Blood Collection
Maternal – Prenatal & Perinatal
Child – Early Childhood, Middle Childhood, Adolescence

Collection, Processing and Storage Protocol
Site or Home Collection

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A. Overview

- The blood collection procedures apply to mothers in prenatal and perinatal lifestages, and children in early childhood, middle childhood, and adolescence life stages. Collection of blood in each trimester from mothers in prenatal lifestage is recommended. Refer to the ECHO wide Cohort Data Collection Protocol (EWCP) for more details.
- For perinatal lifestage collection, collect maternal blood upon admission to labor and delivery otherwise attempt to collect within 8 hours of delivery. If not collected within this recommended time frame, note the deviation on the Specimen Tracking Form (STF).
- Instructions in this document are for site and home-visit collection.
- Aliquots of whole blood, plasma, buffy coat, serum and red blood cells will be saved from each participant. The buffy coat will be used for obtaining a source of DNA. In addition, whole blood will be applied to a blood spot card prior to centrifuging.
- If a participant refuses or is unable to provide a blood specimen via venipuncture, ask the participant to provide a blood specimen on a blood spot card using a lancet. Refer to the Blood Spot Card Collection, Processing and Storage Protocol.
- If a participant refuses or is unable to provide a blood specimen via venipuncture for obtaining a source of DNA, ask the participant to provide a saliva specimen. Refer to the Saliva Collection, Processing and Storage Protocol.

B. Guidelines

- The goal is to collect up to 15 mL whole blood, 5 mL each in two K2 EDTA tubes (lavender top) and 5 mL in a Serum tube (red top), from each participant. In case a participant is unable to provide 15 mL and/or a cohort must retain some portion of the specimen for their cohort-specific aims, a minimum of 10 mL (5 mL in an EDTA tube and 5 mL in serum tube) should be saved for EWCP.
  - The aliquots should be prepared in the order provided in the Processing section.
    - If you do not have sufficient volumes to prepare all aliquots, the small ones take priority over the large.
  - Filled Serum tube (red top) and EDTA (lavender top) tubes should be stored vertically before processing.
  - The Serum tube (red top) will be set aside at room temperature for a minimum 30 minutes and up to 60 minutes prior to centrifuging/processing. If delays in processing are unavoidable, at 60 minutes, the Serum tube should be placed at
2–8°C (refrigerated temperature; water ice bath or freezer gel packs) until processed and record the deviation on the STF.

- The EDTA (lavender top) tubes will also be placed at 2–8°C (refrigerated) upon collection and for a maximum 30 minutes prior to centrifuging/ aliquoting. If there is a delay in processing the lavender top tube, keep it on refrigerated temperature until processing and record the deviation on the STF.

- If EDTA (lavender top) is not placed at refrigerated temperature immediately upon collection, record the deviation on the STF and store the specimen at refrigerated temperature as soon as possible and then follow the procedure for processing.

- Note: **Use of dry ice prior to centrifuging/aliquoting is not recommended.**

- Five (5) 50 µL blood spots are placed on the Whatman blood spot cards from the EDTA tube. Allow 4 hours for blood spots to dry before closing the card. If 4 hours of drying time is not feasible, allow blood to dry for a minimum of 15 minutes to as long as possible, close the card and seal in a biohazard bag, and then reopen as soon as possible to allow it to dry completely.

- If tubes are centrifuged and aliquots are prepared off-site, store the cryovials and Whatman blood card on dry ice in an insulated shipper for no longer than 48 hours. If Whatman card cannot be placed in −20°C and cryovials in −80°C, then record deviation on the STF.

**•** This document includes procedures for blood draw with the participant in a seated position. It is possible for study staff to draw blood in another manner. Generally, blood is drawn from adults in a sitting position and from children in supine position.

**•** Elevations of 2–5% in lipid measures are observed after two minutes of continuous venous occlusion. It can also be uncomfortable for the participant therefore, a total tourniquet time of less than two minutes is required.

**•** Fasting is not required.

**•** Participants should be instructed to avoid vigorous exercise and to refrain from smoking cigarettes or any other substance and alcohol use one hour prior to the blood draw. Participants are allowed to take any required medications as soon as they wake up, but should refrain from taking any other medications prior to the blood draw. Blood should still be drawn if participant does not comply with these instructions and details of the event should be record on the STF.

**•** Skin numbing agents should be avoided as it may interfere with assays. If use is unavoidable, record on the STF.
• All materials provided in the collection and processing kits should be used. If other materials are used, for example, a local laboratory uses their own needle, record deviation on the STF.
• Refrigerated centrifuge is preferred during processing. Record the type of centrifuge (refrigerated, not) on the STF.
• Universal precautions need to be employed during any specimen collection.
• The following order of draw should be followed if collecting additional blood outside the EWCP:
  o Blood culture
  o Sodium citrate (light blue)
  o Serum (red)
  o Heparin plasma (green)
  o EDTA plasma (lavender)
  o Trace element (royal blue)
  o PAXgene RNA

  Note: In this circumstance, if a sodium citrate (light blue top) tube will be the first tube drawn, and a winged collection set is used, blood must be drawn into a discard tube before the light blue top tube. For details see Appendix A.

• The following is the maximum amount of blood that can be collected within a 24-hour period, including blood being collected for EWCP and other protocols:
  o Adults (not pregnant): 5mL/kg body weight
  o Pregnant adults: 40mL
  o Children: 3mL/kg body weight

  o Per 45 CFR § 46.110[f,2] and 21 CFR § 56.110[f,2], for minimal risk research purposes, the collection of blood by finger stick, heel stick, ear stick, or venipuncture from children and pregnant women should not exceed the lesser of 50 mL or 3 mL/kg in an 8-week period and may not occur more frequently than twice per week.

C. Collection Kit, Supplies and Kit Assembly
1. Blood Kit (Fisher BioServices)
   • Two (2) 6 mL K2 EDTA tubes (lavender top)
   • One (1) 6 mL Serum tube (red top)
   • One (1) 21 gauge butterfly safety-lok needle with a holder
• Three (3) twin specimen ID labels for tubes

2. Supplies (Site)
  • Powder-free gloves and laboratory coat
  • One (1) alcohol wipe
  • Two (2) sterile gauze (2 x 2 in)
  • Band-Aid or adhesive bandage
  • Tourniquet
  • Phlebotomy ball
  • Water Ice or freezer gel packs
  • Ice bath pan
  • Tube rack
  • Hand sanitizer for home-visit collection
Biohazard container or bag for home-visit collection
Sharp container or bag for home-visit collection
Portable Centrifuge if specimen is processed off-site
Blood – Specimen Tracking Form (STF)
Transport materials for home-visit collection:
  a. Kit includes an insulated shipper, cardboard box, gel packs, biohazard bag, and absorbent sleeves for transport to the study site.
     https://www.fishersci.com/shop/products/therapak-eas-refrigerated-shipper/22130438?searchHijack=true&searchTerm=22130438&searchType=RAPID&matchedCatNo=22130438
  b. Items can be ordered separately:
     i. Insulated Shipper: 608UPS
        https://www.thermosafe.com/model/608UPS
     ii. Optional: Insulated shipper with outer cardboard box: 609UPS
        https://www.thermosafe.com/subcategory/EPS+Foam+w/Corrugate
     iii. Water Ice or 3 Gel Packs: PP6
        https://www.thermosafe.com/subcategory/Gel+Packs
     iv. Biohazard bag: 6x6
     v. Absorbent sleeves for the tubes:

3. Kit Assembly
   Follow the below steps to assemble the collection kit and prepare for the collection prior to meeting with the participant.
   • Confirm that you have all the collection kit contents and supplies.
   • Print the STF and fill out the headers.
   • Record **KIT STOCK NUMBER** on the STF.
   • There will be two identical labels for each tube. Affix the first label to the EDTA tube (lavender top). Apply the second label to **STEP 1A** in the field labeled **EDTA #1** on the STF.
a. Repeat the labeling step for the second EDTA tube (lavender top), section **EDTA #2**, and Serum tube (red top), section **Serum (red top tube)**.

- Ensure that the labeled tubes and STF are available during collection.

### D. Site Collection

1. Site Pre-Collection Instructions for the Study Staff
   
i. Prepare the ice bath for EDTA lavender tubes by placing water ice or freezer gel packs in the bath pan.

   ii. Have the following items available:
       
       a. Three labeled tubes
       
       b. Tourniquet
       
       c. Phlebotomy ball
       
       d. One alcohol wipe
       
       e. Two sterile gauzes
       
       f. Band-Aid or adhesive bandage
       
       g. Blood - STF

   iii. Inform the participant that you will draw approximately 15 mL blood (1 tablespoon) in three tubes.

   iv. Ask the participant if he/she has a history of fainting. If yes, consider doing blood draw in a reclining position.

   v. Ask the participant when was the last time he/she ate or drank anything other than plain water. Record the answer in **STEP 0: PRE-COLLECTION** section on the STF.

2. Site Collection Instructions for the Study Staff
   
i. Find a place for the participant to sit while the blood draw is being done. The participant should be able to rest his or her selected arm comfortably while holding it straight at the elbow, palm up on a flat surface. There also should be room for the specimen collection contents.

   Note: The phlebotomist should consult with the participant their preference for the arm to draw blood from. If there is no preference, phlebotomist should consider using a non-dominant arm to reduce risk of hematoma. Do not draw blood from an arm that has a rash, open sore, is swollen, or shows signs of a recent venipuncture or hematoma. Do not draw blood from an arm that contains an arterial access such
as a fistula or shunt. Any institutional policy should always be followed. Note: Generally, blood is drawn from adults in a sitting position and from children in supine position.

ii. Cleanse hands thoroughly with water and soap and put on gloves and a laboratory coat.

iii. Organize the blood tubes in the appropriate draw order. Refer to the guidelines section.

iv. Prepare the site for a blood draw:
   - Have the participant extend his or her arm, palm up and straight at the elbow.
   - Position the arm on the work area so that the veins are readily accessible. Be sure the arm is in a downward position, with the elbow lower than the heart.
   - Inspect the selected arm for venipuncture. The veins of choice are those located in the antecubital area.
   - Apply the tourniquet several inches above the elbow and palpate for a suitable vein.
   - Select a vein that is palpable and well-fixed to surrounding tissue. Palpate even when the vein can be seen. Note: If the veins do not distend rather quickly, the following techniques may be used:
     - Massage the arm from wrist to elbow. This forces blood into the veins.
     - Tap the area sharply with the index and second finger two or three times; this causes the veins to dilate.
     - The arm to be used for venipuncture may be hung at the participant’s side without a tourniquet. This allows the veins to fill with blood to their capacity.
     - Examine the other arm. Sometimes the veins in one arm are larger than in the other.
     - Check carefully for scar tissue or the presence of tendons near the vein and avoid these areas.
     - If the tourniquet has been applied for more than 2 minutes while searching for a vein, release the tourniquet. Prolonged obstruction of blood flow by the tourniquet is unnecessary and
uncomfortable for the participant and may alter certain results. After 2–3 minutes, reapply the tourniquet but not too tightly.

- Ask the participant to squeeze the phlebotomy ball or make a tight fist.

  v. Open the 21 gauge butterfly needle assembly unit pre-attached to the tube holder.

  vi. Cleanse the area with an alcohol wipe. Hold the alcohol wipe with two fingers on one side so that only one side of the alcohol wipe touches the area of the puncture site. Cleanse the area using a circular motion beginning with a narrow radius and moving outward so as not to cross over the already-cleansed area. Allow area to air dry. Do not palpate the vein after cleansing the skin.

  vii. Remove the cover from the needle.

  viii. The vein should be fixed or held taut during the puncture. Place the left thumb about 1 inch below the point of entry and pull the skin gently in a downward motion (this stretches the skin and anchors or fixes the vein).

  ix. Hold the needle in line with the vein, with the bevel up and at a 15° angle with the skin, about 1/2-inch below the proposed point of entry to the vein.

  x. Push the needle firmly and deliberately into the vein. When firmly in the vein, blood appears in the tubing of the needle assembly past the end of the needle. This blood flow into the tubing verifies that the needle has adequately penetrated the selected vein.

  xi. Quickly push the first tube onto the needle in the holder, puncturing the center of the stopper. If blood is collected for EWCP only, the first tube should be Serum tube (red top).

  xii. Tips:

- If no blood enters the tube and no bruise is forming, probe the vein until entry is indicated by blood flowing into the tube.

- If no blood enters the tube and a bruise is forming, remove the needle immediately. Do not keep probing as this could cause severe bruising. Place a gauze pad over the puncture site and ask the participant to apply firm pressure to the puncture site for 3 minutes. Remove the tourniquet.

- Ask the participant if a second attempt may be made. If he or she agrees, make a second attempt on the other arm with new sterile collection supplies and new tubes. A second attempt is allowed only after verbal consent from the participant. No more than two attempts should be made.
- Release the tourniquet after the flow is established in the tube or if the participant becomes uncomfortable. The participant may open his or her fist once blood flow in the first tube is established.

xiii. When the first tube is filled to capacity, remove it from the holder and place the **second tube** in the holder. If blood is collected for EWCP only, the second tube should be **EDTA (lavender top)**.

  - If the first tube was Serum tube (red top), gently invert 5 times immediately upon removing and place upright on a tube rack at room temperature for 30 minutes.

xiv. When the second tube is filled to capacity, remove it from the holder and place the **third tube** in the holder.

  - If the second tube was EDTA (lavender top), gently invert 8–10 times immediately upon removing and place on water ice/freezer gel packs bath while filling the next tube.

xv. Repeat until all the desired tubes are filled.

  - If blood is collected for EWCP only, the **third tube** should be **EDTA (lavender top)**. Gently invert 8–10 times immediately upon removing and place on ice/freezer gel packs bath.

xvi. Do not allow the specimens to stand in direct sunlight or become exposed to extreme temperatures.

xvii. When the last tube is filled, carefully withdraw the needle, covering the puncture site with a sterile gauze pad. Remove the needle in a smooth and quick motion. NEVER apply pressure to the gauze until the needle is clear of the puncture site and away from the arm. Applying pressure during the needle withdrawal causes the needle tip to scratch the skin under the gauze.

xviii. Have the participant hold the gauze pad with mild pressure and raise the arm in the air for 2 minutes without bending the arm. This procedure helps prevent the formation of a hematoma.

xix. Immediately slide the needle safety guard forward to prevent an accidental needle stick. Place the entire used needle assembly in the sharps container. **Discard the entire assembly.** DO NOT reuse the needle holder for multiple blood draws.

xx. Check the venipuncture site. If it is adequately clotted, remove the gauze and apply a Band-Aid or adhesive wrap. Instruct the participant to remove it in no less than 45 minutes if the bleeding has stopped. Also, suggest that he or she sit quietly for
a few minutes. Observe the participant during this time for adverse effects. If bleeding continues, keep direct pressure on the site for 5 minutes or more.

3. Site Post-Collection Instructions for the Study Staff
   i. Ensure that the work area is reusable and contamination such as a spill, has not occurred.
      • If a spill has occurred, clean with a bleach wipe. Discard in the biohazard container. Note: put on gloves prior to performing any clean-up procedures.
   ii. After performing clean-up procedures, remove your gloves, discard them in the biohazard container, and wash your hands with soap and water.
   iii. Complete STEP 1 and 1A on the STF. Add date and time tubes were placed at refrigerated/room temperature in STEP 2A.
   iv. After up to 30 minutes on ice/freezer gel packs, transfer the EDTA (lavender top) tubes to the refrigerated centrifuge. Note: If there is a delay in processing, record on the STF.
   v. After minimum 30 minutes and up to 60 minutes, transfer the Serum tube (red top) to the refrigerated centrifuge. Note: If there is a delay in processing, the Serum tube (red top) should be placed at 2–8°C (refrigerated) temperature at 60 minutes until processed.
   vi. Complete STEP 2A on the STF.

E. Home-Visit Collection

1. Home Pre-Collection Instructions for the Study Staff
   i. If longer than 60 minutes are anticipated before tubes can be returned to the site for centrifuging/processing, study team/phlebotomists should take all the collection as well as processing supplies to the home visit including one of the portable centrifuges.
   ii. The EDTA (lavender top) tubes should be placed at 2–8°C (refrigerated temperature; water ice bath or freezer gel packs) immediately upon collection for maximum 30 minutes prior to centrifuging. If there is a delay in centrifuging or placing it in refrigerated temperature, record the deviations on the STF.
   iii. The Serum tube (red top) should be kept at room temperature for minimum 30 minutes and up to 60 minutes, prior to centrifuging. If Serum tube cannot be
processed at 60 minutes, place the tube at 2–8°C (refrigerated) temperature until processing.

**Note: Use of dry ice prior to centrifuging/aliquoting is not recommended.**

iv. If there is a delay in centrifuging any tubes, record specimen condition and time in **STEP 1A** and **STEP 2A** on the STF.

v. If tubes are centrifuged and aliquots are prepared off-site, ensure that you have supplies indicated in the processing section. Store the cryovials and Whatman blood card on dry ice in an insulated shipper for no longer than 48 hours. If Whatman card cannot be placed in −20°C and cryovials in −80°C, then record deviation on the STF.

vi. Remember to take freezer gel packs/ water ice and/or dry ice, depending on what procedures will be performed off-site, in an insulated shipper and close the lid. Ensure that you have a biohazard bag and absorbent sleeves.

Note: If using freezer gel packs, they must be placed in the freezer 24 hours or longer prior to the home visit.

2. Home Collection Instructions for the Study Staff
   
i. **If centrifuging will be performed at home, it is recommended that the study staff sets up the centrifuge prior to the blood draw.**
   
   ii. Inform the participant that you are will be drawing approximately 15 ml (1 tablespoon) blood in three tubes.
   
   iii. Ask the participant if he/she has a history of fainting. If yes, consider doing blood draw in a reclining position.
   
   iv. Ask the participant when was the last time he/she ate or drank anything other than plain water. Record the answer in **STEP 0: PRE-COLLECTION** on the STF.
   
   v. Draw blood using steps indicated in site collection instructions (Section D.2).
   
   vi. After centrifuging and aliquoting, specimen can be placed on dry ice until placed in the −80°C freezer. When placing tubes on dry ice, wrap them in the absorbent sleeves, place in biohazard bag, and then put on dry ice in an insulated shipper.

3. Home Post-Collection Instructions for the Study Staff
   
i. Ensure that the area is clean.
   
   ii. Remove your gloves, discard them in the biohazard bag, and cleanse your hand with a hand sanitizer.
iii. Fill **STEP 1, 1A, 2 and 2A** (as applicable).

iv. Be sure to pack the biohazard bag appropriately for transport to the site. The biohazard bag must be disposed of properly at the site. Transport specimen to the site.

**F. Processing**

1. **Blood Kit (Fisher BioServices)**
   - Two (2) 2 mL FluidX (metals-free) cryovials (orange capped) – Whole Blood
   - Ten (10) 1 mL cryovials & ten (10) orange cap inserts – Plasma
   - Three (3) 2 mL cryovials & three (3) orange cap inserts – Plasma
   - Two (2) 1 mL cryovials & two (2) green cap inserts – Buffy coat
   - Two (2) 2 mL cryovials & two (2) red cap inserts – Red Blood Cells
   - Five (5) 1 mL cryovials (white capped) – Serum
   - Two (2) 2 mL cryovials (white capped) – Serum
   - One (1) pipette
   - One (1) Whatman blood spot card, biohazard bag, 2 desiccants
   - One (1) twin specimen ID labels for Whatman blood spot card
   - Twenty-six (26) twin specimen ID labels for cryovials

![Blood Kit Images](image-url)
2. Processing Supplies (Site)
   - Powder free gloves, eye wear, laboratory coat
   - −80°C Freezer
   - −20°C Freezer
   - Volumetric pipette and 5 tips
   - Tube rack
   - Refrigerated (preferred) Centrifuge (1500 g)
   - Biohazard container
   - Cryobox(es) for 1 mL and 2 mL cryovial storage (5 x 5 x 2 in; 81 cell; 9 x 9 grid)
   - Storage Container for Whatman spot card
     a. Recommendation: 5 x 5 x 3 in. box (30 Whatman cards per box)
   - Blood – Specimen Tracking Form (STF)
3. Pre Processing

- **Home-Visit Collection:** Upon receipt of specimen to the site, complete **STEP 2** on the STF.
- Ensure that you have all processing kit contents and supplies.
- When ready to process, gather the specimen collection tubes and complete **STEP 2A** on the STF.
- Each cryovial must be labeled and the identical label placed on the appropriate section of the STF (**STEP 3, 3C–3F, 4, and 4A**).
- Specimen ID label should be affixed to the back side of the Whatman spot card and the matching label is placed in **STEP 3A** on the STF. Do not write on card.

4. Processing

- The following illustration provides an overview of the centrifuge and aliquoting steps:

***R = Remainder of sample: may not be full aliquot volume***

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**ECHO Blood Collection, Processing and Storage Protocol**

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• Wear gloves, eye wear and a laboratory coat.

• The following steps should be followed to centrifuge and prepare as many aliquots as possible. It is possible your number of aliquots do not match the aliquots in the illustration. If only one EDTA (lavender top) and one Serum tube (red top) is available for ECHO-wide Cohort Data Collection Protocol, follow the same order and prepare as many aliquots as possible.

  i. Pre-cool the centrifuge at 4°C. The shields should be refrigerated if they are removed from the centrifuge, so that they will be chilled prior to spinning blood. Maintain the temperature of 4°C by keeping the centrifuge door closed and locked until ready for loading.

  ii. **Before centrifuging the tubes**, from one EDTA (lavender top) tube, transfer each 0.5 mL whole blood into two 2 mL metals-free cryovials using the pipette provided in the kit.

  iii. Then, using volumetric pipette and a tip, draw up **50 μL of whole blood** from the same EDTA (lavender top) tube and apply to the first spot on the Whatman blood spot card. Do not touch the pipette tip to the card. Refer to the Blood Spot CPS Protocol for details.

  • Repeat this procedure an additional 4 times to complete the remainder of the 4 spots on the blood spot card.

  • Note: It is recommended to allow 4 hours for blood spots to dry before storing. If 4 hours of drying time is not feasible, allow blood to dry for a minimum of 15 minutes to as long as possible, close the card and seal in a biohazard bag, and then reopen as soon as possible to allow it to dry completely.

  iv. Set the centrifuge TIMER control to 15 minutes and the speed to 1500 g.

  v. Balance the load.

  Warning: A balanced load is essential for the safe operation of all centrifuges. An unbalanced load produces vibration and can damage the unit, therefore, always make sure that the rotor is loaded symmetrically with a full complement of accessories, and with a full (or paired) set of tubes. Tube adapters should also be installed symmetrically corresponding in size, shape, and relative position of parts or opposite sides. To obtain good dynamic balance, the opposite loads must not only be equal in mass, but must also have the same center of gravity. Tubes filled with water (or 50/50 water/alcohol) can be used to equal the volume
of processing specimens. Failure to balance can result in injury or loss of specimen or both.

vi. Load the rotor, being careful to place balanced tubes directly opposite each other.

vii. Recheck the centrifuge preset parameters for temperature, speed, time and brake position.

viii. Close cover and lock.

ix. Push the RUN button to begin the run cycle. Complete **STEP 3B: CENTRIFUGING** on the STF.

x. The centrifuge will stop automatically at the pre-selected time and the stop indicator will light. The centrifuge brake can be used to slow down the centrifuge, but must be engaged slowly to avoid disturbing the cell layer. If the brake is used, use of the lowest braking speed possible is recommended to avoid needing to re-centrifuge. Re-centrifuge the specimen if the cell layer is disturbed by braking. Remove specimens one at a time and carefully place them in the tube rack.

xi. To remove the stopper from the tube you are working with, use gauze to remove the stopper directing the inside of the stopper away from your face.

xii. The EDTA (lavender top) and Serum tube (red top) tubes should look similar to this after centrifuge:

![Image of centrifuged tubes]

xiii. Visually inspect serum and plasma for the presence of hemolysis prior to aliquoting. Record on the STF the degree of hemolysis observed as none, mild, moderate, or severe according to color grading scheme below:
xiv. For aliquoting, remember to use a new pipette tip for each blood part: plasma, buffy coat, red blood cells.

xv. Arrange cryovials in the tube rack to prepare for aliquoting.

xvi. Use caution not to disturb the next cell layer cells when drawing plasma into a pipette tip. Insert the tip as far into the tube as possible, tilting the tube slightly and moving the pipette down as the pipette tip is filled to obtain maximal volume.

xvii. Prepare the following aliquots from EDTA (lavender top) tubes:

Note: In STEP 3C of the STF, indicate the EDTA tube that the 120 µL plasma aliquots are prepared from.

- **10x 120 µL plasma** in 1 mL cryovials (orange capped) – take these from a single EDTA tube, using the tube with the least hemolysis.
- **Up to 3x 1 mL plasma** in 2 mL cryovials (orange capped) – for a given aliquot, do not combine plasma from more than 1 EDTA tube
- **2x Buffy coat** in 1 mL cryovials (green capped)
  - Note: The buffy coat is a thin layer between the plasma (clear layer above) and red blood cells (red fluid below).
- **2x 1.8 mL red blood cells** in 2 mL cryovials (red capped)

xviii. Prepare the following aliquots from Serum tube (red top):

- **5x 120 µL serum** in 1 mL cryovials
- **2x1 mL serum** in 2 mL cryovials
ECHO Blood Collection, Processing and Storage Protocol

xix. Cap cryovials immediately after aliquoting the specimen before proceeding to the next specimen to prevent evaporation of specimens.
xx. Dispose blood collection tubes in biohazard container, re-capped with their original stoppers.
xxi. Remove gloves, discard in a biohazard container, and wash hands thoroughly.

5. Post-Processing
   - Ensure that 1 mL and 2 mL cryovials are placed in a labeled cryobox (5 x 5 x 2 in; 81 cell; 9 x 9 grid).
     - Both 1 and 2 mL cryovials can be placed in the same box, as can cryovials containing aliquots of different blood components.
   - Once the Whatman blood spot card is dried, place it in a biohazard bag and include 2 desiccants. Place the bag in a labelled storage container.
   - Ensure that STEP 3–3F, 4 and 4A on the STF are filled except storage date and time.

G. Storage
   - Place labeled cryoboxes with blood cryovials immediately in a −80°C freezer, otherwise as soon as possible and preferably within 90 minutes of blood collection.
   - Place labeled storage container containing Whatman biohazard bag with in a −20°C freezer.
   - Enter storage date and time in STEP 3–3F, 4 and 4A on the STF.
   - Data from STF should be entered into Bio-Track immediately or within 48 hours of storing the specimen.

H. Shipping to Biorepository
   This information is available in the Laboratory Manual of ECHO-wide Cohort Data Collection Protocol.

I. Supporting Documents
   1. Blood – Specimen Tracking Form (STF)
   2. Blood – Specimen Tracking Form (STF) Completion Instructions
APPENDIX A: Vacutainer Order of Draw for Multiple Tube Collections

- It is possible that ECHO-wide Cohort Data Collection Protocol blood and ECHO cohort-specific blood are drawn in the same venipuncture. If a sodium citrate (light blue top) tube is to be drawn for the ECHO cohort specific research, it should be the first tube filled.

- Note the following footnote information (with slight editorial insertions in square brackets) regarding the need for a discard tube in this circumstance if a winged blood collection set (“butterfly”) is used:
  - When using a winged blood collection set for venipuncture and a coagulation (citrate) tube is the first specimen tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection set tubing’s "dead space" with blood but the discard tube does not need to be completely filled. This important step will ensure proper blood-to-additive ratio. The discard tube should be a nonadditive or coagulation tube.

BD Vacutainer® Order of Draw for Multiple Tube Collections

<table>
<thead>
<tr>
<th>Closure Color</th>
<th>Collection Tube</th>
<th>Mix by Inverting</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Vacutainer® Blood Collection Tubes (glass or plastic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*</td>
<td>Blood Cultures - SPS</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>Citrate Tube*</td>
<td>3 to 4 times</td>
</tr>
<tr>
<td>or</td>
<td>BD Vacutainer® SST™ Gel Separator Tube</td>
<td>5 times</td>
</tr>
<tr>
<td></td>
<td>Serum Tube (glass or plastic)</td>
<td>5 times (plastic) none (glass)</td>
</tr>
<tr>
<td></td>
<td>BD Vacutainer® Rapid Serum Tube (RST)</td>
<td>5 to 6 times</td>
</tr>
<tr>
<td>or</td>
<td>BD Vacutainer® PST™ Gel Separator Tube With Heparin</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>Heparin Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>EDTA Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td>or</td>
<td>BD Vacutainer® PPT™ Separator Tube K$_2$EDTA with Gel</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>Fluoride (glucose) Tube</td>
<td>8 to 10 times</td>
</tr>
</tbody>
</table>
“dead space” with blood but the discard tube does not need to be completely filled. This important step will ensure proper blood-to-additive ratio [in the citrate tube]. The discard tube should be a nonadditive [e.g., red top] or coagulation [citrate] tube.

- The following can be used as a discard tube:
  - 3.0 mL clear top tube (BD Vacutainer 13 x 75 mm tube, Fisher catalog #02-657-30)