# Best Practices for Human Milk Collection for COVID-19 Research

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**Running title:** Collecting Human Milk for COVID-19 Research

**Word count for abstract:** 232

**Word count for text:** 3500

**Number of tables:** 3

**Number of figures:** 0

**Key words:** human milk | breastmilk | COVID-19 | methods | collection | pathogen

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**Sources of Support:** There was no committed funding for this project.

**Abstract**

In addition to providing life-giving nutrients and other substances to the breastfed infant, human milk can also represent a vehicle of pathogen transfer. As such, when an infectious disease outbreak, epidemic, or pandemic occurs – particularly when it is associated with a novel pathogen – the question will naturally arise as to whether the pathogen can be transmitted via breastfeeding. Until high-quality data are generated to answer this question, abandonment of breastfeeding due to uncertainty can result. The COVID-19 pandemic, which was in full swing at the time this document was written, is an excellent example of this scenario. During these times of uncertainty, it is critical for investigators conducting research to assess the possible transmission of pathogens via milk, whether by transfer through the mammary gland or contamination from respiratory droplets, skin, breast pumps, and milk containers, and/or close contact between mother and infant. To promote the most rigorous science, it is critical to outline optimal methods for milk collection, handling, storage, and analysis in these situations, and investigators should openly share their methods in published materials. Otherwise, the risks of inconsistent test results from pre-analytical and analytical variation, false positives, and false negatives are unacceptably high and the ability to provide public health guidance poor. Here we provide "best practices" for collecting human milk samples for COVID-19 research with the intention that this will also be a useful guide for future pandemics.

**Background**

Human milk is a complex emulsion consisting of a vast array of constituents providing not only nutrition but also protection from pathogens. Concentrations of these constituents vary within an individual, across the lactation period, and even within a feed. Although we do not understand all the factors driving this variability, we know a substantial amount regarding how some factors influence concentrations of some milk constituents. For instance, total lipid content of milk is affected by time postpartum, time of day, time since last feeding, portion of an individual feed (fore- vs. hindmilk), maternal body fat level, and in some cases maternal diet.[1-4] To complicate matters, lipids and cells can adhere to some types of collection containers which can impact research results,[5] and some milk constituents (e.g., viral particles and RNA) can be entrapped in the lipid fraction or other compartments such as exosomes.[6-10] Researchers studying human milk composition should, therefore, consider these factors when designing their protocols for collecting

human milk to study its composition. For example, collecting a foremilk sample in the morning using an inappropriate collection container may easily lead to inaccurate quantification of milk's lipophilic compounds. Another example is host RNA which, although in relatively high concentrations in milk, is quickly degraded by intrinsic RNases;[3,11] as such, milk must be immediately processed or snap frozen for accurate quantification of host RNA. For other constituents (e.g., iron and lactose), concentrations in milk are less prone to variation;[12,13] in these situations, sample collection and storage protocols can be less stringent. Milk composition may even vary between breasts – especially regarding immune factors; this fact has been particularly important in the study of HIV transmission via breastfeeding.[14] For other milk components, such as microbiota, there exists very little research characterizing modifiable factors (e.g., time of day, time within feed) related to variation; in these situations, best practices and standardization (albeit not optimization) are typically employed to ensure that samples are collected in a way that reduces risk of contamination and allows data to be compared across studies.

In summary, because human milk composition is highly variable within and among women and can be influenced by many biological and methodological factors, it is fundamentally important that researchers consider and report core aspects of milk collection, handling, and storage when studying it. These aspects include expression mode (electric pump or manual expression), time of day, time postpartum, complete vs. partial expression (and if the latter, whether foremilk or hindmilk was collected), breast preparation (was the breast cleaned and if so with what), collection container material (and whether it was sterile), and storage conditions (e.g., time until refrigeration or freezing, temperature and duration of storage). In addition, sometimes chemical preservatives are utilized, and these should be carefully evaluated as to whether they might impact the researcher's ability to detect the milk constituent of interest.

The primary purpose of this document is to, using evidence gleaned from the literature and expert opinion, delineate a "best practices" framework related to human milk collection, handling, and storage for COVID-19 research related to breastfeeding. Although we recognize that each microbe is unique, it was our hope that this framework will also be applicable to other pathogenic RNA viruses, DNA viruses, bacteria, and maybe even other organismal taxa. In addition to including information related to the study of presence/absence and viability of these types of pathogens, we provide information on how one might best collect milk for the study of immunoglobulins, cytokines and other soluble

factors, and immune cells as these components are typically studied in this context. Investigators collecting milk for research during an outbreak, epidemic, or pandemic are urged to consider this framework and best practices both in designing their methods and in reporting their findings. Depending on the research question, not all elements of the framework may be relevant; nor may each element be feasible given the patient population and environmental context. However, it is critical for the interpretation of results and to guarantee comparability of findings across studies that key elements of milk collection, handling, and storage be described in published materials. It is noteworthy that this framework and associated best practices will undoubtedly shift as new data emerge related to the nature of the pathogen and how collection and storage conditions do or do not impact the ability to detect and quantify them. Indeed, "best practices" will need to be periodically revised to reflect the evolving state of the science.

**Basic Working Definitions Related to Human Milk Research**

To help investigators navigate the somewhat unique vocabulary of human milk and lactation research, selected terms have been briefly defined and are provided in **Table 1**. Many of these definitions are adapted from those provided in LactaPedia,[15] which is an excellent resource in this respect.

**Framework**

Here, we briefly review the literature describing whether or not selected factors known to impact the concentration and/or stability of some milk components impact a set of components particularly pertinent to research related to potential transmission of pathogens from mother to infant via human milk and/or breastfeeding. These components and attributes include viral DNA and RNA, bacterial DNA, microbial viability, immunoglobulins, cytokines and other soluble components, and immune cells. This information is important because it informs what should be considered, controlled for, or at least reported when human milk is being collected, handled, and stored for this type of research. **Table 2** provides a summary of these factors and provides guidance as to whether they should be controlled for and/or reported in studies related to transmission of a pathogen in milk. It is noteworthy that the state of the science for many of these factors is insufficient, and additional research is urgently needed to fill these knowledge gaps.

*Time postpartum* Whereas little is known regarding whether time postpartum *per se* impacts viral RNA, DNA, and viral viability, some evidence suggests that bacterial profiles and load in milk change over time and particularly

between colostrum and mature milk.[16-18] In addition, myriad studies have documented an effect of time postpartum on concentrations of immunoglobulins, cytokines soluble factors, and immune cell populations.[19-21] For instance, IgA concentrations in milk decline precipitously from birth to 2 weeks postpartum;[22] macrophages, lymphocytes, and lactoferrin continue to decrease through 3 months postpartum;[23] and lysozyme increases.[20] Immune cell concentrations also increase during involution, which is generally associated with later times after parturition,[23] and during mastitis, which is most common in the first several weeks of lactation.[24,25] As such, an attempt should be made to standardize and/or control for time postpartum and breast health (subclinical/clinical mastitis versus none) when comparing data across cohorts (e.g., infected vs. noninfected breastfeeding women), and time postpartum when milk was collected should always be reported in publications. In addition, because composition of milk can be affected by premature delivery, whether the infant was born premature or full-term should be noted.

*Time of day* There is very little published literature rigorously investigating if there is diurnal variation in concentrations of bacterial and viral RNA and DNA, bacterial and viral viability, immunoglobulins, and cytokines and other soluble factors in milk. Limited data, however, suggest that antibody and cytokine concentrations may vary over the course of a day.[26,27] A variety of hormones (many of which are known to impact immune function) also vary throughout the day and night.[28] Cell content of human milk may also influenced by the circadian cycle of cortisol, but very little is known about this.[29] As such, if collecting repeated milk samples from a woman over time, researchers might consider standardizing the time of day the samples were collected. Alternatively, researchers could employ the "gold standard" approach of collecting complete breast expressions for a 24-hour period and analyzing a representative (composite) sample. If this 24-hour collection methodology is not used, researchers should consider recording time of sample collection in the metadata.

*Foremilk vs hindmilk* There is substantial evidence that the lipid content of milk is lower in foremilk than in hindmilk,[2,30-32] and that this is likely related to the time since last feeding.[33] Whereas some studies have documented lower protein content in foremilk than hindmilk,[34-36] others found no difference[33] or the opposite.[37,38] Limited evidence also suggests that cell content is higher in hindmilk than foremilk.[39] To our knowledge, there are no published data using molecular methods relating foremilk vs. hindmilk to variation in detectable microbial communities (or their viability),

although Rodríguez-Cruz and colleagues found no difference in microbial profiles between whole milk and skim milk.[38] Nonetheless, if the microbe of interest is lipophilic it is possible that it might be found in lower abundance if only foremilk is collected. Because of the potential for differential milk composition within a feed (expression) and the dearth of data related to this factor and microbes, antibodies and other soluble factors, and cells in milk, researchers should report whether and how a complete expression was collected, and if not whether foremilk or hindmilk was primarily obtained, and time since last feed.

*Expression mode* Breast pump parts (including tubing) can be contaminated. Consequently, if breast pumps are used, they must be thoroughly disinfected and rinsed to remove all viral/bacterial DNA and RNA and disinfectant. Since foremilk is generally lower in fat than hindmilk, expression mode (hand versus other and complete expression versus partial) might also impact ability to detect a pathogen if it compartmentalizes to the lipid fraction of milk. Nonetheless, Rodríguez-Cruz and colleagues found no difference in microbial profiles between collected via manual expression and that collected with a pump.[39] If hand expression is employed, subjects should thoroughly wash their hands and/or wear clean gloves. Researchers should report if milk was collected using a manual pump, electric pump (and type), or hand expression. Importantly, both researchers and study participants should follow recommended infection prevention and control measures during the collection and handling of the milk. Depending on the cultural norm, women may be comfortable using breast pumps or using hand expression to express milk; but if not, instruction should be provided by a qualified lactation consultant or personnel with suitable expertise.

*Inter-breast variation and inflammation* For some milk constituents there can be inter-breast variation, and mammary inflammation is known to drive some of these differences. For instance, levels of HIV RNA can differ in milk produced by each breast,[40] largely due to differences in mammary inflammatory status.[41] Almost nothing is known about inter-gland difference in other types of pathogens. Pannaraj and colleagues compared milk microbiomes between healthy human breasts and found no difference;[42] although studies of dairy cows clearly show that mastitic and healthy glands produce milk with different microbiomes.[43] It is likely that whether there are differences in milk microbiome between mammary glands can depend on mammary health. Because of potential differences in microbial proteins in milk produced by each breast (likely due to inflammation), researchers should ideally collect milk from both breasts; if

that is not possible, they should aim to evaluate mammary inflammation either visually (e.g, redness) or chemically in the milk sample (e.g., Na/K ratios, cytokines, somatic cell count).

*Breast preparation* Depending on whether the pathogen is primarily blood-borne, respiratory-borne, or environmental, it is possible that some pathogens may be present on the breast skin. Nonetheless, whether the breast should or should not be cleaned is related to the question at hand. If the question relates to whether the pathogen is incorporated into milk in the mammary gland, then the breast should be cleaned prior to milk collection. If the question relates to whether the infant might be exposed to the pathogen through breastfeeding or the consumption of pumped milk, then the breast should not be cleaned. If the question relates to antibody or cytokine content of milk, breast cleaning is irrelevant. Researchers should design their collection methods to suit their research question and describe whether/how the breast was cleaned if it was.

*Collection containers* Some milk components (e.g., lipids and cells) can adhere to certain materials common to collection containers. Others (e.g., IgA) have been demonstrated to be stable in both glass and plastic containers.[44] Given the inadequate state of the science regarding the importance (or lack-thereof) of collection container materials to studying microbes in milk, however, there is no recommendation as to what sort of collection container should be used. This information should be reported, however, in any publication.

*Temperature and storage* Refrigeration, freezing, thawing, and application of heat can all affect the stability of many milk components. [45-47] In addition, some subpopulations of cells can only be isolated from fresh milk, as they are destroyed or altered by freezing and/or thawing. Conversely, HIV RNA levels have been shown to be remarkably stable in whole milk after three freeze-thaw cycles and for up to 30 hours at room temperature,[48] and some data suggest that milk can be stored at 4 °C for up to 48 hours or at -20 °C or -80 °C for at least 6 months without losing its immunological properties.[49] For bacterial DNA, Doyle et al. found very little impact of refrigeration temperature (2, 4, or 6 °C) and storage duration (up to 96 hours) on bacterial profiles (via 16S rRNA analysis) in bovine milk.[50] There are similar findings for human milk.[51,52] Although findings are somewhat mixed,[53-55] there is substantial evidence that some soluble factors and characteristics (e.g., antioxidant capacity) in milk can be influenced by refrigeration and freezing; whereas others

192 (e.g., HMO) are extraordinarily stable.[56-58] It has been shown that prolonged storage at 4°C reduces the infectious titer of
193 hepatitis C and Zika virus that has been spiked into milk.[59-61]

194 To date, little is known about the stability of SARS-CoV-2 during cold storage, although work from our group
195 suggests that SARS-CoV-2 RNA in milk may be stable for 2 days at 4 °C and 7 days at -20 °C and can withstand several
196 freeze/thaw cycles.[62,63] It is unclear what the impact of freezing and thawing on infectivity would be. However, Walker
197 and colleagues[64] provide evidence that Holder pasteurization (but not cold storage) inactivates SARS-COV2; Unger et
198 al.[65] and Chambers et al.[66] have also shown that Holder pasteurization inactivates SARS-CoV-2 and the former that
199 holding milk at room temperature for 30 minutes also reduces infectious viral titers.

200 Because they can be destroyed and/or inactivated by temperature changes, care should also be taken to avoid
201 repeat free thaw cycles of milk collected to measure the antibodies to SARS-CoV-2.[67,68] In considering the cytopathic
202 effects of viruses (e.g. SARS-CoV-2, Ebola), researchers need to be aware that the multitude of immune components in
203 human milk with significant antiviral activity may immediately impact cytopathic activity when milk is held a room
204 temperature or 4 °C.[64,69]

205 In summary, researchers are encouraged to consider whether the milk component of interest is stable under the
206 available storage conditions and, like all important factors, report the temperature at which milk was stored prior to
207 analysis. If in doubt, it is always safest to analyze fresh milk or freeze it at the lowest temperature possible as soon as
208 possible and keep it frozen until it is analyzed. Creating aliquots of the sample is often advised to avoid freeze-thaw
209 cycles of samples.

210 *Milk fraction* When detecting SARS-CoV-2 RNA via qPCR in spiked milk samples, defatted milk yielded better
211 recovery rates than did whole milk.[63] Conversely, up to one-third of HIV RNA in milk produced by infected women may
212 be sequestered in the lipid fraction.[7] Rodríguez-Cruz and colleagues found no difference in bacterial profiles between
213 whole milk and skim milk.[39]

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## Best Practices for Milk Collection and Storage

Below, we provide "best practices" for research purposes in various settings, recognizing that what is possible, ethical, and desirable depends greatly on the research question, context, and capabilities of each research group as well as maternal and infant factors.

**Step 1. Breast Cleaning** – If the research question is related to exposure of the infant to the pathogen via the complex process of breastfeeding ("breastfeeding transfer") or consuming pumped milk, it is unnecessary to clean the breast. However, if the research question is related to whether the pathogen is transmitted through the mammary gland into milk ("milk transfer"), the breast should be thoroughly cleaned prior to milk collection – particularly if the pathogen may be transmitted into milk via respiratory droplets. After donning face covering and a glove on the hand which will clean the breast, research personnel or the mother (depending on cultural acceptability) should clean the "study breast" thoroughly with soap and water or aseptic wipes. The purpose of this step is to physically remove skin pathogens.

**Step 2. Milk Collection** – Using one of the methods detailed below, collect milk from the chosen breast. Depending on which milk constituent is of interest, this milk can be foremilk, hindmilk, or a combination, thereof. If, for some reason, not enough milk can be expressed from the chosen breast, it is generally acceptable to combine milk from both breasts and document how the composite sample was created. If this is needed, the "second" breast should be cleaned (as appropriate to the research question) as described in Step 1 prior to collecting milk. The following options ***are equally*** acceptable.

<u>*Option A:*</u> *Hand expression*: With a newly gloved hand, the mother should express the needed volume of milk into the sterile collection container. The goal is to obtain a "clean catch" sample that drips or squirts directly from the nipple into the sterile container.

<u>*Option B:*</u> *Electric or manual pump:* Using a sterile or thoroughly cleaned and disinfected pump (including attachments, and tubing), have the mother express the needed amount of milk into the sterile collection container.

**Step 3. Milk Partitioning and Storage**

*Option A (preferred): To be used when refrigeration/cold box is available at site and freezer is available in nearby laboratory*

- Place milk immediately in refrigerator, ice, ice box, or cold box. If the research question is related to isolating infectious virus, the sample might need to be snap frozen or analyzed immediately.
- If possible, aliquot milk; transfer milk using sterile pipet into sterile storage containers.
- As soon as possible, freeze milk at -20 °C or (preferable) -80 °C.

*Option B: To be used when refrigeration/freezing is not available*

- Within 30 minutes of milk collection, treat milk with appropriate and validated chemical preservative (e.g., Norgen Biotek Corporation's Milk DNA Preservation and Isolation Kit). Preservatives should have been tested and validated for use with human milk to ensure that they do not destroy or destabilize milk components of interest or interfere with assays. These preservatives likely impact viral/bacterial viability.
- Store in a cool place, at ambient temperature, or as described in manufacturer's instructions.

*When milk supply is limited* Special care should be taken in situations when milk supply is limited and/or when the infant's health is at risk, for example in the very early postpartum period (colostrum samples) and when the infant is very preterm and/or at risk for developing necrotizing enterocolitis. In these situations, researchers are encouraged to modify the methods described above so that the volume of milk available to the infant is not jeopardized. In general, only very small amounts (typically < 1 mL) of colostrum should be collected. Sufficient milk may sometimes be obtained from a sterile swab to enable testing of SARS-CoV-2 gene targets via RT-qPCR testing.[70] In many clinical settings, enteral feeds for very preterm or hospitalized infants are prepared in a central milk preparation room in a batch to last 12 to 24 hours. Often a small volume of milk that would otherwise be discarded can be collected for research purposes and uniquely reflects what an infant would receive the following day.

*Ethical considerations* All procedures should be approved by local, regional, and/or national ethics boards (as appropriate) to protect participants' rights and ensure that subjects' identities are not linked to resultant data. For instance, samples and data should always be deidentified from subjects' names. Each mother should provide informed consent for milk collection, understand the purpose of the study, and be reassured that samples will be neither used for

other purposes nor sold. Not all the mothers will have the same educational level, so it may be necessary to take extra care when communicating the purpose of the study. Particularly in studies with indigenous populations, researchers must understand how participants may view the collection of human milk and its use for biochemical analysis within the prevailing worldview.

*Regarding safety* Researchers working directly with infected breastfeeding women should always follow all infection control and safety recommendations put forth by local, national, and international organizations – including the use of masks, gowns, and gloves. In addition, samples should be processed in an appropriate biosafety cabinet, if available.

*Checklist for collecting human milk in light of an infectious disease* A checklist of important factors that should be considered, documented, and reported when collecting human milk to study potential transmission of a pathogen via breastfeeding is provided in **Table 3**.

**Regarding the State of the Science and Urgency**

As briefly described in this document, there are myriad gaps in knowledge related to studying the presence/absence of pathogens in human milk; their origin, when they are present; and their ability to be adequately characterized in terms of load and viability. This dearth of knowledge makes quickly assessing risk vs. benefit of breastfeeding during a pandemic difficult. In these situations, we encourage researchers to work collaboratively and quickly to develop specialized protocols as needed and openly share information with other researchers so that accurate answers are gleaned in a timely fashion. To facilitate this, granting agencies are encouraged to make emergency funding available to engaged and qualified research groups, and to facilitate contact between groups in the interest of collaboration.

**Author disclosure:** The authors have no real or perceived conflicts of interest to disclose.

**Acknowledgements:** We greatly appreciate the careful review of this manuscript by Dr. Aleksandra Wesolowska, Dr. Jennifer Jourkavitch, Melinda Boss, Dr. Melanie Martin, and Dr. Janet Williams.

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Table 1: Definition of selected terms relevant to collection of human milk for analysis of its composition

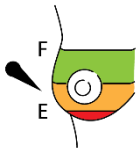
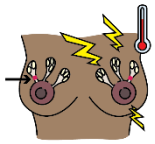
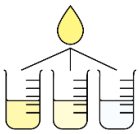
| | | |
|---|---|---|
|  | ***Foremilk vs. hindmilk*** | **1 Foremilk:** Milk removed from the mammary gland at the beginning of a feed or just before the commencement of a breastfeed or breast expression. This milk often has lower fat and higher cellular contents than milk secreted at the end of a feed.<br>**2 Hindmilk:** Milk removed from the mammary gland at the end of a feed or immediately after the completion of a breastfeed or breast expression. This milk often has higher fat and lower cellular contents than milk secreted at the beginning of a feed. |
|  | ***Complete breast expression*** | The process of obtaining nearly all the milk within the mammary gland at a particular pumping or hand expression session. A complete breast expression contains foremilk, hindmilk, and all the milk in between. Obtaining a complete breast expression provides the most representative milk sample possible and is often ideal when there are no data as to whether its composition changes from foremilk to hindmilk for a particular milk component. When complete breast expressions are obtained, researchers should return excess milk to the mother so she can provide it to her infant. |
|  | ***Colostrum vs. transitional milk vs. mature milk*** | **Colostrum:** The usually yellowish viscous secretion of the breast synthesized during the first 48 hours postpartum. It is synthesized by the lactocytes of the mammary gland in small volumes (about 30 mL in the first 24 hours after birth). Compared to mature milk, colostrum has high concentrations of sodium, chloride, protein (particularly IgA), and low concentrations of lactose and citrate.<br>**Transitional milk:** A description of milk as it shifts from colostrum to mature milk after secretory activation (also referred to as "milk coming in). Transitional milk is yet to be defined objectively, but it is generally considered to extend from about 48 hours after birth to 2 to 3 weeks postpartum.<br>**Mature milk:** The secretion produced by the mammary gland following secretory activation (also referred to as "milk coming in"). Human milk is currently considered to be mature after about 2-3 weeks postpartum (provide a ref for this window b/c some reports extend to six weeks). |
|  | ***Exclusive breastfeeding vs. partial breastfeeding vs. complementary feeding*** | **Exclusive breastfeeding:** When the infant receives only human milk via breastfeeding and/or expressed human milk (own mother's or from a donor). This definition often allows the infant to receive drops, syrups (vitamins, minerals, medicines) but does not allow the infant to receive anything else, including water.<br>**Partial breastfeeding:** When an infant receives both human milk and any other food or liquid including water, nonhuman milk, and formula before about 6 months of age.<br>**Complementary feeding:** Nutrient-containing first foods given during the transition from exclusive breastfeeding to family foods while breastfeeding is maintained. Complementary breastfeeding commences during weaning. |
|  | ***Hand expression*** | The process whereby milk is obtained using manual breast expression by hand alone without the use of a manual or electronic breast pump. |
|  | ***Subclinical mastitis vs. clinical mastitis*** | **Subclinical mastitis:** Asymptomatic inflammation of the mammary gland(s) that is not noticeable to the woman or to an observer. Often characterized by a sodium to potassium (Na/K) ratio in milk ≥ 1.<br>**Clinical mastitis:** Inflammation of the breast accompanied by pain, swelling, heat/redness, and/or fever. |
|  | ***Milk fraction*** | Milk has several collections of components, including cells, lipids, and lactoserum (the aqueous, cell-free phase, whey). These are often referred to as "milk fractions." |

Table 2: Milk components often evaluated when studying transmission of a pathogen in milk, and whether various factors are known to impact their concentrations in milk

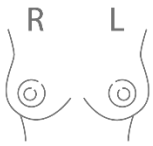
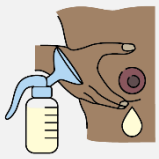
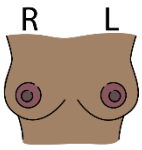
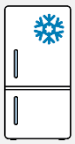
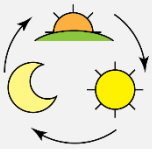
| Factor that can impact milk composition | Viral DNA/RNA, bacterial DNA and microbial viability | Antibodies | Cytokines and other soluble factors | Immune Cells |
|---|---|---|---|---|
|  **Time of day** | Very little known | Very little known | Very little known | May follow diurnal pattern |
|  **Time postpartum** | Bacterial profiles likely vary in early lactation | Varies substantially over lactation | Varies substantially over lactation | Varies substantially over lactation |
|  **Hand expression vs pump** | Contamination can occur | Very little known | Very little known | Very little known |
|  **Foremilk vs hindmilk vs complete expression** | If pathogen is lipophilic it might be more abundant in hindmilk | Very little known | Very little known | Host cells might be higher in hindmilk |
|   **Left vs right breast** | May vary with inflammation | May vary with inflammation | May vary with inflammation | May vary with inflammation |
|  **Cleaning breast** | DNA and RNA might be on the breast | Not important to control for document | Not important to control for document | Not important to control for document |
|  **Collection container material** | Very little known | Very little known | Very little known | Very little known |

Table 3: Metadata to Consider, Collect, and/or Report when Studying Human Milk in Light of an Infectious Disease

| | | |
|---|---|---|
| **Related to milk collection and handling methods** | | |
| ✓ Type of milk collected |  | Foremilk, hindmilk, complete breast expression, composite |
| ✓ Mode of collection |  | Hand expression, electric pump, manual pump |
| ✓ Breast(s) collected |  | Right, left, both |
| ✓ Collection/storage containers |  | Glass, polypropylene, sterile, etc. |
| ✓ Preservative added? |  | If yes, what kind? |
| ✓ Storage conditions |  | Temperature, duration, freeze/thaw cycles |
| **Additional metadata that should be collected and reported if possible** | | |
| ✓ Time postpartum, and term vs. preterm |  | Colostrum, transitional milk, mature milk, gestational age (preterm vs. term) |
| ✓ Time of day |  | Morning, afternoon, evening |
| ✓ Time since last feed |  | Hours since last feeding or pumping session |
| ✓ Breastfeeding practices |  | Exclusively breastfed at the breast, fed pumped milk, mixed feeding |

| | | |
|---|---|---|
| ✓ Inflammatory state of breast |  | Na/K ratio, cytokine profile, somatic cell count, breast redness, breast pain |

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