Breast Milk Collection
Infancy

Collection, Processing and Storage Protocol
Site Collection

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A. Overview

- These Breast milk collection procedures apply to collection at site during the first post-partum visit.

B. Guidelines

- The following stages correspond to milk maturity: colostrum (days 2–3); transitional milk (days 8–12); mature milk (days 26–30), typically complete by 6 weeks postpartum; and weaning (varies by mother).
- Breast milk collection is recommended in the mature milk stage, at 1–3 months postpartum; however, colostrum or transitional milk is acceptable if a mother is scheduled for an early first post-partum visit.
- The goal is to collect a minimum of 25 mL and up to 33 mL of breast milk for the ECHO-wide Cohort Data Collection Protocol (EWCP). Cohorts that collect breast milk for their cohort-specific aims are expected to collect additional specimen per their protocol.
- If colostrum is provided, collect up to 1 mL.
- The participant can collect breast milk by squeezing out the breast milk by hand or by using the Medela pump provided. If using the Medela pump, do not wash any components.
- Two Whatman spot cards will be prepared from each participant. Five 50 µL breast milk spots will be placed on each card prior to aliquoting.
- Specimen should be aliquoted and stored immediately in a −80°C freezer.
- In case delays are unavoidable, place the specimen in refrigerator for up to 24 hours.
- The inside of the collection container must not come into contact with any part of the body, clothing or external surfaces.
- Exposure of breast milk to air should be minimized.

C. Collection Kit, Supplies and Kit Assembly

1. Breast Milk Site-Collection Kit (Fisher BioServices)
   - One (1) Medela Harmony manual breast pump
   - Two (2) bottles and lids (only one set is used for collection)
   - One (1) sterile gauze packet
   - One (1) triplet specimen ID labels
2. Collection Supplies (Site)

- Powder-free gloves
- Breast Milk – Specimen Information Form (SIF)
- Breast Milk – Specimen Tracking Form (STF)

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 the collection kit includes a Medela pump, two bottles, two lids, sterile gauze, and triplet specimen ID labels. **NOTE: Only one bottle and lid will be used for collection. The other set can be provided to the mother as a gift.**
- Print SIF and STF and fill out the headers.
- There will be three identical labels for each specimen ID. Affix the first label to the bottle. Place the second matching label to the field labeled `SPECIMEN LABEL` on the STF and the third matching label to the field labeled `SPECIMEN LABEL` on the SIF.
  
  Note: Study staff must complete the above steps before providing the materials to the participant in order to prevent misplacement or confusion about the forms/collection materials.
- Record `STOCK NUMBER` on the STF. Mark `KIT DISTRIBUTION` section as not applicable.
- Pump, bottle, lid, and sterile gauze will be used during collection.
- Retain the STF at the site.

**D. Site Collection**

1. **Pre-Collection Instructions for the Study Staff**
   - Review the collection kit contents and collection instructions with the participant.
   - Confirm if the participant will provide breast milk or colostrum.
   - Inform the participant that:
     - Breast milk can be collected by squeezing out the breast milk by hand or using the Medela pump provided.
     - She should collect up to 33 mL of breast milk or 1 mL of colostrum. It is acceptable to collect less than these amounts, if necessary.
     - She can provide the breast milk from one breast only.
     - She should wash the breasts using warm tap water only and use the sterile gauze to dry.
     - She should not use any soaps, cleansers, lotions or creams on the breast or nipple prior to the specimen collection.
     - The inside of the bottle must not come into contact with any part of the body, clothing or external surfaces.
   - Provide her the Breast Milk Specimen Information Form (SIF) to complete after the collection.

2. **Collection Instructions for the Participant and Study Staff**
   - Ask the participant if she would like to use the Medela pump or use hand expression.
• Allow time and privacy to the participant to wash her breasts using warm tap water and gauze to dry.
• If the participant chooses not to use the breast pump, provide her with gloves to wear. Hand her the bottle and ask to squeeze out the breast milk by hand, collecting directly into the bottle provided. Close the lid immediately. Collect the bottle from the participant.
• If the participant chooses to use the Medela pump, lay out the collection materials: gloves, Medela manual breast pump and bottle.
• Put on sterile gloves and remove Medela manual breast pump from its package. Check to make sure that the bottle is properly attached to the pump.
• Carefully attach the breast shield to the pump body, holding only the outer surface of the plastic as to not contaminate the inner part of the breast shield.
• Ask the participant to place the breast shield on the nipple. The nipple should be centered in the tunnel of the shield.
• Ask the participant to start pumping in the stimulation mode, by pressing down on the milk drops symbol on top of the pump handle. Press and release quickly until you see milk flow begin.
• Once milk starts to flow, ask the participant to switch to the expression mode, by pressing the bottom of the pump handle. Collect about 33 mL of breast milk into the bottle.
• Collect the manual breast pump from the participant with the attached bottle.
• Detach the bottle and close the lid immediately.

3. Post-Collection Instructions for the Study Staff
• Collect the completed SIF from the participant.
• Remove gloves and discard supplies.
• Complete STEP 1: COLLECTION and STEP 3: PROCESSING sections of the STF. Mark STEP 2: TRANSPORT AND RECEIPT section as not applicable.
• Take the specimen to the processing station as soon as possible for aliquoting. If delays in processing is expected, place the collection container in the refrigerator for up to 24 hours.

E. Site Processing
1. Processing Kit (Fisher BioServices)
• Eight (8) 2 mL FluidX (metals-free) cryovials (orange capped) – breast milk
• Three (3) 10 mL cryovials – (white capped) – breast milk
• Ten (10) 1 mL cryovials & ten (10) white cap inserts – colostrum
2. Processing Supplies (Site)
   - Powder free gloves, eye protection glasses and a laboratory coat.
   - Tube rack
   - Polyethylene sheet (recommended)
   - Storage Container (Whatman Card)
     a. Recommendation: 5 x 5 x 3 in. box (30 Whatman cards per box)
   - Cryobox for 2 mL cryovial storage (5 x 5 x 2 in; 81 cell; 9 x 9 grid)
• Cryobox for 10 mL cryovial storage (5 x 5 x 3 in; 36 cell; 6 x 6 grid)
• Cryobox for 1 mL cryovial storage (5 x 5 x 2 in; 81 cell; 9 x 9 grid)
• HEPA-filtered Laminar Flow hood (preferred) or fume hood
• −20°C Freezer
• −80°C Freezer
• Breast Milk - Specimen Tracking Form (STF)

3. Pre-Processing Instructions for the Study Staff
• Take the specimen to the processing station as soon as possible for aliquoting.
• If specimen was placed in the refrigerator, record time specimen was removed from the refrigerator in STEP 3: PROCESSING.
• Use the same pipette provided to perform all aliquoting for one participant’s specimen, then discard.
• Each cryovial must be labeled and the identical label placed in the appropriate fields in the STEP 3 sections of the STF. Complete this section of the STF during processing.
• Affix label to the back side of the Whatman spot cards. Do not write on the card.

![Image with label instructions]

• Use the same pipette provided to perform all aliquoting for one participant’s specimen, then discard.
• Perform all processing under a hood.
• The number of aliquots depends on the specimen volume. Below is a summary and order of aliquots:
  a. Colostrum:
     i. 0.1 mL into each of the ten 1 mL cryovials
  b. Breast milk (mature or transition):
     i. Five 50 µL breast milk spots on each of the two Whatman spot cards.
     ii. 1 mL breast milk in each of the eight 2 mL cryovials.
iii. Up to 8 mL aliquots in each of the two 10 mL cryovials.
iv. If another 2–8 mL breast milk is available, prepare another aliquot with remaining milk in a 10 mL cryovial

4. Processing Instructions for the Study Staff

- Wear gloves, eye protection glasses and a laboratory coat.
- If colostrum was provided, swirl the container gently, taking care not to create bubbles or froth.
  - Using the pipette provided, aliquot 0.1 mL into each of the ten 1 mL cryovials.
  - Add cap inserts to the cap, the cryovials and place in the appropriate labeled cryobox.
  - Complete STEP 3D: ALIQUOTING COLOSTRUM section of the STF.
- If breast milk was provided, prepare Whatman spot cards:
  a. Gently swirl the collection container, taking care not to create bubbles or froth.
  b. Draw up 50 μL of breast milk using the pipette provided from the collection container and apply to the first spot on the Whatman spot card. Do not touch the pipette tip to the card.
  c. Repeat this procedure 4 more times to complete the remainder of the 4 spots on the card.
  d. Repeat the process to place spots on the second Whatman spot card.
    i. Set the cards flat on the back portion of the lab counter, preferably on a polyethylene sheet, in an area that will not be disturbed and that is away from sunlight. Do not place the flap over the spots. Dry the cards for at least 4 hours or longer. Overnight drying is recommended. Do not heat, stack, or allow spot cards to touch other surfaces during the drying process and do not set anything on top of the cards while drying. If 4 hours of drying time is not feasible, allow spots 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. Complete STEP 3A: ALIQUOTING BREAST MILK TO WHATMAN CARD(s) section of the STF.
  f. Once the cards are completely dry, tuck the flap of the cards as indicated on the card.
  g. Insert each folded card into a separate biohazard bag and add 2 desiccant packs. Seal the bag and place in a cryobox.
- Using a new pipette provided, transfer 1 mL each into eight 2 mL cryovials.
- Aliquot additional 8 mL breast milk into each of the two 10 mL cryovials.
If another 2–8 mL of breast milk is remaining, aliquot into a 10 mL cryovial.
Cap the cryovials and place in the appropriate labeled cryobox.
Discard used supplies in the biohazard container.
Record data in STEP 3 sections of the STF.

F. Storage

- Place labeled cryobox(es) with breast milk or colostrum cryovials in −80°C freezer. In case a −80°C freezer is not available, cryobox can be stored in a −20°C freezer.
- When the Whatman card is dried, place it in a biohazard bag with 2 desiccants. Store the bag containing the Whatman card in a labeled storage container and place in a −20°C freezer.
- Fill out storage date and time in STEP 3 sections on the STF.
- Data from the SIF and STF should be entered into Bio-Track immediately or within 48 hours of storing the specimen.
- Retain the SIF and STF at the site.

G. Shipping to Biorepository

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

H. Supporting Documents

1. Breast Milk – Specimen Information Form (SIF)
2. Breast Milk – Specimen Tracking Form (STF)
3. Breast Milk – Specimen Tracking Form (STF) Completion Instructions