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ABM Clinical Protocol #8: Human Milk Storage Information for Home Use for Full-Term Infants, Revised 2017

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A central goal of The Academy of Breastfeeding Medicine is the development of clinical protocols, free from commercial interest or influence, for managing common medical problems that may impact breastfeeding success. These protocols serve only as guidelines for the care of breastfeeding mothers and infants and do not delineate an exclusive course of treatment or serve as standards of medical care. Variations in treatment may be appropriate according to the needs of an individual patient.

Background

Breastfeeding mothers may encounter unforeseen reasons for separation from their infants, but more often women express and store milk for planned events, lifestyle flexibility, and returning to work. Knowledge of appropriate human milk handling and storage is essential for breastfeeding success in these situations. One study indicated that although most women store their milk as recommended, ~12% heated their milk in a microwave, and 17% rinsed bottle nipples/teats with only water before reuse,[1] which may reduce the milk's biological properties and increase risk of contamination, respectively. Another study showed that neonatal nurses' knowledge and practice of breast milk collection and storage were adequate, however, there was inadequacy related to discarding, storing, and thawing breast milk.[2]

Human milk is a fresh, living food with many antioxidant, antibacterial, prebiotic, probiotic, and immune-boosting properties in addition to nutrients. Although some of these nutrients and health properties change with storage, there is good evidence that human milk storage can be safe, allowing provision of optimal nutrition to the child when breastfeeding or immediately expressed milk is not available. When direct breastfeeding is not possible, stored human milk maintains unique qualities, such that it continues to be the gold standard for infant feeding.

Preparation for Human Milk Storage

1. Washing: Women should wash their hands with soap and water, or a waterless hand cleanser if their hands don't appear dirty, before milk expression. Unclean hands may transmit viruses and bacteria, some of which can cause illness. Studies show that human milk containing fewer bacteria at the time of expression develops less bacterial growth during storage and has higher protein levels compared to milk that has an abundance of bacteria.[3–5] Additional hand hygiene and cleaning of the breasts before expression are not necessary.[6] (IIB) (Quality of evidence [levels of evidence IA, IB, IIA, IIB, III, and IV] is based on levels of evidence used for the National Guidelines Clearing House[7] and is noted in parentheses.)
2. Hand or Pump: Milk expression can be achieved by hand or by a pump. As long as the appropriate steps are taken for hand cleansing and cleaning of pump parts as per the pump manufacturer's instructions, there does not seem to be a difference in milk contamination with pumping versus hand expression.[8,9] (IIB, IV) There is no need to discard the first few drops of milk with initiating milk expression. This milk is not more likely to be contaminated than milk that is subsequently expressed.[7] One study found that milk expressed at home appears to have more bacterial contamination than milk expressed at the hospital, possibly related to equipment at home or transport, not related to personal hygiene.[6] (IIB)
3. Storage Container Choice: Several studies have been done to evaluate a range of available storage containers. There is a significant reduction in percent of fat and an increase in total protein and carbohydrate concentrations with either glass or polyethylene, polypropylene, polycarbonate, or polyethersulfone bottles or bags.[10] Glass and polypropylene containers appear similar in their effects on adherence of lipid-soluble nutrients to the container surface,[11] the concentration of immunoglobulin

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A (IgA), and the numbers of viable white blood cells in the stored milk.[12] Use of polyethylene containers was associated with a marked drop (60%) of IgA[12] and milk's bactericidal effect when compared to Pyrex, a type of tempered glass.[13] Steel containers were associated with a marked decline in cell count and cell viability when compared to polyethylene[14] and glass.[15] (IIB)

There has been concern about possible contamination of milk stored in polypropylene bags because of the risk of contamination by puncturing the plastic.[16] (IV) However, one study showed no difference between contamination and fat loss when comparing hard and soft polypropylene containers.[17] Therefore, plastic bags used for human milk storage should be sturdy, sealed well, and stored in an area of the freezer where damage to the bag would be minimized. (IIB) Containers made with bisphenol A, which is found in several plastic containers including baby bottles, should be avoided based on strong evidence of its adverse effects as an endocrine disruptor.[18] There should be caution about the use of bottles with bisphenol S, a bisphenol A alternative, as it may also have deleterious effects, although this is not well established in the literature.

Human milk should not be stored in hospital plastic specimen storage containers such as those used for urine or other bodily fluids because there is insufficient evidence regarding their chemical safety and effects on infants' health;[19] only food grade plastic containers should be used for human milk storage. (IV)

4. Care of Containers: Containers for human milk storage and breast pump milk collection kits must be completely dismantled, washed in hot soapy water and rinsed or washed in a dishwasher,[8] and should always be thoroughly air dried or dried with paper towels.[20] They do not need to be sterilized. If soap is not available, then boiling water is preferable. (IIB) Chemical disinfection is not ideal, as the disinfectant can be easily deactivated and could expose infant to unnecessary risk of both inadequately clean containers and residual chemical disinfectant.[20] (IV)

Storage of Human Milk

1. Freshly expressed human milk may be stored safely at room temperature (10–29°C, 50–85°F) for some period of time. Studies suggest different optimal times for room temperature storage because conditions vary greatly in the cleanliness of milk expression technique and the room temperature. Warmer ambient temperatures are associated with faster growing bacterial counts in stored milk. For room temperatures ranging from 27°C to 32°C (29°C = 85°F), 4 hours may be a reasonable limit.[5,21,22] For very clean expressed milk with very low bacterial counts, 6–8 hours at lower room temperatures may be reasonable, but it is best to chill or refrigerate as soon as possible if the milk will not be used during that time.[4,23–25] (IIB)
2. Ice packs: Very few studies have evaluated milk storage safety at 15°C (59°F), which would be equivalent to an ice pack in a small cooler. Hamosh et al.[21] suggested that human milk is safe at 15°C for 24 hours, based on minimal bacterial growth noted in the samples from their study. (IIB)
3. Refrigeration: Several studies have demonstrated the safety of refrigerating human milk (4°C, 39.2°F), either by evaluating the bactericidal capacity of stored milk as a marker for milk quality or by measuring bacterial growth in the stored milk samples. Bactericidal capacity of stored refrigerated human milk declines significantly by 48–72 hours.[26–28] However, studies of expressed human milk with little contamination at the time of expression demonstrate safe, low levels of bacteria growth in milk at 72 hours[24] and even after 4–8 days of refrigeration.[3,4,29]

   Few studies have been done on the change in milk composition during refrigerator storage. One study found that lipid composition and lipase activity remained stable up to 96 hours in the refrigerator.[30] Lactoferrin levels are stable in the refrigerator for 4–5 days.[31,32] Many immunologic factors in colostrum such as IgA, cytokines, and growth factors are not diminished with refrigeration for 48 hours.[33] (IIB)
4. Freezing expressed human milk (–4°C to –20°C = 24.8°F to –4°F) has been demonstrated to be safe for at least 3 months. Evidence indicates that thawed human milk, previously frozen for at least 6 weeks at –20°C (–4°F), has the same bacterial viability and diversity as it did when it was freshly expressed.[34] The basic principles of freezing dictate that frozen foods at –18°C (0°F) are safe indefinitely from bacterial contamination, although enzymatic processes inherent in food could persist, with possible changes in milk quality.[35]

   Fat, protein, and calories decrease in human milk when frozen for 90 days compared to fresh human milk.[36] Frozen human milk has a significant increase in acidity by 3 months, likely due to ongoing lipase activity, that increases free fatty acids in the milk.[37] Based on a few studies with very small samples sizes, vitamin E appears stable in frozen milk over time, and vitamin C levels decrease significantly after 1–5 months of storage.[38,39] There is a paucity of research on how freezer storage affects nearly all vitamins and minerals in human milk.[38–40]

   Bioactive factors in human milk variably diminish with freezing. Lactoferrin levels and bioactivity are significantly lower in human milk frozen at –20°C for 3 months.[13,31,32] However, several cytokines, IgA and growth factors from colostrum are stable for at least 6 months at –20°C (–4°F).[10,33] One trial evaluating milk frozen for 9 months found a progressive decline in pH and in bacterial counts, and increases in nonesterified fatty acids. Other macronutrients, osmolality, and immunoactive proteins remained unchanged in this study after 9 months.[41] Frozen human milk should be stored in the back of the freezer to prevent intermittent rewarming due to freezer door opening, and should be kept away from the walls of self-defrosting freezers. All containers with human milk should be well sealed to prevent contamination. (IIB)
5. Smell of stored milk: Refrigerated and frozen human milk may have an odor different from fresh milk due to lipase-mediated triglyceride breakdown, releasing fatty acids. The odor likely comes from oxidation of these fatty acids.[42,43] This lipolysis process has antimicrobial effects preventing the growth of microorganisms in thawed refrigerated milk.[44] There is no evidence to

suggest that infants often reject human milk due to this odor. Many foods that humans eat, such as eggs, cheese, and fish, have an unpleasant odor that does not affect taste. One study demonstrated that freezing human milk to −80°C (−112°F) leads to less change in smell as compared to conventional freezing to −19°C.[43] Heating milk to above 40°C to deactivate lipase is not advised because this may destroy many of the immunologically active factors in human milk. (IIB)

6. Expansion while freezing: When filling a container with human milk, space should be left at the top to allow for expansion with freezing. All stored containers of human milk should be labeled with the date of milk expression and the name of the child if the milk will be used in a child-care setting. It is typical for infants in daycare to take 60–120 mL (2–4 ounces) of human milk at one feeding. Therefore, storing human milk in a variety of small increments such as 15–60 mL is a convenient way to prevent waste of thawed human milk.
7. Mixing milk: Freshly expressed warm milk should not be added to already cooled or frozen milk, to prevent rewarming of the already stored milk. It is best to cool down the newly expressed milk first before adding it to older stored milk.

A summary of milk storage guidelines is given in Table 1.

Using Stored Human Milk

1. Cleaning of feeding devices: Containers and feeding devices used to feed the infant should be cleaned with soap and water and air dried or dried with a paper towel before/after every use. They do not need to be sterilized for a healthy infant. (IIB)
2. Using fresh milk first: Fresh milk is of higher quality than frozen milk. Fresh milk contains current maternal secretory IgA antibodies that may be relevant to the dyad's recent infectious exposures.[45] Freshly expressed milk is highest in antioxidants, vitamins, protein, fat, and probiotic bacteria compared to refrigerated or frozen milk.[27,36,38,39] Fresh human milk also has the greatest immunologic activity compared to refrigerated or frozen milk.[10,31,46] (IB)
3. Thawing frozen milk: There are several ways to thaw frozen human milk: by either placing the container in the refrigerator overnight; by running it under warm water; by setting it in a container of warm water; or by using a waterless warmer. Slow thawing in the refrigerator causes less fat loss than thawing in warm water.[47] (IIB)
4. Warming human milk: Most infants drink milk cool, at room temperature, or warmed; infants may demonstrate a preference. Warming thawed human milk to body temperature is best done over a period of 20 minutes in lukewarm water (at most 40°C). Even warming the milk just to 37°C brings the fat to its melting point, promoting changes from solid fat, which is present at 4°C refrigerator temperature, to liquid or oil fat. Oil fat appears to adhere to the side of the container at 37°C more than it does at 4°C, therefore lowering the fat content of the milk. One study compared tepid water warming at 37°C and waterless warming and found there was no difference between them in regards to changes in fat, protein, lactoferrin, and secretory IgA.[44]

   Milk placed in hot water bath (80°C, which is not uncommon in the real setting) creates islets of high temperature milk due to lack of stirring.[48] Overheating during the warming process causes denaturation and inactivation of milk's bioactive proteins and decreased fat content. (IIB)
5. Microwaving: Studies done on defrosting human milk in a microwave demonstrate that controlling the temperature in a microwave is difficult, causing the milk to heat unevenly.[49] Although microwaving milk decreases bacteria in the milk much like pasteurization does, it also significantly decreases the activity of immunologic factors, which may reduce its overall health properties for the infant.[50,51] (IIB)
6. Using thawed milk: Once frozen milk is brought to room temperature, its ability to inhibit bacterial growth is lessened, especially by 24 hours after thawing.[52] Previously frozen human milk that has been thawed for 24 hours should not be left out at room temperature for more than 2 hours.[44] (IIB)
7. Refreezing: There is little information on refreezing thawed human milk. Bacterial growth and loss of antibacterial activity in thawed milk will vary depending on the technique of milk thawing, duration of the thaw, and the amount of bacteria in the milk at the time of expression. At this time no recommendations can be made on the refreezing of thawed human milk.
8. Using previously fed milk: Once an infant begins drinking expressed human milk, some bacterial contamination occurs in the milk from the infant's mouth. The length of time the milk can be kept at room temperature once the infant has partially fed from the cup or bottle would theoretically depend on the initial bacterial load in the milk, how long the milk has been thawed, and the ambient temperature. There has been insufficient research done to provide recommendations in this regard. However, based on related evidence thus far, it seems reasonable to discard the remaining

Table 1. Milk Storage Guidelines

Location of storage	*Temperature*	*Maximum recommended storage duration*
Room temperature	16–29°C (60–85°F)	4 hours optimal 6–8 hours acceptable under very clean conditions
Refrigerator	~4°C (39.2°F)	4 days optimal 5–8 days under very clean conditions
Freezer	<–4°C (24.8°F)	6 months optimal 12 months acceptable

milk within 1–2 hours after the infant is finished feeding. (IV) To avoid wasting or discarding unfed milk, mothers may consider storing milk in a variety of increments such as 15, 30, or 60 mL.

9. Handling: Expressed human milk does not require special handling (such as universal precautions), as is required for other bodily fluids such as blood. It can be stored in a workplace refrigerator where other workers store food, although it should be labeled with name and date.[53] (IV) Mothers may prefer to store their milk in a personal freezer pack or cooler, separate from communal refrigerator areas.
10. Infections: Uncontaminated human milk naturally contains nonpathogenic bacteria[54,55] that are important in establishing the neonatal intestinal flora. These bacteria are probiotics—they create conditions in the intestine that are unfavorable to the growth of pathogenic organisms.[55] If a mother has breast or nipple pain from a bacterial or yeast infection, there is no evidence that her stored expressed milk needs to be discarded. Human milk that appears stringy, foul, or purulent should, however, be discarded and not be fed to the infant. (IV)

Areas for Future Research

The evidence for some aspects of human milk storage is lacking. Many studies are older, and because of differences in methodology, are difficult to compare. The studies vary in many respects, such as technique of milk collection, cleanliness and types of containers, duration of storage, method of thawing and warming milk, temperature and type of storage unit, and culture techniques of milk samples. Large high-quality studies evaluating human milk storage in a variety of circumstances over a longer duration of time are needed. Standards for evaluating milk quality, such as culture techniques, need to be established. Although it is ideal to have a universal international guideline for human milk storage, it may be impossible for one guideline to represent unusual or limited circumstances in some cultures.

Human milk naturally has both prebiotic and probiotic activity that is essential in establishing the infant gut microbiome. Human milk's prebiotic components are nondigestible factors such as oligosaccharides that promote the growth of beneficial microorganisms in the intestines. Human milk's probiotic components are commensal organisms. Because of the impact of refrigeration, freezing, thawing, and warming on the bactericidal activity of human milk, feeding an infant stored human milk may have different consequences on infant intestinal health compared to breastfeeding, and this should be investigated further. Along the same lines, stored human milk changes in quality over time, as demonstrated by many of the referenced articles included in this protocol. The effect of stored human milk versus fresh human milk on the health of a child should be studied.

There is also no agreed-upon definition of unsafe milk. Several studies describe the degree of milk contamination over a period of time under certain temperature and storage time conditions, typically described as the number of colony-forming units per milliliter. There is no accepted limit at which point milk should not be consumed, although 1×10^4 colony-forming units/mL has been suggested. Other studies have investigated the bactericidal capacity of stored human milk, which would reflect its immunologic effectiveness for the infant and the risk of the milk becoming contaminated over time during storage. The percentage loss of bactericidal activity that would render human milk unfit has not been determined. A definition for adequate milk quality should be established, with guidelines on what would constitute unsafe milk or lower-quality milk that would necessitate discarding of stored milk.

There is only one study investigating human milk quality after 6 months of freezing. This is particularly concerning, given that a few very small studies have demonstrated a decline in some vitamins after 3 months of freezing. Because some infants rely entirely on frozen human milk for nutrition, studies should be done to confirm that this is nutritionally safe.

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ABM protocols expire 5 years from the date of publication.

Content of this protocol is up-to-date at the time of publication. Evidence-based revisions are made within 5 years or sooner if there are significant changes in the evidence.

The 2004 and 2010 editions of this protocol were authored by Anne Eglash.

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