Breast Milk Collection
Infancy

Collection, Processing and Storage Protocol
Home Collection

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A. Overview

- These breast milk collection procedures apply to home collection.
- The study staff can distribute the Breast Milk Home Collection kit to the participant via mail, provide it during a site visit, or take it to the participant’s home.
- Once collected, the participant can return the breast milk specimen by mail, bring it to the site, or study staff can pick it up from the participant’s home.

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.
- 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.
- Participants will use their standard breastfeeding routine when collecting breast milk specimen at home. For example, if mother freezes breast milk after collection, she will use the same process. If she refrigerates, she will use the same process. If she nurses the baby directly from her breasts only, she would use hand expression to provide the specimen.
- Specimen should be collected Monday–Wednesday at home and shipped to the site no later than Wednesday. Participants are expected to mail the specimen on the same day it is collected. Specimen should be mailed on freezer gel packs via FedEx Express Overnight.

C. Collection Kit, Supplies and Kit Assembly

1. Breast Milk Home-Collection Kit (Fisher BioServices)
2. Collection Supplies (Site)

- Breast Milk – Home Collection – Collection Instruction Sheet (CIS)
- Breast Milk – Specimen Information Form (SIF)
- Breast Milk – Specimen Tracking Form (STF)
- Transport materials for home collection
  a. Kit includes an insulated shipper, cardboard box, gel packs, biohazard bag, and absorbent sleeves for transport. [https://www.fishersci.com/shop/products/therapak-eas-refrigerated-shipper/22130438?searchHijack=true&searchTerm=22130438&searchType=RAPID&matchedCatNo=22130438](https://www.fishersci.com/shop/products/therapak-eas-refrigerated-shipper/22130438?searchHijack=true&searchTerm=22130438&searchType=RAPID&matchedCatNo=22130438)
  b. Items that can be ordered separately:
     i. Insulated shipper with outer cardboard box: 609UPS [https://www.thermosafe.com/subcategory/EPS+Foam+w/Corrugate](https://www.thermosafe.com/subcategory/EPS+Foam+w/Corrugate)
     ii. Pack of 3 Gel Packs: PP6 [https://www.thermosafe.com/subcategory/Gel+Packs](https://www.thermosafe.com/subcategory/Gel+Packs)
     iv. Absorbent Sheet 3x6
3. Kit Assembly

Follow the steps below to assemble the collection kit and prepare for the collection, prior to meeting with the participant or distributing the kit.

- Confirm that the collection kit includes a collection container and triplet specimen ID labels.
- Print SIF and STF and fill out the headers.
- There are three identical labels for each specimen ID. Affix the first label to the collection container. Apply the second matching label to the field labeled SPECIMEN LABEL on the SIF and the third matching label to the field labeled SPECIMEN LABEL on the STF.
  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.
- Collection container and SIF is provided to the participant.
- Retain the STF at the site.

4. Home Collection: Kit Distribution and Specimen Receipt

Use the following steps to prepare for distribution of the collection kit and return of specimen to the site via mail.

- Place kit contents assembled in the previous step in a mailing box.
- Include the CIS.
- Include 3 gel packs, zip lock bag, absorbent sheet, insulated shipping box, cardboard box, and prefilled and prepaid FedEx Express Overnight air bill for return of specimen to the site
- Ship the mailing box to the participant’s home.
- Complete KIT DISTRIBUTION section on the STF.

D. Home Collection

1. Collection Instructions for the Participant
- Refer to the Breast Milk (Home Collection) Collection Instruction Sheet (CIS).

2. Post-Collection Instructions for the Study Staff
- Upon receipt of the collection at the site, check the collection container label against the SIF label.
ECHO Breast Milk – Home Collection – Collection, Processing and Storage Protocol

- Complete **STEP 1: COLLECTION, STEP 2: TRANSPORT AND RECEIPT** and **STEP 3: PROCESSING** sections on the STF.
- 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
   - Two (2) Whatman spot cards
   - Two (2) biohazard bags for Whatman cards
   - Four (4) desiccants
   - Two (2) pipettes
   - Twenty-three (23) twin specimen ID labels
2. Processing Supplies (Site)

- Powder free gloves, eye protection glasses, and a laboratory coat
- Tube rack
- Polyethylene sheet (recommended)
- 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)
- Storage Container (Whatman Card)
  - a. Recommendation: 5 x 5 x 3 in. box (30 Whatman cards per box)
- 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**.
- 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 the label to the back side of the Whatman spot cards. Do not write on the card.

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.
  
  o Using the pipette provided, aliquot 0.1 mL into each of the ten 1 mL cryovials.
  
  o Add cap inserts to the cap, cap the cryovials and place in the appropriate labeled cryobox.
  
  o 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 cryobox 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 – Home Collection – Collection Instruction Sheet (CIS)
2. Breast Milk – Specimen Information Form (SIF)
3. Breast Milk – Specimen Tracking Form (STF)
4. Breast Milk – Specimen Tracking Form (STF) Completion Instructions