

BIOSPECIMEN 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.....	3
3.1	Precautions for handling blood specimens.....	3
4.	Participant and exam room preparation.....	3
4.1	Preparation for processing	3
5.	Detailed procedures.....	4
5.1	Processing.....	4
5.1.1	General	4
5.1.2	Description of blood collection tubes	4
5.1.3	Immediate processing.....	4
5.1.4	Aliquots per sample type:	4
5.1.5	Centrifugation of EDTA plasma samples.....	5
5.1.6	Making EDTA plasma aliquots.....	5
5.1.7	Centrifugation of serum samples	6
5.1.8	Making serum aliquots	6
5.1.9	Freezing	6
5.1.10	Completed forms	7
5.2	Summary of processing time limitations	7
6.	Quality assurance	7
6.1	Training requirements	7
6.2	Certification requirements	8
6.3	Quality assurance checklist	8
Appendix 1	Sample Label Sheet (Bar Codes)	9
Appendix 2	Label Orientation on Cryovial.....	12
Appendix 3	Laboratory Processing Data Collection Form	13
Appendix 4	Sample Processing Checklist.....	14
Appendix 5	Freezer Box diagrams	15
Appendix 6	Sample Storing Checklist	16

1. Background and rationale

This year of the Health ABC study involves the collection of approximately 20 mL of blood from participants. The blood is collected in two types of tubes for specialized processing of different blood components. Specimens will temporarily be stored at the University of Pittsburgh.

2. Equipment and supplies

Necessary processing supplies include:

- Centrifuges capable of spinning at 3000 g-minutes
- -80° Freezer space is required
- Refrigerator space
- Dry Ice
- Pipets and tips: 0.5 mL volume
- Lab coat and gloves
- Biohazardous waste disposal container
- Balance tubes for the centrifuge
- Lab mat
- 10% bleach solution
- Freezer racks
- Rubber bands

2.1 Sample ID labels

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

Each cryovial label also has a 2-digit extension (01 to 20) that serves as a unique identifier for each cryovial within a sample ID. See Appendix 2 for proper orientation of the barcode label.

Beneath the human-readable ID number, cryovial labels also have one to three lines of text that is intended to increase accuracy in labeling and filling the cryovials.

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 extra. They all have 1-3 lines of text indicating which specimen container they are intended for, including the stopper color and volume, if applicable.

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

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.*

3. Safety issues and exclusions

3.1 Precautions for handling blood specimens

In accordance with the OSHA regulations on blood borne pathogens (see copy on file in laboratory), the study recommends the following laboratory safety protocol for the field center laboratories:

- Non-permeable lab coats, latex gloves, and face shields should be used when handling any blood in any situation where splashes, spray, spatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonably anticipated.
- 'Universal Precautions' should be followed when handling any blood products.
- Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leakproof container. Never recap or break needles.
- Hepatitis B vaccine should be offered to all unvaccinated technicians handling blood and documentation of vaccination or technician's declining to be vaccinated should be kept.

4. Participant and exam room preparation

4.1 Preparation for processing

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

Aliquot racks will be set up to correspond to each blood collection tube rack. Rack setup is completed the previous day. All tubes and vials are labeled with sample ID bar codes (see Label Orientation diagram in Appendix 2) and arranged in appropriate working order. After labeling draw tubes and cryovials, there will be 4 labels left: 2 "Backup" labels, 1 "Phlebotomy Form," and 1 "Laboratory Processing Form" label. These can be separated into 2 mini-sheets: The "Backup Vacutainers," "Phlebotomy Form" and "Laboratory Processing Form" labels should be clipped to the corresponding blood collection tray.

5. Detailed procedures

5.1 Processing

5.1.1 General

Tube #1 should be mixed (for about 30 seconds) and immediately placed on ice. 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 sample is hemolyzed, fill the bubble marked H. If the tube is only partially filled, fill the bubble marked P. If the tube is both hemolyzed and partially filled, fill the bubble marked B. If the tube is not filled at all, fill the bubble in the second from last column (“N”) for “not filled.”

5.1.2 Description of blood collection tubes

Each draw tube is color coded to aid in handling.

Tube #1 is a 10 mL lavender stoppered tube containing EDTA as the anticoagulant. After drawing, the tube should be mixed and immediately placed on ice. Immediately after the blood draw is complete, this tube will be spun. The plasma supernatant will be aliquoted into cryovials. The plasma will be used for archival purposes.

Tube #2 is a 10 mL 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). The serum will be aliquoted into cryovials and archived.

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 ice bath should contain tube #1. The rack should contain tube #2.

5.1.4 Aliquots per sample type:

The following is a summary of how to handle each collection tube. Detailed instructions follow (volume indicates sample size, not cryovial size).

EDTA plasma: Immediately after the draw and placement in ice bath, these tubes are spun. The plasma from tube #1 is aliquoted into 10 0.5-mL cryovials (#11-20). The total number of aliquots is 10
10 x 0.5 mL sample volume

Serum: The serum from tube #2 is held at room temperature for at least 40 minutes before being spun (maximum 90 minutes). The serum from these tubes is aliquoted into 0.5 mL cryovials (#01-10). The total number of aliquots is: 10
10 x 0.5 mL

The total number of aliquots per participant is 20. A detailed listing of aliquots can be found on the Laboratory Processing form (Appendix 3).

5.1.5 Centrifugation of EDTA plasma samples

Tube #1 is centrifuged at 4° C for 10 minutes at 3000 G. (A total of 30,000 g-minutes). Be sure to balance the centrifuge either with another plasma tube from another participant or with a balance tube filled with an equal volume of water.

5.1.6 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 a blank area of the Laboratory Processing Form. Keep the collection tube (#1) on ice until aliquoting can occur.

Aliquots: 10 x 0.5 mL plasma use 0.5-mL cryovials

- 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 it on ice. Follow the outline on the Laboratory Processing form for aliquoting the plasma samples. Fill in the bubble next to each cryovial that is filled, whether the cryovial is filled partially or totally. If the tube is not filled at all, fill the bubble in the second from last column (“N”) for “not filled.” If the tube is only partially filled, also fill the bubble marked P. If a sample is hemolyzed, fill the bubble marked H. If the tube is both hemolyzed and partially filled, fill the bubble marked B (only one P, H, or B bubble should be filled for each cryovial, if applicable).
- Pipet the plasma with the *proper volume pipet*. Do not use the cryovial to estimate volume.
- Recap aliquots after each sample tube has been pipetted.

5.1.7 Centrifugation of serum samples

Tube #2 should be left at room temperature for at least 40-45 minutes (maximum 90 minutes; longer duration gives higher serum yield) 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.

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.

5.1.8 Making serum aliquots

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

Serum (Tube #2) Color coded Red

Aliquots: 10 x 0.5 mL serum use 0.5-mL cryovials

- Follow the outline on the Laboratory Processing form for aliquoting the serum samples. Fill in 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 a sample is hemolyzed, fill in the bubble marked H. If the tube is both hemolyzed and partially filled, fill the bubble marked B (only one P, H, or B bubble should be filled for each cryovial, if applicable). If the tube is not filled at all, fill the bubble in the second from last column (“N”) for “not filled.”
- 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.

5.1.9 Freezing

Upon completion of the processing steps, the serum and EDTA plasma must be frozen at -80° or on dry ice within a maximum of 30 minutes.

After aliquoting is complete, the rack containing the serum and plasma is removed from the ice bath and placed upright in the freezer at -80° C for at least half an hour (preferably until the end of the day). Make sure the aliquots are not wet when placed in the freezer to avoid ice formation on the outside of the cryovials which can interfere with scanning of the barcode labels. If a freezer is not immediately available, place the rack of samples on dry ice.

5.1.10 Completed forms

The completed Phlebotomy and Laboratory Processing forms can be set aside in a daily work folder. Originals are scanned into the data system and filed in the participants' charts.

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 5).
- Re-stock blood collection trays with supplies.
- Label the next day's draw tubes and cryovials.
- Arrange draw tubes and aliquots in their proper racks.
- Wipe down all work areas with 10% Clorox solution.

5.2 Summary of processing time limitations

From end of venipuncture to start of processing:

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

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

6. Quality assurance

6.1 Training requirements

Clinical experience with processing of blood samples is strongly recommended. Additional training should include:

- Read and study manual
- Attend Health ABC training session on techniques (or observe processing by experienced examiner)
- Discuss problems and questions with local expert or QC officer

6.2 Certification requirements

- Complete training requirements
- Process samples from volunteer or participant while being observed by QC officer using QC checklist

6.3 Quality assurance checklist

Preparation

- Aliquot racks correctly set up
- Cryovials correctly labeled
- Hepatitis B vaccination given or offered to all personnel handling blood

Processing EDTA whole blood and plasma

- Time checked to ensure that tube #1 is processed within 15 minutes of completion of phlebotomy
- Tube #1 centrifuged at 4° C for 10 min at 3000 G
- Plasma correctly aliquoted
- No cells contaminating aliquots

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.
- Serum correctly aliquoted

Freezing

- Aliquots checked to ensure they are not wet
- Rack placed upright in -80° C freezer or samples placed on dry ice
- Phlebotomy and Laboratory Processing forms placed in daily work folder
- Aliquots 01-20 placed in freezer boxes and stored in -80° C freezer

Appendix 1 Sample Label Sheet (Bar Codes)

<p>##### Draw Tube 1 Purple top Plasma EDTA 10 mL</p>	<p>##### Draw Tube 2 Red top Serum 10 mL</p>	<p>##### Back-up Vacutainer</p>
<p>##### Back-up Vacutainer</p>	<p>##### Phlebotomy Form</p>	<p>##### Laboratory Processing Form</p>
<p>Place this end on vial first</p>  <p>#####-01 Serum 0.5</p>	<p>Place this end on vial first</p>  <p>#####-02 Serum 0.5</p>	<p>Place this end on vial first</p>  <p>#####-03 Serum 0.5</p>
<p>Place this end on vial first</p>  <p>#####-04 Serum 0.5</p>	<p>Place this end on vial first</p>  <p>#####-05 Serum 0.5</p>	<p>Place this end on vial first</p>  <p>#####-06 Serum 0.5</p>

Place this end on vial first  #####-07 Serum 0.5	Place this end on vial first  #####-08 Serum 0.5	Place this end on vial first  #####-09 Serum 0.5
Place this end on vial first  #####-10 Serum 0.5	Place this end on vial first  #####-11 W/Plasma EDTA 0.5	Place this end on vial first  #####-12 W/Plasma EDTA 0.5
Place this end on vial first  #####-13 W/Plasma EDTA 0.5	Place this end on vial first  #####-14 W/Plasma EDTA 0.5	Place this end on vial first  #####-15 W/Plasma EDTA 0.5
Place this end on vial first  #####-16 W/Plasma EDTA 0.5	Place this end on vial first  #####-17 W/Plasma EDTA 0.5	Place this end on vial first  #####-18 W/Plasma EDTA 0.5

Place this end
on vial first



#####-19
W/Plasma
EDTA 0.5

Place this end
on vial first

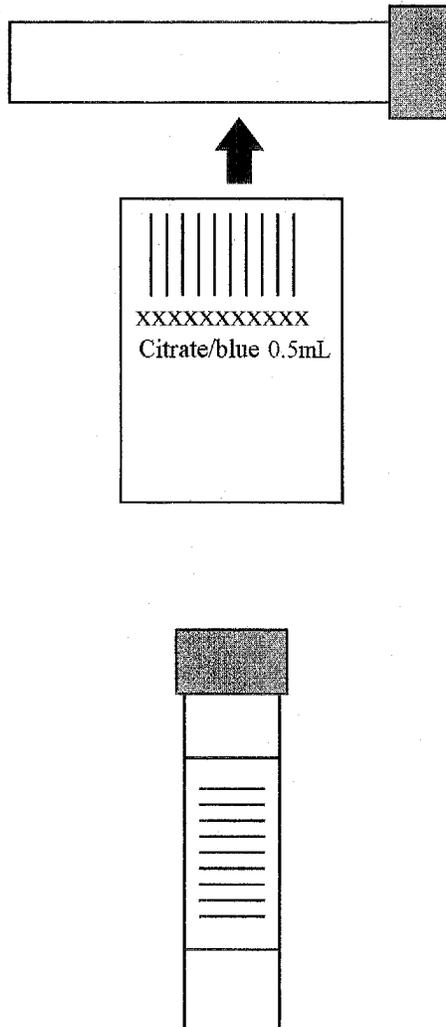


#####-20
W/Plasma
EDTA 0.5

Appendix 2 Label Orientation on Cryovial

HEALTH ABC STUDY

Label Orientation on Cryovial



Appendix 3 Laboratory Processing Data Collection Form



HABC Enrollment ID #	Acrostic	Visit Year	Date Visit Completed	Staff ID#
H <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 16	<input type="text"/> / <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Month Day Year	<input type="text"/> <input type="text"/> <input type="text"/>

LABORATORY PROCESSING

Time at start of processing: O am O pm
Hours Minutes

Bar Code Label

Collection Tubes	Cryo #	Sample vol.	Filled? Yes/No	Problems	Collection Tubes	Cryo #	Sample vol.	Filled? Yes/No	Problems
#2 Serum	1	0.5 mL	OY ON	OHOPOB	#1 Plasma (EDTA)	11	0.5 mL	OY ON	OHOPOB
	2	0.5 mL	OY ON	OHOPOB		12	0.5 mL	OY ON	OHOPOB
	3	0.5 mL	OY ON	OHOPOB		13	0.5 mL	OY ON	OHOPOB
	4	0.5 mL	OY ON	OHOPOB		14	0.5 mL	OY ON	OHOPOB
	5	0.5 mL	OY ON	OHOPOB		15	0.5 mL	OY ON	OHOPOB
	6	0.5 mL	OY ON	OHOPOB		16	0.5 mL	OY ON	OHOPOB
	7	0.5 mL	OY ON	OHOPOB		17	0.5 mL	OY ON	OHOPOB
	8	0.5 mL	OY ON	OHOPOB		18	0.5 mL	OY ON	OHOPOB
	9	0.5 mL	OY ON	OHOPOB		19	0.5 mL	OY ON	OHOPOB
	10	0.5 mL	OY ON	OHOPOB		20	0.5 mL	OY ON	OHOPOB

H-Hemolyzed; P=Partial; B=Both

Appendix 4 Sample Processing Checklist

- Crushed ice in ice bucket or plastic tub
- Pipets: 0.5 mL volume
- Transfer pipets
- Labeled cryovials in rack
- Lab coat and gloves
- Biohazardous waste disposal
- Refrigerated centrifuge capable of spinning at 3000 g-minutes
- Balance tubes for the centrifuge
- 10% bleach solution
- 2" Freezer boxes with 9 x 9 grids
- Rubber bands

Appendix 5 Freezer Box Diagrams

Freezer Box Diagram for Plasma and Serum Samples

Numbers = cryovial #01-20
20 total blood sample cryovials per participant

start #1

Top

Ppt #1 01	02	03	04	05	06	07	08	09
10	11	12	13	14	15	16	17	18
19	20	Ppt #2 01	02	03	04	05	06	07
08	09	10	11	12	13	14	15	16
17	18	19	20	Ppt #3 01	02	03	04	05
06	07	08	09	10	11	12	13	14
15	16	17	18	19	20	Ppt #4 01	02	03
04	05	06	07	08	09	10	11	12
13	14	15	16	17	18	19	20	

Bottom

End#81

Continue to next box. . .

Label outside of box: Serum, EDTA/Plasma Box #1 Date: ____ / ____ / ____

Appendix 6 Sample Storing Checklist

- Absorbent material
- 2" Freezer boxes with 9x9 grids
- Copies of Completed Phlebotomy/Processing Forms
- Rubber bands for boxes