

BIOSPECIMEN COLLECTION**TABLE OF CONTENTS**

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BIOSPECIMEN COLLECTION

1. Background and rationale

Approximately 20 mL of blood will be collected from participants on the day of the muscle tissue collection. Since the study depends on the voluntary return of participants over an extended period of time, every effort must be made to make the entire procedure as easy and painless as possible both for the participants and for the field center personnel.

A standard informed consent has been prepared for this study. With regard to laboratory procedures, the consent statement informs study participants that there is a small risk of bruising at the spot on the arm where the blood is taken and about what amount of blood is drawn.

2. Equipment and supplies

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).

Two labels are to be used to label the draw tubes and two extra for backup vacutainers.

There are also 2 barcoded labels with the ID number, one called “Phlebotomy Form,” which is placed on the Phlebotomy Form (Appendix 2), and the other called, “Laboratory Processing Form” which is placed on the Laboratory Processing Form. *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.*

There are 20 labels intended for labeling cryovials. These have extensions -01 through -20 (see Biospecimen Processing chapter).

Blood drawing trays are prepared in advance for the following day. Each tray is stocked with a full supply of blood drawing equipment for two participants and holds an ice bath and the individual blood collection tube rack for each participant.

2.2 Blood collection tray. The collection tray itself is made of hard plastic, which is unbreakable and can be easily cleaned. The tray has ten individual compartments which are filled with the following supplies:

Alcohol swabs	Smelling salts
Band-Aids	Timer/stopwatch
Gauze	Scissors
Tourniquets (2)	Adhesive tape
Vacutainer holders	Pencils/pens
Needle/sharps container	Latex gloves
Styrofoam ice bath filled ~10 min before draw	
21G Butterfly needles with Luer adapter	

2.3 Blood collection rack: labeling and setup

A separate tube rack containing the necessary draw tubes is set up for each participant. They are arranged according to the priority of the draw. This rack will fit into the blood collection tray. The two main blood collection tubes should be pre-labeled with sample ID labels. After the labels have been used for setting up the blood collection rack and the aliquot rack (see Lab Specimen Processing chapter), there will be 4 labels left: 2 “Backup” labels, 1 “Phlebotomy Form” label, 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.

2.4 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.

Tube #2 is a 10 mL glass 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 is used for archiving.

3. Precautions

3.1 Precautions for handling blood specimens

In accordance with the OSHA regulations on blood borne pathogens (see OSHA regulations that are kept in the laboratory), the central lab 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 technicians' declining to be vaccinated should be kept.

3.2 Participant precautions, exclusions, and refusal

3.2.1 Participant phlebotomy questionnaire

Following the questionnaire format on the Phlebotomy Form, each participant is asked a series of questions. Questions 1 and 2 relate to rare, but important exclusions. If a participant has had a radical mastectomy, including removal of the axillary (armpit) lymph nodes, any damage to veins on the side from which the lymph nodes were removed could result in chronic edema and clotting problems. Therefore, it is safest to use the arm from which lymph nodes were not removed. If a participant has had a bilateral radical mastectomy, it is safest not to do the blood draw at all. If they aren't sure whether their mastectomy was radical or a modified procedure, it is safest to treat it like a radical mastectomy.

Similarly, if a participant has had a graft or shunt implanted to allow kidney dialysis, application of the tourniquet and venipuncture in the area of the graft could seriously compromise the graft. Again, it is safest to use the arm without the graft or, if the participant has had grafts in both arms, not to do the blood draw at all.

Participants are also asked if they have a bleeding disorder (Question 3). If they have had any problems with excessive bleeding or bruising at a venipuncture site, use your own judgment to decide whether or not a clinic physician or nurse supervisor should be consulted.

If the participant has experienced fainting spells during phlebotomy (Question 4), ask the participant the frequency of fainting spells. If the participant frequently faints, again, use your own judgment to determine whether or not a consultation with the clinic physician or nurse supervisor is necessary. Provide smelling salts, basin, and a cold cloth if needed. See section below on precautions when a participant feels faint.

3.2.2 PRECAUTIONS WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD DRAWING.

- Have the participant remain in the chair, if necessary have them sit with their head between their knees.

- Provide the participant with a basin if they feel nauseated.
- Have the participant stay sitting until the color returns and they feel better.
- Place a cold wet cloth on the back of the participant's neck.
- If the participant faints, use smelling salts to revive by crushing the ampule and waving it under the participant's nose for a few seconds.
- If the participant continues to feel sick, contact a medical (nursing) staff member who will advise you on further action.

3.2.3 Participant refusal of phlebotomy

Rarely, a participant will refuse phlebotomy. Please keep a list of Health ABC Enrollment ID #s of any of these participants and identify which test they refused.

4. Participant and exam room preparation

4.1 Phlebotomy room

The blood drawing should take place in an isolated room or participants should be separated by room dividers. The room should be equipped with all of the necessary blood drawing supplies. A separate counter or work table should be equipped with all of the materials and vials that are used in the blood handling and processing. The centrifuge, refrigerator, and freezer should be nearby.

4.2 Preparation for phlebotomy

Preparation for phlebotomy is done in the following manner. Early morning, before any participants arrive:

- Check to make sure that blood collection tray is properly equipped. Every item on the checklist (Appendix 3) must be ready before proceeding.
- Check that each vacutainer tube is properly labeled with sample ID labels and numbered 1 and 2.
- Check that the sample processing station is properly equipped (see Biospecimen Processing chapter).
- Make sure the phlebotomy room is tidy and stocked with extra smelling salts, basin, and disposable wash cloths, and that blood mixer is functional.

Approximately 10 minutes before scheduled participant arrival:

- Fill styrofoam ice bath 3/4 full with crushed ice.

4.3 Preparation of participants for phlebotomy

It should be stressed that this study requires the voluntary cooperation of the participants. These people are donating both time and blood on a purely voluntary basis, with no reward other than the knowledge that they are contributing to progress in medicine. Thus, the whole experience must be made as pleasant as possible. Two tubes of blood are collected, containing a total of approximately 1 tablespoon of blood. Any participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is only 1 tablespoon, although it may look like more. The phlebotomist may also assure participants that they donate more than 20 times as much blood (470 mL) when they donate a unit of blood.

5. Detailed procedures

5.1 Forms

5.1.1 Phlebotomy form

An example of the Phlebotomy form is in Appendix 2. The purpose of this form is to provide a vital link between the sample ID# and the participant ID# and to facilitate the collection of plasma and serum samples from participants. The collection must be done in a rapid and efficient manner, with maximum protection for the participant. In addition, the process must facilitate the monitoring of phlebotomy and other quality assurance parameters. All forms must be completed in ink.

The Phlebotomy form has the following purposes:

1. Assure the most efficient and safest possible venipuncture for participants
2. Allow the monitoring of the quality of the above procedures
3. Provide information critical to the interpretation of the assay results

The participant will arrive at the phlebotomy station with their Health ABC participant ID# already filled in on their Phlebotomy and Laboratory Processing forms. The sample ID# will be determined by the set of pre-labeled tubes used to collect their samples. It is vital that this same sample ID be matched up with the participant ID on both the Phlebotomy and the Laboratory Processing forms (see Biospecimen Processing chapter). There will be a small sheet of labels clipped to the rack of vacutainers. On it is a “Phlebotomy Form” label, to be affixed to the upper right corner of the Phlebotomy Form, and a “Laboratory Processing Form” label, which should be affixed to the upper right corner of the Laboratory Processing Form. This should be done before drawing any blood, to insure that this critical task is not forgotten.

There are actually two parts to the Phlebotomy form associated with blood drawing. The first section contains questions that are important for participant safety; these questions should be asked immediately before phlebotomy and deal with any propensity to bleed or faint, plus

questions about fasting. The second part deals with details of the phlebotomy procedure, whether it went smoothly, how long it took, etc.

5.2 Phlebotomy

5.2.1 General

Blood drawing is standardized for the sitting position.

The venipuncture is performed with a 21-gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. A 23-gauge needle may be used, if necessary, for a difficult draw, *but this must be noted on the Phlebotomy form under "Comments on phlebotomy."* The butterfly has a small, thin-walled needle, which minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. If the participant is concerned about the venipuncture, they may be reassured to know such care is taken. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with the technician who draws the blood and their general attitude and competence.

If the participant is nervous or excited, the technician briefly describes the procedure. Sample script: *"I am going to be drawing about 1 tablespoon of blood. We hope to be able to use the results of these tests to better understand health and disease in older people."*

5.2.2 Handling participants who are extremely apprehensive about having blood drawn

Do not under any circumstances force the participant to have blood drawn. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. If the participant has "good veins," the phlebotomist can reassuringly say, "Oh, you have good veins; there should be no problem." Elderly participants are often aware of the difficulty they pose to phlebotomists and should receive extra consideration and detailed explanations as required.

5.2.3 Venipuncture procedure

- Wear Latex gloves and a lab coat.
- Arrange draw tubes in order of draw (see Section 5.2.9) on the table top within easy reach. Assemble butterfly apparatus and vacutainer holders, gauze, and alcohol prep prior to tourniquet application.
- Apply tourniquet.
- Examine participant's arms for the best site for venipuncture. Generally the antecubital vein is preferred, if feasible. Release tourniquet.

- Cleanse venipuncture site. Prepare area by wiping with alcohol swab in a circular motion from center to periphery. Allow area to dry.
- Reapply tourniquet and start timer. Note the start time on the Phlebotomy form.
- Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches below the venipuncture site.
- With the needle bevel upward, enter the vein in a smooth continuous motion.
- Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.
- Grasp the flange of the vacutainer holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.
- Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly.
- Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure or reintroduce pressure after completion of the draw.
- Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a vacutainer tube fills only partially, remove the vacutainer and attach one of your extra, backup tubes of the same type without removing the needle from the vein. Be sure to place one of the "Backup Vacutainer" labels on that tube after completing phlebotomy.
- Remove the tourniquet as soon as you connect tube #2. Once the draw has started, do not change the position of the tube until it is withdrawn from the needle. If blood flow ceases after the tourniquet is removed, it may be reapplied for another 2 minutes. Note on the Phlebotomy form the total length of time the tourniquet was on.
- After tube #1 is removed, mix by gently inverting before placing tube on the mixer. Note: do NOT mix red top tube # 2. (See section on Blood Mixing During Venipuncture below).
- When the blood flow ceases, remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted.
- Average venipuncture time is 2 to 3 minutes, but any difficulties may increase this time to 10 minutes. Be sure to note the time venipuncture is completed on the Phlebotomy form.

5.2.4 Removing the needle

- To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle into puncture-proof sharps container.
- Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.
- Allow tube #2 to sit at room temperature for at least 45 minutes.

5.2.5 Bandaging the arm

Under normal conditions:

- Slip the gauze pad down over the site, applying mild pressure.
- Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
- Tell the participant to leave the bandage on for at least 15 minutes.

If the participant continues to bleed:

- Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
- Wrap a gauze bandage tightly around the arm over the pad.
- Tell the participant to leave the bandage on for at least 15 minutes.

5.2.6 Completing the blood drawing procedure

- Dispose of needle and tubing in the appropriate biohazard needle sharps containers.
- Complete the Phlebotomy form. This includes rating the venipuncture as clean or traumatic and writing any comments about any difficulties with the phlebotomy under “Comments on Phlebotomy.”
- Clean up the venipuncture area (if necessary).
- Bring blood collection tray to the processing area with the filled vacutainer tubes and Laboratory Processing form.

5.2.7 Procedures for difficult draw

If a blood sample is not forthcoming, the following manipulations may be helpful.

- If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.
- If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.
- Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.
- DO NOT attempt a venipuncture more than twice unless a participant encourages you to do so.
- Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on their part.
- If venipuncture is unsuccessful, participant should be rescheduled at a later date, preferably with a different Field Center phlebotomist.
- Document any problems with venipuncture and sample collection on the Phlebotomy form. In particular, note whether a vein other than one of the antecubital veins was used.

5.2.8 Other possible problems

1) Not all tubes are collected (blood flow ceases, difficult venipuncture, etc.): *Always fill the collection tubes in the order specified.* Make notations of difficulties on the Phlebotomy form. If the participant is willing, another attempt should be made to complete the draw.

2) Collection tube does not fill: First, try another tube of the same type. Partially filled plasma tubes are not acceptable if less than 2/3 full. Partial tubes for serum are okay, but will result in a reduced number of aliquots. Check “No” for Question 12a (not filled to capacity).

5.2.9 Priority of tubes

A total of approximately 20 mL of blood will be drawn from each participant in 2 tubes. Tubes are numbered 1 and 2 and arranged in the rack to be drawn in the following order of priority:

- | | | |
|----|-------------|--------------|
| 1. | EDTA 10 mL | lavender top |
| 2. | Serum 10 mL | red top |

5.3 Blood mixing during venipuncture

Each tube should be treated as follows:

- #1 EDTA: place on mixer for ~30 seconds, then place in ice bath
- #2 Serum: do NOT mix; place in rack at room temperature for AT LEAST 40 minutes

6. Alert values/Follow-up

Most of the blood collected in Year 16 will be used for exploratory metabolic research purposes. No analysis are being done immediately, except for blood draw in the first visit as part of the muscle biopsy screening process which are not described here.

7. Quality assurance

7.1 Training requirements

Clinical experience with phlebotomy is mandatory. Additional training should include:

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

7.2 Certification requirements

- Complete training requirements
- Explain what to do for difficult venipuncture
- Recite measures to take for fainting participant
- Conduct phlebotomy on volunteer or participant while being observed by QC officer using QC checklist

7.3 Quality assurance checklist

Preparation:

- Blood collection trays properly prepared
- Blood draw tubes properly labeled
- Questions on Phlebotomy form asked
- Hepatitis B vaccination given or offered to all personnel handling blood

Venipuncture properly carried out:

- Script properly delivered
- Non-permeable lab coats, gloves, and face shields used
- Preparation of venipuncture site correctly done
- Venipuncture smoothly done
- Tubes filled in proper priority order

- Tourniquet removed at the start of tube 2
- Needle removed and arm bandaged correctly
- Needle and tubing appropriately disposed

Tubes mixed and handled correctly after filling:

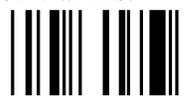
- Tube 1 mixed for at least 30 seconds, placed in ice bath
- Tube 2 NOT mixed, placed on rack at room temperature

Phlebotomy form properly filled out:

- Sample ID barcode label affixed to upper right corner (and to upper right corner of Lab Processing form)
- Time at start of venipuncture entered
- Time at end of venipuncture entered
- Total elapsed time with tourniquet entered
- Quality of venipuncture checked
- Total fasting time correctly calculated

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>

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

Place this end
on vial first



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

Place this end
on vial first



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



HABC Enrollment ID #	Acrostic	Visit Year
H [] [] [] [] [] []	[] [] [] [] [] []	● 16

PHLEBOTOMY

3. Do you bleed or bruise easily?
 Yes No Don't know Refused

4. Have you ever experienced fainting spells while having blood drawn?
 Yes No Don't know Refused

5. **Examiner Note: If participant already had the muscle tissue collection procedure, transcribe this information from the Muscle Tissue Collection Form, Page P19, Question #7. Otherwise ask:**
 What is the date and time you last ate anything?

a. Date of last food:	[] [] / [] [] / [] [] [] []
	Month Day Year
b. Time of last food:	[] [] : [] [] <input type="radio"/> am <input type="radio"/> pm
	Hours Minutes
c. How many hours have passed since the participant last ate any food?	[] [] hours (Question 8 minus Question 5b. Round to nearest hour.)

6. Which arm(s) can safely be used for phlebotomy?
 (Examiner Note: Refer to Phlebotomy Form, Page P25, Questions #1 and #2.)
 Right Left Either Neither

7. Date of blood draw:: [] [] / [] [] / [] [] [] []
 Month Day Year

8. Time venipuncture started: [] [] : [] [] am pm
 Hours Minutes

9. Time venipuncture completed: [] [] : [] [] am pm
 Hours Minutes

10. 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: _____ _____ _____
--



HABC Enrollment ID #	Acrostic	Visit Year
H [] [] [] [] []	[] [] [] [] []	● 16

PHLEBOTOMY

11. 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:)* _____

12. Was any blood drawn?

- Yes No, unable to draw blood No, participant refused

Please describe why not: _____

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

Tube	Volume	Filled to Capacity?		Comment
		Yes	No	
1. Plasma (EDTA)	10 mL	<input type="radio"/>	<input type="radio"/> →	_____
2. Serum	10 mL	<input type="radio"/>	<input type="radio"/> →	_____

Appendix 3 Phlebotomy Checklist

Blood Collection Tray Checklist

Per Tray:

- 10 21G Butterfly needles with Luer Adapters
- 10 Alcohol Swabs
- 15 Band-Aids
- 15 Gauze pads
- 7 Vacutainer holders
- Complete set of extra, unlabeled collection tubes
- 2 Tourniquets
- 1 Smelling salts
- 1 Timer or stopwatch
- 2 Pencils/pens
- Latex gloves
- 1 Hemostats
- 1 Adhesive tape
- 1 Scissors

~10 min before draw:

- 1 styrofoam ice bath filled with ice

Per participant:

- 1 Blood tube rack with 2 draw tubes labeled and numbered.

At the Phlebotomy Station:

- Basin
- Cold cloth
- Tube mixer
- Biohazard containers
- Needle/Sharps container
- Paper towels