Cord Blood Collection
Perinatal

Collection, Processing and Storage Protocol

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A. Overview (all delivery specimens: maternal blood, cord blood, placenta)

- Fresh cord blood and placental tissue will be collected for the ECHO-wide Cohort Data Collection Protocol (EWCP) and processed (aliquoted) at the site prior to shipping to the biorepository for storage.
- Cohorts are expected to fulfill placental and cord blood collection requirements for cohort-specific aims and contribute remaining specimens to the EWCP.
- All sites are expected to follow their local or institutional policies when handling placenta and cord blood specimens.
- All or selected collection and processing procedures in this document should be followed based on the cohort’s expertise and availability of resources. The following tables provides an overview of the delivery collections in relation to time of delivery:

<table>
<thead>
<tr>
<th>Admission to Labor and Delivery</th>
<th><em>Maternal Blood</em> Perinatal: Recommended</th>
<th><strong>Cord Blood</strong> Recommended</th>
<th>Placenta Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collect specimen upon admission to Labor and Delivery</td>
<td>Option 1 - Collect cord blood directly from the cord: Study/clinical staff collects 15 mL cord blood in tubes directly from the cord and reclamps cord. Prepare blood spot card.</td>
<td>Photograph (pre-biopsies)</td>
<td>Clean away blood clots and blood. Photograph fetal and maternal surfaces of the placenta.</td>
</tr>
<tr>
<td>Delivery</td>
<td>Option 2 - Collect Cord blood from placental vessels: If 15 mL cord blood was not already collected, study/clinical staff collects blood from placental vessels using syringe within 30 minutes of delivery (preferably after taking the first set of fetal photographs if collecting placenta). Prepare blood spot card, if not already done above.</td>
<td>Biopsies:</td>
<td>If collected within 3 hours, store tissue for epigenetics in RNAlater and for environmental analysis.</td>
</tr>
<tr>
<td>Collect if specimen has not been collected at admission to Labor and Delivery. Attempt to collect within 8 hours of delivery. If not collected within recommended time, note deviation on Specimen Tracking Form (STF).</td>
<td></td>
<td>If collected within 12 hours, store tissue for environmental analysis only.</td>
<td></td>
</tr>
</tbody>
</table>
B. Cord Blood Collection 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 the cord. In case a 15 mL collection is not possible and/or a cohort must retain some portion of the specimen for their cohort-specific aims, cohorts are encouraged to collect whatever volume is feasible for the EWCP. The volume collected should be split equally into EDTA (lavender top) and serum (red top) tubes.
  - 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 Specimen Tracking Form (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 at refrigerated
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temperature until processing and record the deviation on the STF.

- If the 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.
  - Use of dry ice or liquid nitrogen prior to centrifuging/aliquoting is not recommended.
  - A refrigerated centrifuge is preferred during processing. Record the type of centrifuge (refrigerated, not) on the STF.

- When study team is informed that a mother is about to deliver/has delivered, ask the nurse/clinical staff to re-clamp the cord following any clinical blood collections.

- Option 1: Study/clinical staff collects 5 mL cord blood into each of 3 tubes, two EDTA and one serum, using drip method in the delivery room. Post-delivery room, site staff places five full blood spots on a Whatman spot card from a K2 EDTA tube using a syringe.

- Option 2: If 15 mL cord blood is not collected in option 1, study staff draws blood from placental vein using a syringe prior to clotting, typically within 30 minutes of delivery. Site will try to fill 5 mL into each of 3 tubes, two EDTA and one serum. Do not combine blood into one tube if two options are used. Site staff will also place five full blood spots on a Whatman spot card from a K2 EDTA tube using a syringe.

- 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.

C. Collection Kit, Supplies and Kit Assembly

1. Cord Blood Kit (Fisher BioServices)
   - Two (2) 6 mL K2 EDTA tubes (lavender top)
   - One (1) 6 mL serum tube (red top)
   - One (1) 22 gauge needle
   - One (1) 20 mL luer-Lok sterile syringe
   - One (1) Whatman blood spot card, biohazard bag, 2 desiccants
• Four (4) twin specimen ID labels

2. Supplies (Site)
• Powder-free gloves, eyewear, and a laboratory coat
• Blue absorbent underpad (chux)
• Water ice or freezer gel packs
• Ice bath pan/bucket
• Tube rack
• Sterile clamp and hemostat(s)
• Sharps container
• Biohazard container
• −20°C freezer for Whatman spot card
• Storage container for Whatman spot card
  a. Recommendation: 5 x 5 x 3 in. box with no grid (30 Whatman cards per box)
• Cord blood – Specimen Tracking Form (STF)
• Portable centrifuge if specimen is processed off-site
• Transport materials for home/satellite site 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 steps below to assemble the collection kit prior to collecting cord blood in delivery room or post-delivery.
   • Print the Cord Blood STF and fill out headers.
   • Record **KIT STOCK NUMBER** on the STF.
   • There will be two identical labels for each tube. Affix the first label to the serum tube and apply the second label in **STEP 1A** of the STF, under serum (red top tube) field.
     o Repeat the labeling step for the two EDTA (lavender top) tubes.
     o Ensure that all tubes are labeled and matching labels are applied to the STF prior to collection.
• Affix another label to back side of Whatman spot card. Do not write on card. Place duplicate label in **STEP 3A** on the STF.

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**D. Option 1: Cord Blood Collection from the Cord in Delivery Room**

• Provide two labeled K2 EDTA tubes (lavender top) and a serum tube (red top), and a tube rack to the study/clinical staff in the delivery room.

• Upon delivery, study/clinical staff drips 5 mL cord blood directly into each tube from the umbilical cord.
  a. Cap and gently invert the serum tube 5 times and place upright on a tube rack.
  b. Cap and gently invert the EDTA tubes 8 to 10 times and place upright on a tube rack.

• Study/clinical provides the tube rack to the study staff for processing and informs them about the time specimen was collected.

• Study staff completes **STEP 1**, records date and time of collection and other information as available in **STEP 1A** on the STF.

• Whatman Spot Card:
  a. Attach syringe to the needle and withdraw air into syringe to assure plunger is free to move.
  b. Using the syringe, draw approximately 5 full drops of blood.
     i. Place a drop to the first spot on the Whatman blood spot card. Do not let the needle tip touch the card.
  c. Repeat this procedure an additional 4 times to complete the remainder of the 4 spots on the blood spot card. The spot will contain 5 spots total.
d. 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.
e. Fill out STEP 3A on the STF.
   - Keep K2 EDTA tubes at 2–8°C (refrigerated) temperature (water ice bath or freezer
gel packs) temperature for 30 minutes and record the time in STEP 2A on the STF.
   - After 30 minutes, transfer the EDTA tubes to the refrigerated centrifuge. Note: If there
     is a delay in processing, record on the STF.
   - Keep serum tube at room temperature for 30 minutes. After a minimum of 30 minutes
     and up to 60 minutes, transfer the serum tube to a refrigerated centrifuge. Note: If
     there is a delay in processing, the serum tube should be placed at 2–8°C (refrigerated)
temperature (water ice bath or freezer gel packs) for 60 minutes until processed. Fill
     out fields in STEP 2A on the STF.

E. Option 2: Cord Blood Collection from the Placenta Vessels Post-Delivery
   Room
   - If 15 mL cord blood is not collected in the delivery room, recover blood from placental
     vessels on fetal surface as soon as possible after delivery.
   - If collecting placental tissue, follow instructions on taking pictures of placental
     fetal surface in the Placenta CPS protocol and then perform cord blood
     collection using instructions below. After cord blood collection, photograph
     maternal surface, perform biopsies, re-photograph maternal and fetal surface,
     and fixate placenta in formalin as described in the Placenta CPS protocol.
   - Wear gloves, eyewear, and a laboratory coat.
   - Place placenta on a chux.
   - Remove amnion from the chorionic plate (fetal) surface of the placenta, if not already
     removed for photography of the fetal and maternal surfaces.
     - Best to lift the amnion from the chorionic plate. Cut the membrane and place
       your finger or forceps to separate and lift amnion from the entire chorionic
       plate. You will need to cut the amnion from the umbilical cord.
   - Attach syringe to the needle and withdraw air into syringe to assure plunger is free to
     move.
• Select a bulging placental vein on the chorionic plate surface.
• Have a hemostat nearby to clamp vessel when withdrawing needle.
• Insert needle into the vein and withdraw blood to meet the 15 mL collection recommendation and some extra blood for the blood spot card. We recommend collecting 20 mL in the syringe (CC).
  Note: Do not combine cord blood into one tube if both options are used.
  Note: If more blood is required for cohort-specific protocol, clamp vein with the hemostat and insert needle into another placental vein. Once required amount is collected, use another hemostat to clamp the vein.
• If necessary, massage the placenta gently to encourage blood flow and rotate the needle.
• Whatman Spot Card (if not already done):
  o From the syringe, place a full drop of blood on the first spot on the Whatman blood spot card. Do not let the needle tip touch the card.
    ▪ Repeat this procedure 4 times to complete the remaining 4 spots on the blood spot card. The card will contain 5 spots total.
    ▪ Using appropriate precautions to avoid needle stick injury, push the safety cover on the needle and remove the needle from the syringe. Safely dispose the needle in a sharp container.
    ▪ 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 or as long as possible, close the card, seal in a biohazard bag, and then reopen as soon as possible to allow it to dry completely.
    ▪ Fill out STEP 3A on the STF
• From the syringe, add up to 5 mL blood in the serum tube (red top). Gently invert the tube 5 times immediately upon filling and place upright on a tube rack.
• Add up to 5 mL in each of two K2 EDTA tubes (lavender top). Gently invert the tube 8 to 10 times immediately upon filling and place upright on a tube rack.
• Discard the entire syringe assembly
• Complete **STEP 1**, records date and time of collection and other information as available in **STEP 1A** on the STF.
• Place the EDTA tubes at 2–8°C (refrigerated) temperature (water ice bath or freezer gel packs) for maximum 30 minutes prior to transferring to a refrigerated centrifuge, and **record time on STEP 2A on the STF**.
• Keep serum tube at room temperature for 30 minutes. After minimum 30 minutes and up to 60 minutes, transfer the serum tube to a refrigerated centrifuge. Note: If there is a delay in processing, the serum tube should be placed at 2–8°C (refrigerated) temperature for 60 minutes until processed.
• **Use of dry ice prior to centrifuging/ aliquoting is not recommended. Avoid allowing specimen to freeze.**
• Remove your gloves, discard them in the biohazard container, and wash your hands.

F. Home/Satellite Site Collection

• It is possible that cord blood collection occurs outside the central site using option(s) 1 and/or 2 above.
• If longer than 60 minutes is anticipated before tubes can be returned to the site for centrifuging/processing, study teams should take all the collection as well as processing supplies, including a portable centrifuge.
• Remember to take freezer gel packs/water ice if specimen is transported back to the site for processing within 60 minutes.
  o Note: If using freezer gel packs, they must be placed in the freezer 24 hours or more prior to the visit
• If centrifuging and aliquoting is performed, take dry ice.
• After centrifuging and aliquoting, store the cryovials and Whatman card in dry ice until placed in the −80°C freezer (Whatman card in −20°C). When placing tubes on dry ice, wrap them in the absorbent sleeves, place in biohazard bag and close the insulated shipper lid. Store specimen in an insulated shipper for no longer than 48 hours.
• Complete **STEP 2** on the STF to provide information about specimen condition.

G. Post-Collection

• If collecting placental tissue, follow the instructions for taking pictures,
performing biopsies and formalin fixation in the Placenta CPS Protocol prior to processing cord blood.

- After up to 30 minutes on ice/freezer gel packs, transfer the EDTA (lavender top) tubes to the refrigerated centrifuge and record time in STEP 2A of the STF. If no refrigerated centrifuge is available, note type of centrifuge used on the STF.
- After a minimum of 30 minutes and up to 60 minutes, transfer the serum tube (red top) to the refrigerated centrifuge and record data in STEP 2A on the STF. Clotting should occur between 30–60 minutes. Note: If there is a delay in processing, the serum tube should be placed at 2–8°C (refrigerated) temperature for 60 minutes until processed.
- Once the Whatman blood spot card is dried, place it in a biohazard bag and include 2 desiccants. Place the bag in a labeled storage container and store at −20°C. Complete STEP 3A on the STF.

H. Cord Blood Processing

1. Processing Kit (Fisher Bioservices)
   - Two (2) 2 mL FluidX (metals-free) cryovials (orange capped) – whole blood
   - Ten (10) 1 mL cryovials and ten (10) orange cap inserts – plasma
   - Three (3) 2 mL cryovials and three (3) orange cap inserts – plasma
   - Two (2) 1 mL cryovials and two (2) green cap inserts – buffy coat
   - Two (2) 2 mL cryovials and 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
   - Twenty-six (26) twin specimen ID labels for cryovials
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- **Plasma**
  - 2 mL cryovial x 3
  - Orange Cap Inserts x 3
  - Twin Specimen IDs x 3

- **Buffy Coat**
  - 1 mL cryovial x 2
  - Green Cap Inserts x 2
  - Twin Specimen IDs x 2

- **RBCs**
  - 2 mL cryovial x 2
  - Red Cap Inserts x 2
  - Twin Specimen IDs x 2

- **Serum**
  - 1 mL cryovial x 5
  - Twin Specimen IDs x 6

- **Transfer Pipette**
  - 2 mL Fluid X tube x 2
  - Twin Specimen IDs x 2
2. Processing Supplies (Site)
- Powder-free gloves, eyewear, laboratory coat
- −80°C freezer
- −20°C freezer
- Micropipette and tips
- Tube rack
- Refrigerated (preferred) centrifuge (1500 x 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)
- Cord Blood – Specimen Tracking Form (STF)

3. Pre Processing
- Ensure that you have all processing kit contents and supplies.
- When ready to process, gather the specimen collection tubes and ensure that STEP 2A of the STF is completed.
- **Remember to place the colored cap inserts on the appropriate cryovials.**
- It is recommended that 2mL Fluid X metals-free cryovials are filled from the EDTA tube that was collected using the drip method directly from the cord blood OR if syringe method was used, use EDTA tube filled last/second.
  - If only one EDTA tube was collected, it can be used to fill metals-free cryovials.
- Each cryovial must be labeled and the identical label placed on the appropriate section of the STF (STEP 3, 3C–3F, 4, and 4A).

4. Processing
- The following illustration provides an overview of the centrifuge and aliquoting steps:
- Wear gloves, eyewear and a laboratory coat.
- The following steps should be followed to centrifuge and prepare as many of the 26 specified aliquots as possible. If the blood draw yields less than 15 mL of whole blood, it is possible your number of aliquots will be fewer than shown in the illustration. Follow the same order and prepare as many of the 26 specified aliquots as possible.
  - 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.
  - Before centrifuging the tubes, transfer each 0.5 mL whole blood from one EDTA (lavender top) tube into two 2 mL metals-free cryovials using the pipette provided in the kit.
  - Set the centrifuge TIMER control to 15 minutes and the speed to 1500 x g.
  - If your centrifuge does not display RCF values, consult the manual for a conversion chart or use the following formula:

\[
RPM = \sqrt{\frac{(RCF+100,000)}{(1.12+r)}}
\]

where "r", expressed in cm, is the radial distance from the center of the centrifuge head to the bottom of the tube.
- 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.
- Load the rotor, being careful to place balanced tubes directly opposite each other.
- Recheck the centrifuge preset parameters for temperature, speed, time, and brake position.
- Close cover and lock.
- Push the RUN button to begin the run cycle. Complete **STEP 3B: CENTRIFUGING** on the STF.

- 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 recentrifuge. Recentrifuge the specimen if the cell layer is disturbed by braking. Remove specimens one at a time and carefully place them in the tube rack.
- 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.
- The EDTA (lavender top) and serum tube (red top) tubes should look similar to this after centrifuge:

- 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 the color grading scheme below:

- For aliquoting, remember to use a new pipette tip for each blood part: plasma, buffy coat, red blood cells.
- Arrange cryovials in the tube rack to prepare for aliquoting.
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.

Prepare the following aliquots from EDTA (lavender top) tubes:
- **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)

Prepare the following aliquots from serum tube (red top):
- **5x 120 µL serum** in 1 mL cryovials
- **2x 1 mL serum** in 2 mL cryovials

Cap cryovials immediately after aliquoting the specimen before proceeding to the next specimen to prevent evaporation of specimens.

Dispose blood collection tubes in biohazard container, re-capped with their original stoppers.

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 cells; 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.
- If not already done, once the Whatman blood spot card is dried, place it in a biohazard bag and include 2 desiccants. Place the bag in a labeled storage container.
- Ensure that **STEP 3–3F, 4A and 4B** on the STF are completed except for storage date and time.
I. Storage

- Immediately place labeled cryoboxes with cryovials immediately in a −80°C freezer, otherwise as soon as possible and preferably within 90 minutes of 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.

J. Shipping to Biorepository

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

K. Supporting Documents

1. Cord Blood – Specimen Tracking Form (STF)
2. Cord Blood – Specimen Tracking Form (STF) Completion Instructions