LAB SPECIMEN PROCESSING

TABLE OF CONTENTS

1. Background and Rationale ................................................................. 2
2. Equipment and Supplies ................................................................. 2
2.1 Sample ID Labels ............................................................................ 2
3. Safety Issues and Exclusions .............................................................. 4
3.1 Precautions for Handling Blood Specimens .............................................. 4
4. Participant and Exam Room Preparation .................................................. 4
4.1 Preparation for Processing ............................................................... 4
5. Detailed Procedures ............................................................................. 5
5.1 Processing ......................................................................................... 5
5.1.1 General ....................................................................................... 5
5.1.2 Description of Blood Collection Tubes .............................................. 5
5.1.3 Immediate Processing ................................................................. 6
5.1.4 Aliquots per Sample Type ............................................................. 6
5.1.5 Making Whole Blood Aliquots ......................................................... 7
5.1.6 Centrifugation of EDTA Plasma Samples ........................................ 7
5.1.7 Making EDTA Plasma Aliquots ....................................................... 7
5.1.8 Centrifugation of Serum Samples .................................................. 8
5.1.9 Making Serum Aliquots ............................................................... 8
5.1.10 Making Blind Duplicates (if applicable) ........................................... 9
5.1.11 Freezing ...................................................................................... 12
5.1.12 Return Visit Aliquots ................................................................. 12
5.1.13 Completed forms ........................................................................ 13
5.2 End of the Day Procedures ............................................................... 13
5.3 Summary of Processing Time Limitations ............................................ 14
5.4 Shipping the Blood Samples ............................................................. 14
5.4.1 General ....................................................................................... 14
5.4.2 Methods for shipping frozen samples .............................................. 15
5.4.3 Methods for shipping whole blood samples .................................... 17
6. Procedures for Performing the Measurements at Home ......................... 18
7. Quality Assurance ............................................................................... 19
7.1 Blind duplicate aliquots ..................................................................... 19
7.1.1 Rationale .................................................................................... 19
7.1.2 Blind duplicate sample IDs ........................................................... 19
7.1.3 Paperwork .................................................................................. 20
7.1.4 Keeping the blind duplicates “in sync” with the normal samples: .......... 20
7.2 Training Requirements .................................................................... 21
7.3 Certification Requirements ............................................................... 21
7.4 Quality Assurance Checklist ............................................................ 21
APPENDIX 1 Health ABC Cryovials and Processing .................................... 24
APPENDIX 2 Sample Label Sheet (Bar Codes) ............................................. 26
APPENDIX 3 Laboratory Processing Form ................................................ 30
1. Background and Rationale

The Health ABC study involves the collection of approximately 17 mL of blood from participants. The blood is collected in two types of tubes for specialized processing of different blood components. After processing, the specimens will be aliquoted into cryovials to be sent to 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:

- Centrifuges capable of spinning at 30,000 g-minutes
- -20°C Freezer space is required
- Refrigerator space
- Dry Ice
- Blue ice or gel packs
- Pipets and tips: 0.5 and 1.0 mL volumes
- Lab coat and gloves
- Biohazardous waste disposal container
- 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 13) 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 1-3 lines of text. The first line consists of a letter, a word, and a number. This line of text is intended to increase accuracy in labeling and filling the cryovials. The letter refers to the color of the cryovial cap (R=red, W=white, 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 (e.g. “EDTA” for EDTA-treated plasma). The number refers to the cryovial volume (1.5 mL or 0.5 mL), not the volume aliquoted.

There are also 4 labels containing the ID number with no extension. Two are to be used for pre-labeling the 2 draw tubes, with 2 extras for backup vacutainers. These labels have no barcode, and they have 1-4 lines of text indicating which specimen container they are intended for, including the stopper color and volume, if applicable.

There are 2 barcoded labels with the ID number, one with the words “Phlebotomy Form,” which is placed on the Phlebotomy Form (see Blood Collection chapter), and the other with the words “Laboratory Processing Form,” which is placed on the Laboratory Processing Form (Appendix 3). This process of matching the participant-specific Health ABC Enrollment ID# (already on the form brought to the lab by the participant) to the sample-specific ID barcode is crucial to being able to use the data collected from laboratory tests.

Finally, there are 5 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 copy on file in laboratory), 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. Participant and Exam Room Preparation

4.1 Preparation for Processing

All items on the Sample Processing Checklist (Appendix 4) should be on hand before beginning processing.

A aliquot racks will be set up to correspond to each blood collection tube rack. Rack setup is completed the previous day. All tubes and vials are labeled with sample ID bar codes (see Label Orientation diagram in Appendix 2) and arranged in appropriate working order. After labeling draw tubes and cryovials, there will be 9 labels left: 2 “Backup Vacutainer” labels, 1 “Phlebotomy Form,” 1 “Laboratory Processing Form” label, 5 “BDID Form” labels. These can be separated into 2 mini-sheets: The “Backup Vacutainer,” “Phlebotomy Form” and “Laboratory Processing Form” labels should be clipped to the corresponding blood collection tray. “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, except that cryovials #01 and #06 are omitted. To label the blind duplicate cryovials, use a set of labels designated for blind duplicate samples (see Section 7, Quality Assurance, below). The “Laboratory Processing Form” labels (not the “BDID Form” labels) from the same sheet should be affixed to a Laboratory Processing form pre-labeled with a dummy participant ID and Acrostic (See Section 7, below). The “BDID Form” labels should be used to label the upper right corner of the Blind Duplicate Identification Form. The same dummy Health ABC ID #s and acrostics used in Year 1 should be used for the identifiers on the Year 4 dummy Phlebotomy and Laboratory Processing Forms. The dummy Laboratory Processing form and Blind Duplicate Identification Form should be clipped to the aliquot rack until all aliquots are filled.

5. Detailed Procedures

5.1 Processing

5.1.1 General

Tube #1 should be mixed and immediately placed on ice. The other tube (serum tube #2) should be held at room temperature for up to 90 minutes. Personal protective equipment (non-permeable lab coats, double-gloves with at least one latex pair, splatter shields) MUST BE worn for processing.

It is possible that not all tubes will be collected due to problems with phlebotomy. During processing, work in the order specified and make as many aliquots as possible while meeting the volume requirement of each cryovial. On the Laboratory Processing form, fill the bubble next to each cryovial that is filled, whether partially or totally. If the samples is hemolyzed, fill the bubble marked H. To determine whether a sample is hemolyzed, compare its color to the chart provided by LCBR. If the tube is only partially filled, fill the bubble marked P. If the tube is both hemolyzed and partially filled, fill the bubble marked B. If the tube is not filled at all, only fill the last bubble (marked not filled).

5.1.2 Description of Blood Collection Tubes

Each draw tube is color coded to aid in handling.

Tube #1 is a 7 ml lavender stoppered tube containing 15% liquid EDTA as the anticoagulant. After drawing, the tube should be mixed and immediately placed on
ice. 1 mL of whole blood will be collected and stored. This 1 mL will be sent to LCBR for analysis of HgA1c. The cryovial cap is coded white. The remaining blood in the draw tube will be spun. Cryovial caps are coded white. The plasma will be used for archival purposes.

Tube #2 is a 10 ml siliconized red stoppered tube used to collect serum. This tube contains no anticoagulant so that the blood clots to form serum. After drawing, the blood is allowed to clot at room temperature for 40-45 minutes (Maximum = 90 minutes). Cryovial caps are coded red. The serum is used for analysis of fasting glucose, cholesterol, and archiving.

5.1.3 Immediate Processing

Upon reaching the blood processing station, remove the blood drawing rack and ice bath containing tubes from the blood collection tray. The rack should contain tube #2. The ice bath should contain tube #1.

Tube #2 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. Note the time that serum processing started in the space provided on the Laboratory Processing form.

Tube #2 can be held at room temperature for up to 2 hours. These tubes should not be placed on ice. Note the time that cell processing started in the space provided on the Laboratory Processing form. Use standard 12-hour time, and include am/pm.

5.1.4 Aliquots per Sample Type:

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

Whole Blood: 1 mL of whole blood is collected from tube #1 before spinning. This sample is aliquoted into one 1.5 mL cryovial. The total number of aliquots is 1 (Color code = white)

Plasma: The plasma from tube #1 is aliquoted into four 0.5 mL cryovials for archival. The total number of aliquots is 4 (Color code = white)
Serum: The serum from tube #2 is aliquoted into eight 0.5 mL cryovials (One cryovial is sent to LCBR for fasting glucose and a cholesterol test. The remaining aliquots are designated for archival.) The total number of aliquots is: 8 (Color code = red) 8 x 0.5 mL

The total number of aliquots per participant is 13. A detailed listing of aliquots can be found on the Laboratory Processing form

5.1.5 Making Whole Blood Aliquots

Collect 1 mL of whole blood from Tube #1 before spinning this tube down for plasma. Place the 1 mL of whole blood into a 1.5 mL cryovial with a white cap (cryovial #01). Immediately place cryovial #01 in the sample box in the refrigerator. **Do not freeze the whole blood cryovial.**

Aliquots: 1 x 1.0 mL whole blood use 1.5 mL cryovial LCBR

- Follow the outline on the Laboratory Processing form for aliquoting the whole blood samples. Fill the bubble next to the cryovial that is filled, whether partially or totally. If the tube is only partially filled, also fill the bubble marked P. If the tube is not filled at all, fill the bubble in the last column (not filled).

5.1.6 Centrifugation of EDTA Plasma Samples

After 1 mL is collected for the whole blood sample, Tube #1 is restoppered and centrifuged at 4°C for 10 minutes at 3000 G. (A total of 30,000 g-minutes).

While these tubes are spinning:
- 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.

5.1.7 Making EDTA Plasma Aliquots
Once centrifuged, the maximum time allowed before aliquoting the EDTA plasma tube (#1) 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 tube (#1) on ice until aliquoting can occur.

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

- Allow the centrifuge(s) to come to a complete stop. Remove tube 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 plasma samples. Fill the bubble next to each cryovial that is filled, whether partially or totally. If the tube is only partially filled, also fill the bubble marked P. If the tube is not filled at all, fill the bubble in the last column (not filled).
- Pipet the plasma with the proper volume pipet. Do not use the cryovial to estimate volume.
- Recap aliquots after each sample tube has been pipetted.
- If a sample is hemolyzed, fill the bubble marked H. To determine whether a sample is hemolyzed, compare its color to the chart provided by LCBR. If the tube is both hemolyzed and partially filled, fill the bubble marked B.

**Extra plasma 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.**

### 5.1.8 Centrifugation of Serum Samples

Tube #2 should be left at room temperature for at least 40-45 minutes (maximum 90 minutes) after it is drawn. It should be displaying a clot by this time. It is centrifuged at 4° C for 10 minutes at 3000 G.

### 5.1.9 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 (Tube #2) Color coded Red

Aliquots:

- 1 x 0.5 mL serum use 0.5 mL cryovial LCBR
- 7 x 0.5 mL serum use 0.5 mL cryovial McKesson

- Follow the outline on the Laboratory Processing form for aliquoting the serum samples. Fill the bubble next to each cryovial that is filled, whether partially or totally. If the tube is only partially filled, also fill the bubble marked P.
- Pipet the serum with the proper volume pipet. Do not use the cryovial to estimate volume.
- Recap aliquots after each sample tube has been pipetted.
- If a sample is hemolyzed, fill the bubble marked H. To determine whether a sample is hemolyzed, compare its color to the chart provided by LCBR. If the tube is both hemolyzed and partially filled, fill the bubble marked B. If the tube is not filled at all, fill the bubble in the last column (not filled).

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 Making Blind Duplicates (if applicable)

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

Each time sufficient sample exists to fill an extra cryovial, an empty cryovial of the correct type will be selected from the blind duplicate aliquot rack and filled with the appropriate quantity of sample (0.5 mL). The filled cryovial will be placed in the participant’s aliquot rack, which is in the ice bath.

Before doing anything else, a “BDID Form” label must be removed from the participant’s label sheet and affixed to the Blind Duplicate Identification Form in the spot corresponding to the aliquot number that was filled. You must also write the participant’s sample ID number next to the aliquot number in your Blind Duplicate ID log book. This book is simply a notebook with each page devoted to a separate blind duplicate ID number (see Appendix 5 for example).
For example (see figure on page 10), suppose there is sufficient extra plasma from sample ID #123456 to make a 0.5 mL aliquot. Cryovial 04 from the blind duplicate set ID #432890 has not yet been filled. You will pick up the prelabeled cryovial 04 (#432890-04), put 0.5 mL of EDTA 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 04 on the Blind Duplicate Identification Form. You will then write the participant’s ID #123456 next to aliquot 04 in the log book on the page devoted to blind duplicate set #432890.
5.1.11 Freezing

Upon completion of the processing steps, plasma and serum aliquots must be frozen at -70° or on dry ice within a maximum of 30 minutes. **Do not freeze the whole blood cryovial.** Remove that cryovial from the rack immediately after filling and place it in a separate box you will be keeping in your refrigerator (4°C).

After aliquoting is complete, the rack containing the remaining cryovials is removed from the ice bath and placed upright in the freezer at -70° C 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.

5.1.12 Return Visit Aliquots

Occasionally, participants return to the clinic just to have a blood draw. There are separate forms that must be filled out for return visits: the Return Visit Phlebotomy Form and the Return Visit Lab Processing Form (see Appendix 12). Use a new set of sample ID bar code labels. Place the Phlebotomy Form label in the Bar Code Label space on the Return Visit Phlebotomy Form. Place the Laboratory Processing Form label in the Bar Code Label space on the Return Visit Laboratory Processing Form. Use the rest of the labels in the same way as for the regular clinic visit. Be sure to fill out both forms with the header information including the Health ABC Enrollment ID #, Acrostic, Date Form Completed, and Staff ID #.

If the participant returned only because they were not fasting at the clinic visit, they should have had a complete draw during the clinic visit. You will therefore only have to draw a small amount of blood for a fasting serum sample. Do not draw tube #1. For tube #2, substitute a 3- or 5-mL siliconized red-stoppered tube (the 3-ml pediatric tubes may require a different adapter for the centrifuge). You will get more serum than you need for the fasting serum sample, but you should not fill more cryovials for storage. Fill cryovial 06 only. You may also fill one blind duplicate cryovial 06, if needed. The rest of the serum should be discarded.

Only if the participant did not have blood drawn at all during the clinic visit, use the draw tube labels for Draw Tube 1 and 2 and the cryovial labels for cryovials 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, and 13 as usual. If the participant had an incomplete draw, then draw the tube needed to complete the set of cryovials. For
example, if the participant had an EDTA tube drawn at their clinic visit, but a serum sample could not be obtained, draw the regular 10 mL tube #2 and fill cryovials 06-13.

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

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

5.2 End of the Day Procedures

- 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 6)
- Filled blind duplicate cryovials should be temporarily stored in a separate freezer box until the full set has been completed. When all cryovials in the blind duplicate set have been completed, place them in the next available freezer box for McKesson.
- Cryovials (#01 and 06) sent to LCBR in Vermont are each separated out into separate boxes. Note that the labels on these cryovials include the words “To LCBR” to make them easy to identify. These boxes should be numbered consecutively (1, 2, 3, etc.) and should also be labeled with the name of the site (Appendix 6).
- Re-stock blood collection trays with supplies.
- Label the next day’s draw tubes and cryovials.
• Arrange draw tubes and aliquots in their proper racks.
• Wipe down all work areas with 10% Clorox solution.

5.3 Summary of Processing Time Limitations

From end of venipuncture to start of processing:

1. EDTA  7 ml  15 minutes
2. Serum 10 mL  90 minutes

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

5.4 Shipping the Blood Samples

5.4.1 General

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

<table>
<thead>
<tr>
<th>Monday</th>
<th>Memphis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuesday</td>
<td>Pittsburgh</td>
</tr>
</tbody>
</table>

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

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

This shipping protocol follows the procedures mandated by the International Air Transport Association’s Dangerous Goods Regulations-Packaging Instructions 650 and 904. All items from the shipping checklist (Appendix 7) should be kept in stock at all times.

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.
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.

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

Samples should be prepared for shipping as follows:

- Wrap each freezer box in paper towels to absorb possible leakage. Put a rubber band around the towel-wrapped box or bag.
- Put the individual freezer boxes containing the samples into a leakproof zip-lock plastic bag. Seal the zip-lock bags.
- Line the styrofoam mailer with absorbent material (e.g., paper towels).
- Place approximately one third of the dry ice on the bottom of the mailer.
- Carefully place the freezer boxes into the styrofoam mailer. Place no more than a total of 4 L of sample into the styrofoam shipping container. Use two or more styrofoam mailers for the McKesson shipment when necessary. (In this case, label the mailers “1 of 2” and “2 of 2”).
- Place the remaining dry ice (approximately 7 - 14 lbs total) on top and around the samples to fill the styrofoam container.
- Seal the top of the styrofoam container with tape.
- Enclose the styrofoam container in the outer cardboard sleeve.
- Place the copies of the Phlebotomy and Laboratory Processing forms (LCBR) or Laboratory Processing form (McKesson) on top of the styrofoam container before closing up the outer sleeve with tape.

Fill out the FedEx Airbill as follows (Appendix 8):
- Type in your FedEx account number (for both McKesson and LCBR shipments)
- Type the date of the shipment
- Type the name of the person sending the shipment under Section one, where it says ‘From’
- Type in your address and telephone number in Section one.
- Type the recipient’s name, address, and telephone number in Section two. The telephone number is mandatory.
- Type an ‘X’ in the Bill Sender box for the McKesson shipment
• Type an ‘X’ in the Bill Recipient box for the LCBR shipment. Fill in the University of Vermont account number (1531-6949-7) and internal reference number (5-26713) below the account number
• Type an ‘X’ in Priority Overnight under Section 4a
• Type an ‘X’ in the Other Packaging box in Section 5
• Type an ‘X’ in the Deliver Weekday box (Box 2).
• Place an ‘X’ in the “Dry Ice” box in Section five (Box 6). Enter the weight of the dry ice in kilograms as specified and the number of boxes shipped.
• In section 6, place an ‘X’ in the ‘Yes (Shipper’s declaration not required)’ box

Affix the completed airbill to the front side of the package in the plastic pouch (see Appendix 9).

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 9):

• 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 9)
• Diagnostic Specimen Label: placed on top under the return address label.
• Keep Frozen Label (optional): placed on any side

It is necessary to weigh the entire shipping container. The weight of the dry ice in kilograms is written on the Black and White Class 9 Label (Appendix 9) 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:
Patrick Hobson-García
McKesson BioServices
685 Lofstrand Lane
Rockville, MD 20850
(301) 340-1620

FAX the following information to McKesson Bioservices at (301) 340-3275 when a shipment is sent:
- Date of shipment
- Expected arrival date
- Number of styrofoam mailers shipped
- FedEx airbill number

5.4.3 Methods for shipping whole blood samples

The refrigerated samples to be shipped on Monday are those from that day and the previous Thursday and Friday. The samples to be shipped on Wednesday are those from that day and Tuesday. These samples are already packaged in prelabeled boxes stored in the refrigerator.

- Fill out the LCBR Shipping Form for Whole Blood (Cryovial #1) (Appendix 10), listing the sample barcode, participant’s HABCID, the date of the Year 4 clinic visit for each sample in the box. The easiest way to do this is by copying this information from the stack of xeroxed Phlebotomy and Laboratory Processing forms in your daily work folder (see section 5.1.13), making sure you don’t include any cryovials that have already been shipped. Once the Shipping Form is completed, double check against the vials in the box to be shipped, to ensure that all vials in the box are listed on the form and all vials listed on the form are in the box.
- Fax a copy of the LCBR Shipping Form for Whole Blood to Elaine Cornell (Fax # 802-656-8965).
- 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 or frozen gel packs not dry ice into the bottom of the mailer. Add a layer of newspaper.
- Carefully place the sample box into the styrofoam mailer on top of the newspaper, then cover with another layer of newspaper. Place another one or two
ice packs on top and around it. **Do not let the ice pack come into direct contact with the sample box.**

- It may be helpful to pack any remaining empty space with newspaper to prevent the package from shifting during shipment.
- Keep a copy of the shipping form to fax to LCBR with the FedEx tracking number. Enclose one copy with the styrofoam mailer.
- Seal the top of the styrofoam container with tape.
- Enclose the styrofoam container in the outer cardboard sleeve.
- Place the LCBR Shipping Form for Whole Blood (Cryovial #1) on top of the styrofoam container before closing up the outer sleeve with tape. Do not enclose copies of the Plebotomy and Laboratory Processing forms with this shipment, as they will be included in the shipment of frozen samples for the same participants.
- Seal the outer sleeve with tape.
- Be sure to use appropriate styrofoam mailers that LCBR provides.
- Fill out the FedEx Airbill as above, except that the “Dry Ice” box should not be checked in section 5.
- Fax the LCBR Shipping Form for Whole Blood to LCBR at 802-656-8965.

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 procedure is the same for home visits as for clinic visits. The samples will be returned to the lab as soon as possible after the home visit, preferably within 2 hours. Be sure to check the “time blood draw completed” field on the Phlebotomy form and begin processing within the time limits described in Section 5.3. This may not be possible if there was a delay in getting the samples back to the lab. It is therefore doubly important to record the time processing was started on the Lab Processing form.
7. Quality Assurance

7.1 Blind duplicate aliquots

7.1.1 Rationale:
In order to monitor reproducibility of the assays being carried out by LCBR, it is necessary for them to assay 5% of the samples twice, without knowing which samples are duplicates. This year, this applies only to assays that will be run later from archived samples.

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 serum collected from each participant will be aliquoted for immediate assay or storage. The process of producing these blind duplicates is therefore difficult to explain, but easy to carry out once the process is understood.

7.1.2 Blind duplicate sample IDs:

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

To create a “blind duplicate participant” the blood processing staff will need to aliquot into extra cryovials any extra serum 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 01 or 06. This blind duplicate aliquot rack must be kept handy during the processing of all participant samples.

A sheet of blind duplicate sample ID labels will be used to label the cryovials in the blind duplicate aliquot rack exactly as is done for regular cryovials. A “BDID Form” label from the sheet of blind duplicate sample ID labels will be placed in the appropriate box at the top of the “Blind Duplicate Identification Form” (see Appendix 11), 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, a dummy Laboratory Processing form must be made for each blind duplicate ID number used (no dummy Phlebotomy form is needed this year because no blind duplicate samples are being sent directly to LCBR). It is not necessary to waste an entire clinic visit workbook to get this form. The Laboratory Processing form should be photocopied from a blank workbook. The dummy Laboratory Processing forms should be filled out with the same dummy Health ABC Enrollment ID#s and Acrostics used for the first visit. The Coordinating Center has provided a list of Health ABC Enrollment ID#s and Acrostics used in Year 1; if you need another copy, contact Emily Kenyon.

Keep the dummy form with the blind duplicate aliquot rack until all cryovials are filled. McKesson and the Coordinating Center need only the Laboratory Processing form. Once copies of the Laboratory Processing forms are sent to the Coordinating Center 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 #, the bar code, and the visit year 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 Laboratory Processing Forms for the blind duplicates. Once the Laboratory Processing Form has been entered into the data system, it should be filed in this central file.

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

7.1.4 Keeping the blind duplicates “in sync” with the normal samples:
Blind duplicate cryovials should be filled in the same order as regular cryovials. Do not make partial blood aliquots for blind duplicates. If there is not enough sample
left to fill a blind duplicate aliquot to the intended level, discard the remaining sample.

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 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 and they should contact the Health ABC Coordinating Unit. 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 processing 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

**Processing EDTA whole blood, plasma and urine**
- Time checked to ensure that tube 3 is processed within 15 minutes of completion of phlebotomy
- Whole blood correctly aliquoted
- Tube #1 centrifuged at 4°C for 10 min at 3000 G
- Plasma correctly aliquoted

**Processing serum tubes**
- Time checked to ensure that tube# 2 has stood at room temperature for at least 40 minutes, maximum 90 minutes
- Tube 2 centrifuged for 10 minutes at 3000 G.
- Centrifuge correctly balanced with water tube(s)
- Serum correctly aliquoted

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

**Freezing**
- Remaining aliquots checked to ensure they are not wet
- Rack placed upright in -20°C freezer or samples placed on dry ice

**Whole Blood Sample**
- Sample placed immediately in refrigerator after filling.

**Shipment procedure – whole blood**
- Boxes correctly wrapped, etc.
- Styrofoam mailers correctly packed – absorbent material, ice pack or frozen gel pack not dry ice, newspaper, sample, more newspaper, more ice packs, top sealed with tape.
End of day procedure
- Phlebotomy and Laboratory Processing forms placed in daily work folder
- Frozen aliquots removed from rack and placed in appropriate freezer boxes
- Freezer boxes correctly labeled
- Aliquots 01 and 06 stored in separate boxes, each for shipment to LCBR
- Aliquots 02-05, and 07-13 placed in 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
APPENDIX 1  Health ABC Cryovials and Processing

VENDORS:
VWR: 800-932-5000
  www.vwrsp.com
Fisher Scientific: 800-766-7000
  www.fishersci.com
Polyfoam Packers: 800-323-7442
  www.polyfoam.com
Krackler Scientific: 800-334-7725 (NY)
Krackler Scientific: 800-221-6921 (NC)
BD- Vacutainer: 888-237-2762
BD:  Becton Dickson brand, available through VWR, Baxter, and Fisher

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

<table>
<thead>
<tr>
<th>cryovials / caps/ racks</th>
<th># per participant</th>
<th>sample type</th>
<th>vendor: catalog #</th>
<th>$ price/pk</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mL with skirt w/o cap non-sterile</td>
<td>12</td>
<td>serum, plasma</td>
<td>VWR: 20170-209</td>
<td>28.10/500</td>
</tr>
<tr>
<td>1.5 mL with skirt w/ clear cap</td>
<td>1</td>
<td>whole blood</td>
<td>VWR: 20170-213</td>
<td>28.10/500</td>
</tr>
<tr>
<td>colored screw cap: red</td>
<td>8</td>
<td>serum</td>
<td>Fisher: 02-681-361</td>
<td>68.77/500</td>
</tr>
<tr>
<td>colored screw cap: white</td>
<td>5</td>
<td>WB, plasma</td>
<td>VWR: 20170-274</td>
<td>50.21/500</td>
</tr>
<tr>
<td>cryovial rack</td>
<td>1</td>
<td>all</td>
<td>VWR: 30128-346</td>
<td>60.00/5</td>
</tr>
<tr>
<td>cover for cryovial rack</td>
<td>1 optional</td>
<td>all</td>
<td>VWR: 30128-350</td>
<td>39.00/5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood collection supplies</th>
<th># per participant</th>
<th>sample type</th>
<th>vendor: catalog #</th>
<th>$ price/pk</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL vacutainer/ 16 x 100 size</td>
<td>1</td>
<td>serum</td>
<td>VWR:VT6430 / BD: 6430</td>
<td>25.94/100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fisher: 02-685-A</td>
<td>28.81/100</td>
</tr>
<tr>
<td>7 mL EDTA vacutainer / 13 x 75 size</td>
<td>1</td>
<td>plasma</td>
<td>VWR:VT6454 / BD:366454</td>
<td>28.81/100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fisher: 02-685-2A</td>
<td>31.64/100</td>
</tr>
<tr>
<td>vacutainer blood collection set 21G 3/4&quot;</td>
<td>1</td>
<td></td>
<td>VWR: VT7251</td>
<td>60.50/50</td>
</tr>
<tr>
<td>vacutainer needle holders</td>
<td>1</td>
<td>NA</td>
<td>BD: 4893</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fisher: 02-665-110</td>
<td>182.00/10</td>
</tr>
<tr>
<td>transfer pipets (3.2 mL is fine)</td>
<td>~4</td>
<td>all</td>
<td>Fisher: 13-711-7</td>
<td>33.80/500</td>
</tr>
<tr>
<td>Storage/ Shipping Supplies</td>
<td># suggested</td>
<td>sample type</td>
<td>vendor: catalog #</td>
<td>$ price/pkg</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>freezer boxes (from McKesson)(2”)</td>
<td>NA</td>
<td>all</td>
<td>VWR: 55705-424</td>
<td>24.00/12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Krackeler: 114-5144-F12</td>
<td>24.03/12</td>
</tr>
<tr>
<td>box dividers (9X9)</td>
<td>NA</td>
<td>all</td>
<td>VWR: 55701-762</td>
<td>16.00/12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Krackeler: 114-5144-F29</td>
<td>16.19/12</td>
</tr>
<tr>
<td>Leakproof ziplock bags (12 x 12”)</td>
<td>23 per week</td>
<td></td>
<td>VWR: 11217-128</td>
<td>101.51/250</td>
</tr>
<tr>
<td>Polyfoam Packer products(reusable):</td>
<td>4 for start</td>
<td></td>
<td>VWR:</td>
<td></td>
</tr>
<tr>
<td>styrofoam shipping containers: 346</td>
<td></td>
<td></td>
<td>Krackeler</td>
<td>112.90/8</td>
</tr>
<tr>
<td>styrofoam shipping containers: 430</td>
<td></td>
<td></td>
<td>Krackeler</td>
<td>84.70/4</td>
</tr>
<tr>
<td>ice packs: U-TEK reusable packs</td>
<td>12 oz</td>
<td></td>
<td>VWR: 15715-105</td>
<td>21.40/24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Krackeler</td>
<td>26.00/24</td>
</tr>
</tbody>
</table>
#### APPENDIX 2 Sample Label Sheet (Bar Codes) (page 1 of 3)

<table>
<thead>
<tr>
<th>Draw Tube 1</th>
<th>Draw Tube 2</th>
<th>Backup Vacutainer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple top 7 mL</td>
<td>Red top 10 mL</td>
<td></td>
</tr>
<tr>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
</tr>
<tr>
<td>W/1.5 whole blood To LCBR DO NOT FREEZE</td>
<td>W/EDTA 0.5</td>
<td></td>
</tr>
<tr>
<td>W/EDTA 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Backup Vacutainer</th>
<th>Phlebotomy Form</th>
<th>Laboratory Processing Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place this end on vial first</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Lab Specimen Processing, OM 4

Version 1.0
10/10/00
<table>
<thead>
<tr>
<th>Place this end on vial first</th>
<th>Place this end on vial first</th>
<th>Place this end on vial first</th>
</tr>
</thead>
<tbody>
<tr>
<td>#######-04 W/EDTA 0.5</td>
<td>#######-05 W/EDTA 0.5</td>
<td>#######-06 R/Serum 0.5 To LCBR</td>
</tr>
<tr>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
</tr>
<tr>
<td>#######-07 R/Serum 0.5</td>
<td>#######-08 R/Serum 0.5</td>
<td>#######-09 R/Serum 0.5</td>
</tr>
<tr>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
</tr>
<tr>
<td>#######-10 R/Serum 0.5</td>
<td>#######-11 R/Serum 0.5</td>
<td>#######-12 R/Serum 0.5</td>
</tr>
<tr>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
<td>Place this end on vial first</td>
</tr>
<tr>
<td>#######-13 R/Serum 0.5</td>
<td>####### BDID Form</td>
<td>####### BDID Form</td>
</tr>
</tbody>
</table>
HEALTH ABC STUDY

Label Orientation on Cryovial

xxxxxxxxxxxxx
Citrate/blue 0.5mL
## APPENDIX 3 Laboratory Processing Form

### LABORATORY PROCESSING

Time at start of processing: \[\text{[Time]} \pm \text{[Am/PM]}\]

<table>
<thead>
<tr>
<th>Collection Tubes</th>
<th>Cryo #</th>
<th>Vol.</th>
<th>Type</th>
<th>To</th>
<th>Fill in Bubble</th>
<th>Problems</th>
<th>Not Filled</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 whole blood</td>
<td>01</td>
<td>1.0</td>
<td>L/1.5</td>
<td>(L)</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td>#1 EDTA plasma</td>
<td>02</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>03</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>04</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td>#2 serum</td>
<td>06</td>
<td>0.5</td>
<td>L/0.5</td>
<td>L</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>07</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>08</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>09</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H P B</td>
<td>O</td>
</tr>
</tbody>
</table>

L=LCBR; M=McKesson; H=Hemolyzed; P=Partial; B=Both; R=red; W=white

LCBR Use only: Received Date: \[\text{[Date]}\] Time: \[\text{[Time]}\]

Frozen? Yes No

Page Link # [Page 41] Y4CV/W Version 1.0, 6/06/2000 pm
APPENDIX 4 Sample Processing Checklist

☐ Blind duplicate aliquot rack
☐ Crushed ice in ice bucket or plastic tub
☐ Pipets: 1.0 volumes and pipet tips
☐ Labeled cryovials in rack
☐ Lab coat and gloves
☐ Biohazardous waste disposal
☐ Refrigerated centrifuge capable of spinning at 30,000 g-minutes
☐ Room-temperature centrifuge
☐ Balance tubes for the centrifuge
☐ 10% bleach solution
☐ Styrofoam container for freezing cell cryovials
☐ Freezer boxes with 9 x 9 grid
☐ Rubber bands
**APPENDIX 5 Example of Blind Duplicate ID Log Page**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Bar Code #</th>
</tr>
</thead>
<tbody>
<tr>
<td>aliquot 2</td>
<td></td>
</tr>
<tr>
<td>aliquot 3</td>
<td></td>
</tr>
<tr>
<td>aliquot 4</td>
<td></td>
</tr>
<tr>
<td>aliquot 5</td>
<td></td>
</tr>
<tr>
<td>aliquot 7</td>
<td></td>
</tr>
<tr>
<td>aliquot 8</td>
<td></td>
</tr>
<tr>
<td>aliquot 9</td>
<td></td>
</tr>
<tr>
<td>aliquot 10</td>
<td></td>
</tr>
<tr>
<td>aliquot 11</td>
<td></td>
</tr>
<tr>
<td>aliquot 12</td>
<td></td>
</tr>
<tr>
<td>aliquot 13</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 6 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 four participants. 11 total blood sample
cryovials per participant

<table>
<thead>
<tr>
<th>start #1</th>
<th>Top</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppt #1</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Ppt #2</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>09</td>
</tr>
<tr>
<td>Ppt #3</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>07</td>
</tr>
<tr>
<td>Ppt #4</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td>Ppt #5</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td>Ppt #6</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Ppt #7</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Ppt #8</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>06</td>
</tr>
<tr>
<td></td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>08</td>
</tr>
<tr>
<td>Bottom</td>
<td></td>
</tr>
<tr>
<td></td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>
|          | End #81

Lab Specimen Processing, OM 4

Version 1.0
10/10/00
APPENDIX 6
Freezer Box Diagrams
(page 2 of 3)

Freezer Box Diagram for Whole Blood to LCBL

Note: this box must be clearly labeled "Whole Blood -- do not freeze"

Numbers = cryovial #

<table>
<thead>
<tr>
<th>start #</th>
<th>Top</th>
<th>Ppt #1</th>
<th>Ppt #2</th>
<th>Ppt #3</th>
<th>Ppt #4</th>
<th>Ppt #5</th>
<th>Ppt #6</th>
<th>Ppt #7</th>
<th>Ppt #8</th>
<th>Ppt #9</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #10</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #19</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #28</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #37</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #46</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #55</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #64</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Ppt #73</td>
<td></td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
</tbody>
</table>

Bottom

End #81

continue to
Label outside of box: Health ABC Whole Blood Box #1 Date: _____ / _____ / _____
Do not freeze
Freezer Box Diagram for Shipping Serum Samples to LCBR/Vermont

Numbers = cryovial #

<table>
<thead>
<tr>
<th>start #1</th>
<th>Top</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppt #1 06</td>
<td>Ppt #2 06</td>
</tr>
<tr>
<td>Ppt #10 06</td>
<td>Ppt #11 06</td>
</tr>
<tr>
<td>Ppt #19 06</td>
<td>Ppt #20 06</td>
</tr>
<tr>
<td>Ppt #28 06</td>
<td>Ppt #29 06</td>
</tr>
<tr>
<td>Ppt #37 06</td>
<td>Ppt #38 06</td>
</tr>
<tr>
<td>Ppt #46 06</td>
<td>Ppt #47 06</td>
</tr>
<tr>
<td>Ppt #55 06</td>
<td>Ppt #56 06</td>
</tr>
<tr>
<td>Ppt #64 06</td>
<td>Ppt #65 06</td>
</tr>
<tr>
<td>Ppt #73 06</td>
<td>Ppt #74 06</td>
</tr>
</tbody>
</table>

Bottom

End #81 continue to next box....
Label outside of box: Health ABC Serum Box #1 Date: _____ / _____ / _____

TO LCBR
APPENDIX 7 Sample Shipping Checklist

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

Shipping materials can be purchased from: (these materials are also included in Appendix 1)

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 1-800-234-5227
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 8  Federal Express Airbill for Dry Ice Shipment

FedEx USA Airbill

1. From (please print)
   Date: 
   Sender's FedEx Account Number: 
   Sender Name: 
   Phone: 
   Company: 
   Address: 
   City: 
   State: 
   Zip: 

2. To (please print)
   Recipient Name: 
   Phone: 
   Company: 
   Address: 
   City: 
   State: 
   Zip: 

3. Packaging
   Description: 
   Weight: lbs.

4. Special Handling
   Instructions: 
   Attention: 

5. Payment
   Shipper: 
   Prepayment: 
   Third Party: 
   COD/Collections: 
   Other: 

6. Release Signature
   Signature: 
   Date: 

Sender's Copy

FedEx USA Airbill

1. From (please print)
   Date: 
   Sender's FedEx Account Number: 
   Sender Name: 
   Phone: 
   Company: 
   Address: 
   City: 
   State: 
   Zip: 

2. To (please print)
   Recipient Name: 
   Phone: 
   Company: 
   Address: 
   City: 
   State: 
   Zip: 

3. Packaging
   Description: 
   Weight: lbs.

4. Special Handling
   Instructions: 
   Attention: 

5. Payment
   Shipper: 
   Prepayment: 
   Third Party: 
   COD/Collections: 
   Other: 

6. Release Signature
   Signature: 
   Date: 

The World On Time
APPENDIX 9 Dry Ice Label and Labeling Diagram
(page 1 of 2)

Shipper’s Declaration not Required.

- Part B is required

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills/airbills must have the following:

1. "Dangerous Goods - Shipper’s Declaration not required”.
2. Dry Ice; 9; UN 1845; III
3. \( \frac{\text{Number (lbs)}}{\text{Kg } 904} \)

‘DIAGNOSTIC SPECIMENS’
“PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650”
APPENDIX 9
Dry Ice and Labeling Diagram
(page 2 of 2)

Outer Box Labeling

NOTE: Labels must not overlap
APPENDIX 10  LCBR Shipping Form for Whole Blood (Cryovial #1)
# LCBR Shipping Form

**For Whole Blood (Cryovial #01)**

<table>
<thead>
<tr>
<th>Date of Shipment:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>Day</td>
<td>Year</td>
<td></td>
</tr>
</tbody>
</table>

**Field Center:**
- [ ] Memphis
- [ ] Pittsburgh

**Shipment prepared by:**

**Fed Ex Air Bill #:**

<table>
<thead>
<tr>
<th>Bar Code</th>
<th>HABC Enrollment ID #</th>
<th>Date of Year 4 Clinic Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month</td>
</tr>
</tbody>
</table>

Y4 LCBR Shipping Form  Version 1.1, 8/7/00  pjm
APPENDIX 11 Blind Duplicate ID Form

YEAR 4 BLIND DUPLICATE IDENTIFICATION FORM

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

No aliquot 01 duplicate

EDTA plasma

No aliquot 06 duplicate

Serum

Month Day Year

Attach BDID label from Blind Duplicate Label Sheet
APPENDIX 12 Return Visit Laboratory Forms
(page 1 of 4)
**YEAR 4 RETURN VISIT PHLEBOTOMY**

1. Do you bleed or bruise easily?
   - Yes  
   - No  
   - Don’t know  
   - Refused

2. Have you ever experienced fainting spells while having blood drawn?
   - Yes  
   - No  
   - Don’t know  
   - Refused

3. Have you ever had a radical mastectomy? (Female Participants Only)
   - Yes  
   - No  
   - Don’t know  
   - Refused
   
   Which side?
   - Right  
   - Left  
   - Both

   Draw blood on left side  
   Draw blood on right side  
   Do NOT draw blood. Go to Question #10 on page 3.

4. Have you ever had a graft or shunt for kidney dialysis?
   - Yes  
   - No  
   - Don’t know  
   - Refused
   
   Which side?
   - Right  
   - Left  
   - Both

   Draw blood on left side  
   Draw blood on right side  
   Do NOT draw blood. Go to Question #10 on page 3.

*Examiner Note: If the participant is having a repeat blood draw only because they were not fasting during their Year 4 clinic visit, only draw a 3 to 5 ml serum tube. In Question #10.2, mark “Yes” when asked whether the serum tube was filled to capacity (even though the volume is less than 10 ml).*

---

**Page Link #**  

Page 1

Y4RVP Version 1.0, 6/06/00 pm

Lab Specimen Processing, OM 4

Version 1.0  
10/10/00
APPENDIX 12
Return Visit Laboratory Forms
(page 2 of 4)
YEAR 4 RETURN VISIT PHLEBOTOMY

5. Time at start of venipuncture:
   
   
   Hours  Minutes  O am  O pm

6. Time blood draw completed:
   
   
   Hours  Minutes  O am  O pm

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

   Comments on phlebotomy:
   
   

8. What is the date and time you last ate anything?
   a. Date of last food:
      
      
      Month / Day / Year

   b. Time of last food:
      
      
      Hours : Minutes  O am  O pm

   c. How many hours have passed since the participant last ate any food?
      
      
      hours  (Question 6 minus Question 8b. Round to nearest hour.)
APPENDIX 12
Return Visit Laboratory Forms
(page 3 of 4)
YEAR 4 RETURN VISIT PHLEBOTOMY

9 Quality of venipuncture:
   ○ Clean  ○ Traumatic

**Please describe. Mark all that apply:**
   ○ Vein collapse
   ○ Hematoma
   ○ Vein hard to get
   ○ Multiple sticks
   ○ Excessive duration of draw
   ○ Leakage at venipuncture site
   ○ Other (Please specify:)

10 Was any blood drawn?
   ○ Yes  ○ No

   Please describe why not: _______________________________________________________

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

<table>
<thead>
<tr>
<th>Tube</th>
<th>Volume</th>
<th>Filled to Capacity?</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>1. EDTA</td>
<td>7 ml</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>2. Serum*</td>
<td>10 ml</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

*Examiner Note: If the participant had a repeat blood draw only because they were not fasting during their Year 4 clinic visit, mark "Yes" to Question #10.2 when asked whether the serum tube was filled to capacity (even though the volume is less than 10 ml).
APPENDIX 12
Return Visit Laboratory Forms
(page 4 of 4)
### COLLECTION TUBES

<table>
<thead>
<tr>
<th>Collection Tubes</th>
<th>Cryo #</th>
<th>Vol.</th>
<th>Type</th>
<th>To</th>
<th>Fill in Bubble</th>
<th>Problems</th>
<th>Not Filled</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 whole blood</td>
<td>01</td>
<td>1.0</td>
<td>W/1.5</td>
<td>L</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td>#1 EDTA plasma</td>
<td>02</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>03</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>04</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>0.5</td>
<td>W/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td>#2 serum</td>
<td>06*</td>
<td>0.5</td>
<td>R/0.5</td>
<td>L</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>07</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>08</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>09</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.5</td>
<td>R/0.5</td>
<td>M</td>
<td>O</td>
<td>H O P O B</td>
<td>O</td>
</tr>
</tbody>
</table>

*Examiner Note: If the participant had a repeat blood draw only because they were not fasting during their Year 4 clinic visit, only fill cryovial #06.

L=LCBR; M=Mckesson; H=Hemolyzed; P=Partial; B=Both; R=red; W=white

LCBR Use only: Received Date: __________ Time: __________

Frozen? [ ] Yes [ ] No

Version 1.0
10/10/00