (301) 340-9245 when a shipment is sent:

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

5.4.3 Methods for shipping whole blood samples

The refrigerated samples to be shipped on Monday are those from the previous Thursday and Friday. The samples to be shipped on Wednesday are those from Monday, Tuesday, and Wednesday. (It is particularly important to include the

Wednesday samples in the Wednesday shipment if the following Monday is a holiday.) These samples are already packaged in pre-labeled boxes stored in the refrigerator.

- Wrap the box containing the samples in paper towels to absorb possible leakage. Put a rubber band around the box.
- Place the box into a leakproof zip-lock plastic bag and seal.
- Line the styrofoam mailer with absorbent material (e.g., paper towels)
- Place one or two ice packs into the bottom of the mailer
- Carefully place the sample box into the styrofoam mailer and place another one or two ice packs (NOT dry ice) on top and around it
- It may be helpful to pack any remaining empty space with newspaper to prevent the package from shifting during shipment.
- Seal the top of the styrofoam container with tape
- Enclose the styrofoam container in the outer cardboard sleeve
- Seal the outer sleeve with tape

Fill out the FedEx Airbill as follows (Appendix 13):

- Type in your FedEx account number
- Type the date of the shipment
- Type the name of the person sending the shipment under Section one, where it says 'From'
- Type in your address and telephone number in Section one.
- Type the recipient's name, address, and telephone number in Section two. *The telephone number is mandatory.*
- Type an 'X' in the Bill 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 if required. Do NOT check 'Hold for Pickup'
- 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.

The following additional labels are to be attached to each shipping box.

- Return Address Label: placed on top in upper left corner.
- Consignee Address Label: placed on top in bottom right corner.
- Diagnostic Specimen Label: placed on top under the return address label.
- Keep Refrigerated Label (optional): placed on any side

It is necessary to weigh the entire shipping container and record the shipping weight on the Federal Express form. The total declared value is 0.

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

6. Procedures for Performing the Measurements at Home

This examination is not feasible for home visits.

7. Quality Assurance

7.1 Blind duplicate aliquots

7.1.1 Rationale: In order to monitor accuracy 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 and pull out 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 will 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 cryovial 15. This blind duplicate aliquot rack must be kept handy during the processing of all participant samples.

A sheet of blind duplicate 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 ID labels** will be placed **in the appropriate box at the**

top of each page of the “Blind Duplicate Identification Form” (see Appendix 14), *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.* When the Coordinating Center separates out the blind duplicate ID label sheets, they will also create a set of dummy Phlebotomy and Laboratory Processing forms with dummy Health ABC participant numbers. The Coordinating Center will also provide dummy Acrostics. The dummy forms should be used in numerical order from lowest ID number to highest (i.e., low blind duplicate aliquot numbers should be associated with low dummy Phlebotomy and Laboratory Processing form IDs).

Keep the dummy forms with the blind duplicate aliquot rack until all cryovials to be sent to LCBR are filled. Record any comments pertaining to the aliquoting of LCBR samples (cryovials 01, 02, 25, 26, and 46), then check all the rest of the cryovials as filled -- this includes cryovial 15, even though it 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.

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.* 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

7.4 Quality Assurance Checklist

Preparation

- Aliquot racks correctly set up
- Blind duplicate rack correctly set up
- Cryovials correctly labeled
- Hepatitis B vaccination given or offered to all personnel handling blood
- Non-permeable lab coats, gloves, and face shields used

Stage 1

- Time checked to ensure that tubes 2, 3, 4 & 7 processed within 15 minutes of completion of phlebotomy
- Whole blood aliquot removed before centrifuging
- Tubes 2, 3, 4, 7 (& 10) centrifuged at 4° C for 10 min at 3000 G
- Urine correctly aliquoted

Stage 2

- New pipet tip used for each sample type
- Plasma from tubes 2 & 7 pooled before aliquoting
- Plasma correctly aliquoted in correct order
- No cells contaminating aliquots (except buffy coat aliquot)
- Buffy coat correctly aliquoted
- Red cells correctly aliquoted

Stage 3

- Time checked to ensure that tubes 1, 5, 6, (& 9) have stood at room temperature for at least 40 minutes, maximum 90 minutes
- Tubes 1, 5, 6, (& 9) centrifuged at 4° C for 10 minutes at 3000 G.
- Centrifuge correctly balanced with water tube(s)

Stage 4

- Serum from tubes 1, 5, 6, (& 9) pooled before aliquoting
- Serum correctly aliquoted

Stage 5

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

Stage 6

- Aliquot 01 separated out and stored in refrigerator box
- Remaining aliquots checked to ensure they are not wet
- Rack placed upright in -70° C freezer or samples placed on dry ice
- Phlebotomy and Laboratory Processing forms placed in daily work folder

OGTT tube

- Time checked to ensure that tube has stood at room temperature for at least 40 minutes, maximum 90 minutes
- Tube centrifuged at 4° C for 10 minutes at 3000 G.
- Centrifuge correctly balanced with water tube(s)
- Serum correctly aliquoted

End of day procedure

- Frozen aliquots removed from rack and placed in appropriate freezer boxes
- Freezer boxes correctly labeled
- Aliquots 02, 25, 26, and 46 stored in LCBR box
- Aliquots 03-24 and 27-45 placed in blood freezer boxes for McKesson
- Aliquots 47-49 placed in urine freezer boxes for 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

Shipment procedures -- whole blood

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

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

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	33	multiple	20170-209	\$27.03	\$270.27
2.0 mL with Skirt	18	multiple	20170-205	\$27.03	\$270.27
Cap - White	15	EDTA	20170-274	\$48.27	\$482.74
Cap- Natural	1	buffy coat	20170-241	\$48.00	\$480.00
Cap- Red	21	Serum	20170-247	\$48.30	\$482.74
Cap- Blue	4	Citrate	20170-251	\$48.30	\$483.00
Cap-Yellow	4	SCAT-I	20170-243	\$48.30	\$482.74
Cap-Green	1	Urine (acid.)	20170-253	\$48.30	\$483.00
Cap- Orange	2	2-Hr Serum	20170-245	\$48.30	\$482.74
Cap- Violet	2	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	-----

Miscellaneous:

Pipets Capable of pipeting 0.5, 1.0, and 2.0 mL volumes.
For removal of buffy coat (white cell) layer
For collection of 2.0 mL red cells

Test tubes For pooling serum and EDTA plasma before aliquoting.
Plastic (polypropylene) is preferred. Disposable is okay
20 mL minimum capacity

Urine Cups info pending

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

Place this end on vial first. 123456 Draw Tube 1 Red top 10 ml	Place this end on vial first. 123456 Draw Tube 2 Purple top 10 ml	Place this end on vial first. 123456 Draw Tube 3 SCAT-I 5 ml	Place this end on vial first. 123456 Urine cup	Place this end on vial first. 123456 Serum Pool 20 ml	Place this end on vial first. 123456 Plasma Pool 15 ml	Place this end on vial first.  123456-05 W/EDTA 0.5	Place this end on vial first.  123456-06 W/EDTA 1.0	Place this end on vial first.  123456-07 W/EDTA 1.0
Place this end on vial first. 123456 Draw Tube 4 Blue top 4.5 ml	Place this end on vial first. 123456 Draw Tube 5 Red top 10 ml	Place this end on vial first. 123456 Draw Tube 6 Red top 10 ml	Place this end on vial first.  123456 bd ID Form	Place this end on vial first.  123456 bd ID Form	Place this end on vial first.  123456 bd ID Form	Place this end on vial first.  123456-08 W/EDTA 1.0	Place this end on vial first.  123456-09 W/EDTA 1.0	Place this end on vial first.  123456-10 W/EDTA 0.5
Place this end on vial first. 123456 Draw Tube 7 Purple top 10 ml	Place this end on vial first. 123456 Draw Tube 8 Red top 5 ml	Place this end on vial first. 123456 Backup Vacutainer	Place this end on vial first.  123456 bd ID Form	Place this end on vial first.  123456 bd ID Form	Place this end on vial first.  123456-01 W/O.5 whole blood To LCBR DO NOT FREEZE	Place this end on vial first.  123456-11 W/EDTA 0.5	Place this end on vial first.  123456-12 W/EDTA 0.5	Place this end on vial first.  123456-13 W/EDTA 0.5
Place this end on vial first. 123456 Backup Vacutainer	Place this end on vial first.  123456 P/P Form	Place this end on vial first.  123456 P/P Form	Place this end on vial first.  123456-02 W/EDTA 1.0 To LCBR	Place this end on vial first.  123456-03 W/EDTA 0.5	Place this end on vial first.  123456-04 W/EDTA 0.5	Place this end on vial first.  123456-14 W/EDTA 0.5	Place this end on vial first.  123456-15 C/Bufly	Place this end on vial first.  123456-16 W/Cells 2.0

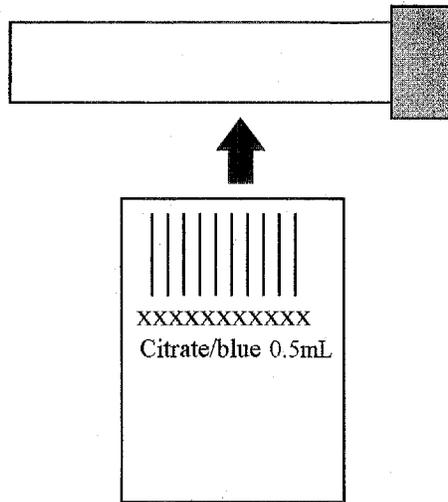
APPENDIX 2
(page 2 of 3)

Place this end on vial first.  123456-17 Y/SCAT 0.5	Place this end on vial first.  123456-18 Y/SCAT 0.5	Place this end on vial first.  123456-19 Y/SCAT 0.5	Place this end on vial first.  123456-29 R/Serum 0.5	Place this end on vial first.  123456-30 R/Serum 0.5	Place this end on vial first.  123456-31 R/Serum 0.5	Place this end on vial first.  123456-41 R/Serum 0.5	Place this end on vial first.  123456-42 R/Serum 0.5	Place this end on vial first.  123456-43 R/Serum 0.5
Place this end on vial first.  123456-20 Y/SCAT 0.5	Place this end on vial first.  123456-21 B/Citrate 0.5	Place this end on vial first.  123456-22 B/Citrate 0.5	Place this end on vial first.  123456-32 R/Serum 0.5	Place this end on vial first.  123456-33 R/Serum 0.5	Place this end on vial first.  123456-34 R/Serum 1.0	Place this end on vial first.  123456-44 R/Serum 0.5	Place this end on vial first.  123456-45 R/Serum 0.5	Place this end on vial first.  123456-46 Q/OGTT 0.5 To LCBR
Place this end on vial first.  123456-23 B/Citrate 0.5	Place this end on vial first.  123456-24 B/Citrate 0.5	Place this end on vial first.  123456-25 R/Serum 0.5 To LCBR	Place this end on vial first.  123456-35 R/Serum 1.0	Place this end on vial first.  123456-36 R/Serum 1.0	Place this end on vial first.  123456-37 R/Serum 1.0	Place this end on vial first.  123456-47 V/Urine 2.0	Place this end on vial first.  123456-48 V/Urine 2.0	Place this end on vial first.  123456-49 G/Acid 2.0
Place this end on vial first.  123456-26 R/Serum 0.5 To LCBR	Place this end on vial first.  123456-27 R/Serum 0.5	Place this end on vial first.  123456-28 R/Serum 0.5	Place this end on vial first.  123456-38 R/Serum 1.0	Place this end on vial first.  123456-39 R/Serum 1.0	Place this end on vial first.  123456-40 R/Serum 0.5			

APPENDIX 2
(3 of 3 pages)

HEALTH ABC STUDY

Label Orientation on Cryovial



APPENDIX 3
Laboratory Processing form



LABORATORY PROCESSING

Staff ID #

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Bar Code Label

Time at start of processing: : am
 pm

Collection Tubes	Cryo #	Vol.	Type	To	Check "X"	Problems	Collection Tubes	N/A	Cryo #	Vol.	Type	To	Check "X"	Problems
#2 Whole blood	01	0.5	W/0.5	L*	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	#1,5,6,9 Serum	<input type="checkbox"/>	25	0.5	R/0.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
#2, 7, (10) EDTA	02	1.0	W/2.0	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	<input type="checkbox"/>	26	0.5	R/0.5	L	<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		27	0.5	R/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		28	0.5	R/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		29	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
	06	1.0	W/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	
	07	1.0	W/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	
	08	1.0	W/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		32	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
	09	1.0	W/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		33	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
	10	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		34	1.0	R/2.0	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		35	1.0	R/2.0	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		36	1.0	R/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
	13	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		37	1.0	R/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
	14	0.5	W/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		38	1.0	R/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	
	#2, 7 Buffy	15	var	C/2.0	M	<input type="checkbox"/>		<input type="checkbox"/> H <input type="checkbox"/> P	39	1.0	R/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
#2, 7 RBC	16	2.0	W/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	40	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		
#3 SCAT-I	17	0.5	Y/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	41	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		
	18	0.5	Y/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	42	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		
	19	0.5	Y/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	43	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		
	20	0.5	Y/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	44	0.5	R/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P		
#4 Citrate	21	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	#8 OGTT	<input type="checkbox"/>	46	0.5	O/0.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	22	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	URINE		47	2.0	V/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	23	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P			48	2.0	V/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
	24	0.5	B/0.5	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	(acidified)		49	2.0	G/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P

L=LCBR; M=McKesson; H=Hemolyzed; P=Partial; W=white; C=clear; Y=yellow; B=blue; R=red; O=orange; V=violet
G=green *DO NOT FREEZE. To be shipped separately on wet ice.

Page Link #



APPENDIX 4**OSHA****Enforcement Procedures for the Occupational Exposure to Bloodborne Pathogens
Standard, 29 CFR 1910.1030
(15 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 overflow.

..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) of 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 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

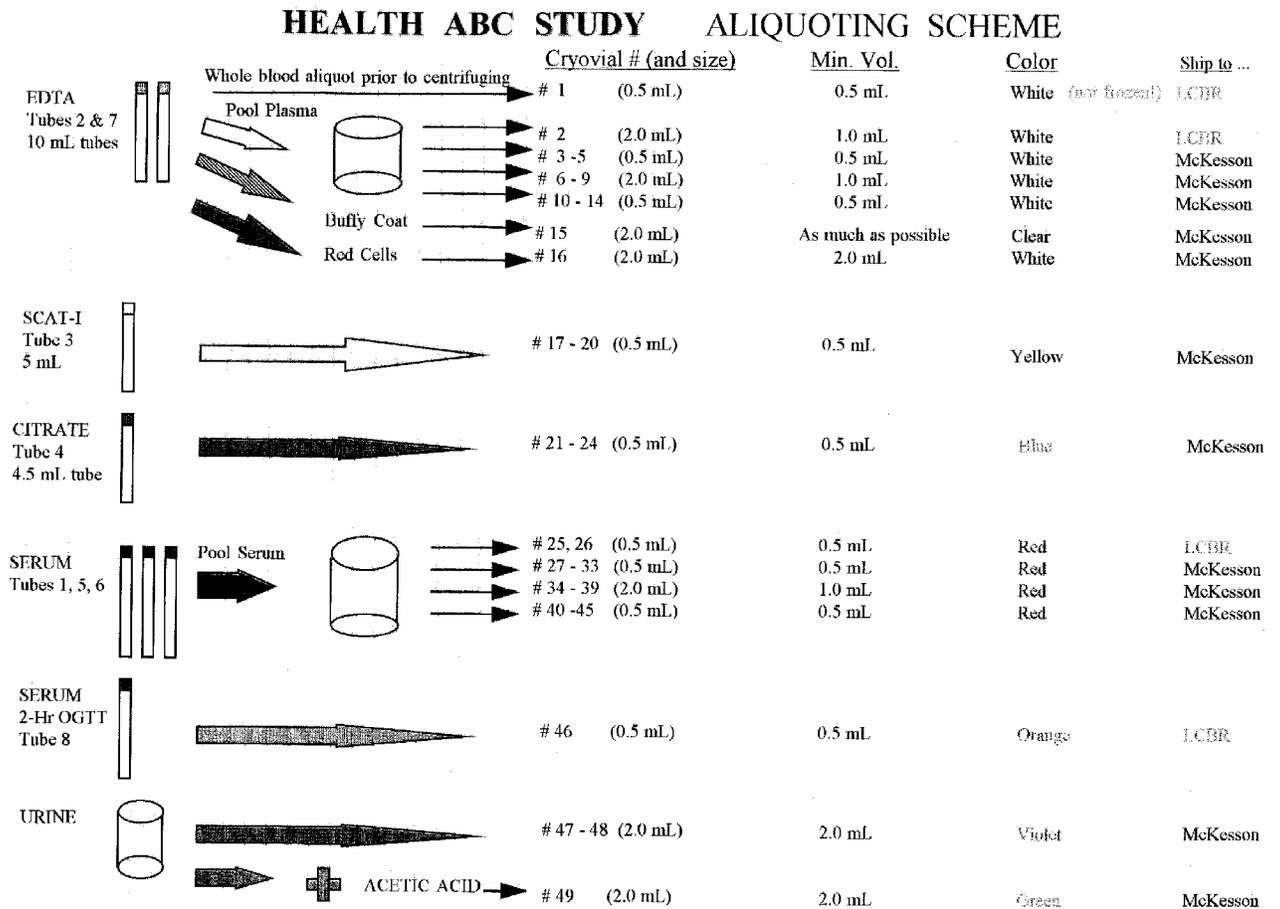
employee's ability to receive vaccination as required by paragraph (f)(2); (C) A copy of all results of examinations, medical testing, and follow-up procedures as required by paragraph (f)(3); (D) The employer's copy of the healthcare professional's written opinion as required by paragraph (f)(5); and (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

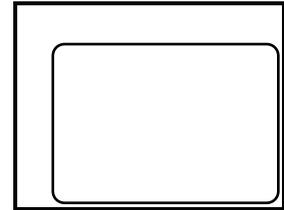
- Ñ Blind duplicate aliquot rack
- Ñ Crushed ice in ice bucket or plastic tub
- Ñ Pipets: 0.5, 1.0, and 2.0 volumes and pipet tips
- Ñ Pasteur pipets
- Ñ Labeled cryovials in rack
- Ñ Lab coat and gloves
- Ñ Biohazardous waste disposal
- Ñ Refrigerated centrifuge capable of spinning at 30,000 g-minutes
- Ñ Tubes for pooling sample
- Ñ Balance tubes for the centrifuge
- Ñ 10% bleach solution
- Ñ Freezer boxes with 9 x 9 grid
- Ñ Rubber bands

APPENDIX 7
Flow Chart/Aliquoting Scheme



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 26	
aliquot 2		aliquot 27	
aliquot 3		aliquot 28	
aliquot 4		aliquot 29	
aliquot 5		aliquot 30	
aliquot 6		aliquot 31	
aliquot 7		aliquot 32	
aliquot 8		aliquot 33	
aliquot 9		aliquot 34	
aliquot 10		aliquot 35	
aliquot 11		aliquot 36	
aliquot 12		aliquot 37	
aliquot 13		aliquot 38	
aliquot 14		aliquot 39	
aliquot 16		aliquot 40	
aliquot 17		aliquot 41	
aliquot 18		aliquot 42	
aliquot 19		aliquot 43	
aliquot 20		aliquot 44	
aliquot 21		aliquot 45	
aliquot 22		aliquot 46	
aliquot 23		aliquot 47	
aliquot 24		aliquot 48	
aliquot 25		aliquot 49	

APPENDIX 9
Freezer Box Diagrams
(page 1 of 3)

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

Numbers = cryovial #

Complete sets of cryovials available for these two participants. 46 total blood sample cryovials per participant / 41 are sent to McKesson Bioservices.

start #1

Top

Part #1								
03	04	05	06	07	08	09	10	11
12	13	14	15	16	17	18	19	20
21	22	23	24	27	28	29	30	31
32	33	34	35	36	37	38	39	40
41	42	43	44	45	Part #2 03	04	05	06
07	08	09	10	11	12	13	14	15
16	17	18	19	20	21	22	23	24
27	28	29	30	31	32	33	34	35
36	37	38	39	40	41	42	43	44

Bottom

End#81

Continue to next box. . .

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

APPENDIX 9
Freezer Box Diagrams
(page 2 of 3)

Freezer Box Diagram for Shipping Urine Samples to McKesson Bioservices

Numbers = cryovial #

start #1 ^			Top					
Part #1 47	48	49	Part #2 47	48	49	Part #3 47	48	49
Part #4 47	48	49	Part #5 47	48	49	Part #6 47	48	49
Part #7 47	48	49	Part #8 47	48	49	Part #9 47	48	49
Part#10 47	48	49	Part#11 47	48	49	Part#12 47	48	49
Part#13 47	48	49	Part#14 47	48	49	Part#15 47	48	49
Part#16 47	48	49	Part#17 47	48	49	Part#18 47	48	49
Part#19 47	48	49	Part#20 47	48	49	Part#21 47	48	49
Part#22 47	48	49	Part#23 47	48	49	Part#24 47	48	49
Part#25 47	48	49	Part#26 47	48	49	Part#27 47	48	49
Bottom						End#81		

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

APPENDIX 9
Freezer Box Diagrams
(page 3 of 3)

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

Numbers = cryovial #

start #1 ^

Top

Part #1 02	25	26	46	Part# 2 02	25	26	46	Part# 3 02
25	26	46	Part # 4 02	25	26	46	Part# 5 02	25
26	46	Part # 6 02	25	26	46	Part# 7 02	25	26
46	Part #8 02	25	26	46	Part# 9 02	25	26	46
Part#10 02	25	26	46	Part#11 02	25	26	46	Part#12 02
25	26	46	Part#13 02	25	26	46	Part#14 02	25
26	46	Part#15 02	25	26	46	Part#16 02	25	26
46	Part#17 02	25	26	46	Part#18 02	25	26	46
Part#19 02	25	26	46	Part#20 02	25	26	46	Part#21 02

Bottom

End#81
continue to
next box....

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

APPENDIX 10
Sample Shipping Checklist

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

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) Please 24 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 to 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 misinformation, 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 \$200. 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 (Next business afternoon) FedEx 2Day* (Second business day)
 FedEx Govt. Overnight (Authorized user only) JETPACK Flight
 FedEx Overnight Freight FedEx 2Day Freight
 (For packages over 150 pounds. Call for delivery schedule.)
 NEW FedEx First Overnight (First next 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 5, UN 1845) (Shipper's Declaration not required) kg 90 CA Cargo Aircraft Only

7 Payment
 Bill to: Sender (Account no. in section 7 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.
 [Signature] **232**
 FORM ID NO. 0200 Rev. Date 10/95 PART #147382 ©1994-95 FedEx • PRINTED IN U.S.A. 68FE 106

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) Please 24 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 to 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 misinformation, 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 \$200. 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 (Next business afternoon) FedEx 2Day* (Second business day)
 FedEx Govt. Overnight (Authorized user only) JETPACK Flight
 FedEx Overnight Freight FedEx 2Day Freight
 (For packages over 150 pounds. Call for delivery schedule.)
 NEW FedEx First Overnight (First next 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 5, UN 1845) (Shipper's Declaration not required) kg 90 CA Cargo Aircraft Only

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

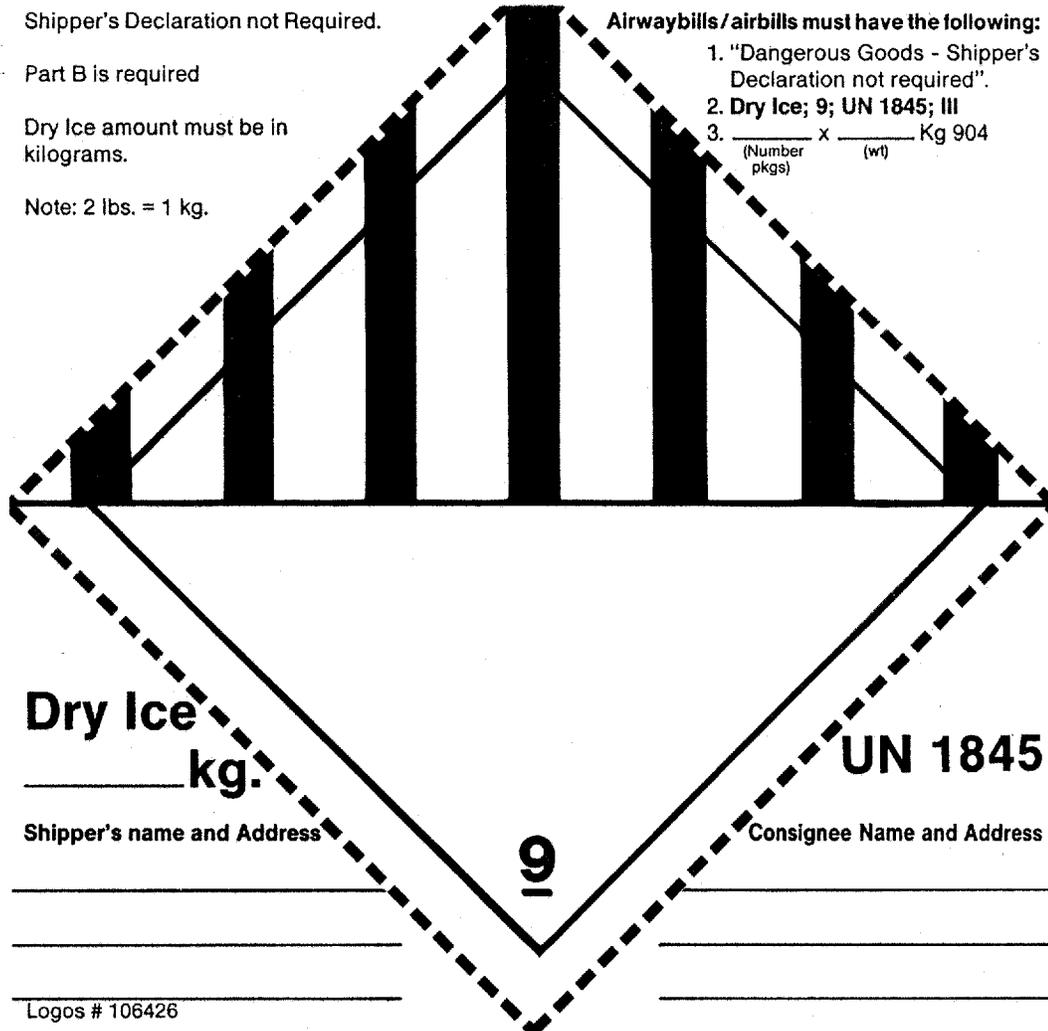
FedEx Account No. **1531-6949-7** Exp. Date **(5-26713)**
 Credit Card No. _____

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.
 [Signature] **232**
 FORM ID NO. 0200 Rev. Date 10/95 PART #147382 ©1994-95 FedEx • PRINTED IN U.S.A. 68FE 106

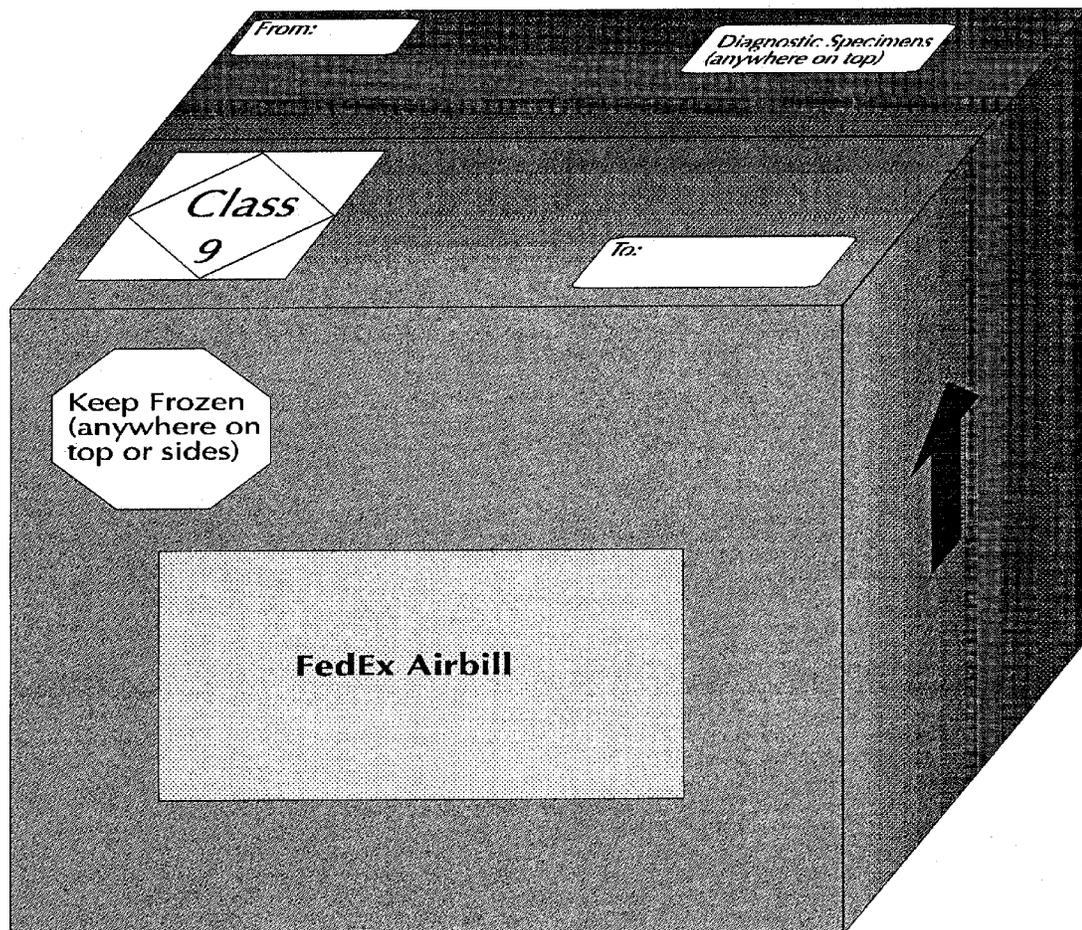
APPENDIX 12
Dry Ice Label and Labeling Diagram
(page 1 of 2)



'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
Federal Express Airbill for Whole Blood Shipment

FedEx USA Airbill

Tracking Number 9260216976

Sender's Copy

1 From (please print)

Date _____ Sender's FedEx Account Number _____

Sender's Name _____ Phone (____) _____ Dept./Floor/Suite/Room _____

Company _____

Address _____

City _____ State _____ Zip _____

2 Your Internal Billing Reference Information
(Optional) (First 24 characters will appear on invoice)

3 To (please print)

Recipient's Name ELAINE CORNELL Phone (802) 656-8963 Dept./Floor/Suite/Room _____

Company UNIVERSITY OF VERMONT - PATHOLOGY

Address 55A SOUTH PARK DRIVE
(Do NOT 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)

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 from delivery, misdelivery, or nonperformance, 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. This includes a declared value for any FedEx Letter and FedEx Pak of \$200. 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

4 Service Delivery commitment may be later in some areas

FedEx Priority Overnight (Next business morning)
 FedEx Standard Overnight (Next business afternoon)
 FedEx 2Day* (Second business day)
 FedEx Court Overnight (Delivered next evening)
 FedEx Overnight Freight
 FedEx 2Day Freight

*For packages over 150 pounds. Call for delivery schedule.

NEW FedEx First Overnight (Earliest next business morning delivery to select locations. Higher rates apply.)

*FedEx Letter/Pak not available. Minimum charge. One pound FedEx 2Day only.

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 (As per attached Shipper's Declaration) Yes (Shipper's Declaration not required)

Dry Ice (Dangerous Goods. Shipper's Declaration not required) CA Cargo Aircraft Only

7 Payment

Bill to: Shipper (Account no. in section 1 will be billed) Recipient (Enter FedEx account no. or Credit Card no. as own) Third Party Credit Card Cash/Check

FedEx Account No. 1531-6949-7 (5-26713) Date _____

Total Packages	Total Weight	Total Declared Value*	Total Charges
		\$.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 full details.

8 Release Signature

Your signature authorizes FedEx of Express to deliver this shipment without obtaining a signature and to agree to indemnify and hold harmless Federal Express from any resulting claims.

FCIM ID NO. 0200

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GSE® 106

APPENDIX 14
Blind Duplicate ID Form
(page 1 of 3)

Health ABC

Date Form Completed	Staff ID#	Attach bd ID label from Blind Duplicate Label Sheet
□□ / □□ / □□	□□□□	

BLIND DUPLICATE IDENTIFICATION FORM

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

Whole blood 01	EDTA plasma 05	EDTA plasma 09	EDTA plasma 13	SCAT-I 17
EDTA plasma 02	06	10	14	18
03	07	11	No aliquot 15 duplicate	19
04	08	12	Red blood cells 16	20

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APPENDIX 14
Blind Duplicate ID Form
(page 2 of 3)



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

BLIND DUPLICATE IDENTIFICATION FORM

Citrated plasma	Serum	Serum	Serum	Serum
21	25	29	33	37
22	26	30	34	38
23	27	31	35	39
24	28	32	36	40

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APPENDIX 14
Blind Duplicate ID Form
(page 3 of 3)



Date Form Completed	Staff ID#	Attach bd ID label from Blind Duplicate Label Sheet
□□ / □□ / □□	□□□□	

BLIND DUPLICATE IDENTIFICATION FORM

Serum	Serum	OGTT	Urine
41	45	46	47
42			48
43			(acidified)
44			49

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APPENDIX 15
Return Visit Laboratory Forms
(page 2 of 3)

	HABC Enrollment ID #	Acrostic	Date Form Completed			Staff ID #
	<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"/>			

RETURN VISIT ORAL GLUCOSE TOLERANCE TEST

1 Has a doctor ever told you that you have diabetes or sugar diabetes?

Yes No Don't know

Do you take insulin or oral hypoglycemic medications to control your diabetes? (Refer to the list to the right)

Yes, insulin
 Yes, oral hypoglycemics
 No
 Don't know

Yes, insulin → Check N/A box next to cryovial #26 on Return Visit Laboratory Processing Form. → Do NOT administer OGTT.

Yes, oral hypoglycemics → Do NOT administer OGTT.

No → Verify from Baseline Health Questionnaire Q#116 or Medication Inventory Form

Don't know → Verify from Baseline Health Questionnaire Q#116 or Medication Inventory Form

Oral Hypoglycemics

Generic Name	Trade Name
Tolbutamide	Orinase
Acetohexamide	Dymelor
Tolazamide	Tolinase
Chlorpropamide	Diabinese
Glipizide	Glucotrol
Glyburide	Diabeta, Micronase
Metformin	Glucophage
Acarbose	Precose
Glicazide	Diamicon
Glimepiride	Amaryl
Troglitazone	Rezulin

2 Was the glucose tolerance test done?

Yes No → Check N/A box next to cryovial #46 on Return Visit Laboratory Processing Form.

Why wasn't the glucose tolerance test done?

Exclusion criteria (above)
 Participant refused
 Could not tolerate glucola
 Other (Please specify): _____

3 Time glucose administered (first sip):

: am
 : pm

→ Add 2 hours to time glucose administered. →

Time blood draw due: :

4 Was entire glucose drink consumed?

Yes
 No, but more than half
 No, less than half - cancel 2-hour blood draw → Check N/A box next to cryovial #46 on Return Visit Laboratory Processing Form.

5 Time glucose tolerance blood drawn:

: am
 : pm

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APPENDIX 15
Return Visit Laboratory Forms
(page 3 of 3)

	HABC Enrollment ID #	Acrostic	Date Form Completed			Staff ID #
	<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	

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	N/A	Cryo #	Vol.	Type	To	Check "X"	Problems
#2 Whole blood	01	0.5	W/0.5	L*	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P	#1 Serum		25	0.5	R/0.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
#2 EDTA	02	1.0	W/2.0	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P			26	0.5	R/0.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
							#8 OGTT		46	0.5	O/0.5	L	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
							URINE		47	2.0	V/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
									48	2.0	V/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P
							(acidified)		49	2.0	G/2.0	M	<input type="checkbox"/>	<input type="checkbox"/> H <input type="checkbox"/> P

L=LCBR; M=McKesson, H=Hemolyzed; P=Partial; W=white; R=red; O=orange; V=violet G=green
*DO NOT FREEZE. To be shipped separately on wet ice.

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