

## Overarching Guidelines for Collecting Human Milk for SARS-CoV-2 Analysis

### INTERIM GUIDELINES – March 27, 2020

#### Key points

- Use PPE to protect assistant (if present) from infection.
- Avoid contamination of milk with SARS-CoV-2 from hands and respiratory droplets of mother and/or assistant and from pumping equipment.
- Collect and store adequate milk to allow multiple future analyses.
- Steps are easy-to-follow for mothers in a home setting without professional support.

STEPS	DESCRIPTION	COMMENTS/JUSTIFICATION
<b>1. UTILIZE PERSONAL PROTECTIVE EQUIPMENT (PPE) (FOR COLLECTION ASSISTANTS)</b>	If an assistant is present during the milk collection, he/she should utilize all appropriate PPE per his/her institutional protocol regarding interaction with a PUI or infected case. Minimum PPE for an assistant in the home setting is face mask and gloves. Mother should also wear a face mask for the collection.	PPE could include a face mask, gloves, surgical gown, eye protection, or any combination thereof as dictated by their home institution. Hand washing should precede this step. The purpose of face masks is to decrease risk of contamination of the milk through respiratory droplets from a cough or sneeze during the collection.
<b>2. CLEAN HANDS</b>	Mother and assistant (if present) should thoroughly clean, rinse, and dry hands for a full 20 seconds prior to beginning milk collection.	Ideally this should be done with warm soapy water for cleaning, warm free-flowing water for rinsing, and single-use, clean paper towels for drying.
<b>3. CLEAN BREASTS</b>	Breast should be thoroughly cleaned, rinsed, and dried with a clean paper towel that has had minimal exposure to the environment.	This is to remove any contaminating microbes from the skin. Ideally this should be done with warm soapy water for cleaning (or several prepackaged soap-containing wipes), warm free-flowing water for rinsing, and single-use paper towels for drying. We recommend supplying the paper towels with the collection kits to avoid environmental contamination. We do not recommend using iodine solutions or alcohol-containing solutions, as use of soap and water or prepackaged wipes are sufficient for cleaning the breast.

<b>4. EXPRESS MILK USING PUMP OR HAND EXPRESSION</b>	Milk should be collected using either a cleaned and sterilized handpump, electric pump and sterile collection kit, or by hand (using gloved hand) into a sterile container. Pumps should be thoroughly sanitized prior to use.	We don't know if one method is better than the other. If using a pump, make sure it is thoroughly sanitized so that it protects the mother and does not contaminate the milk. In any case, possible contamination of the milk is the primary concern for research purposes so milk needs to go directly into the sterile collection vessel.
<b>5. COLLECT MILK</b>	Collect at least 2 mL milk if only SARS-CoV-2 will be assessed; better yet collect at least 10 mL to allow for multiple analyses.	Ideally, get a complete breast expression, but either foremilk or hindmilk is also acceptable (make a note of what type of collection you get). We do not know if the virus is more likely to be found in the foremilk or hindmilk. Hopefully we will figure this out during validation of the methods.
<b>6. ALIQUOT, IF POSSIBLE</b>	If possible, aliquot milk into 1 mL portions (using sterile transfer pipettes and screw top cryovials). If this isn't possible, aliquot in larger portions or just freeze entire sample.	We will likely want to analyze the milk for more than one component (and/or analyze the milk in more than one laboratory). It is likely that repeated freeze/thaw cycles will damage RNA. Do not fill cryovials completely as they may burst upon freezing. Tubes should be placed in a secondary container, such as another tube or a plastic (ziplock) bag.
<b>7. LABEL CONTAINERS</b>	Using waterproof markers or premade labels, clearly mark pertinent information on the aliquots or collection container(s).	Please ensure that all aliquots and/or collection containers are properly and clearly labeled with (at minimum) the subject ID, date, and time the sample was collected.
<b>8. FREEZE AS SOON AS POSSIBLE</b>	Place milk immediately in ice and into a freezer as soon as possible.	When appropriate, subject should contact the researcher to retrieve milk sample. Ideally, the milk should be put in $-80\text{ C}^{\circ}$ , but if not, then $-20\text{ C}^{\circ}$ .
<b>9. CLEAN HANDS</b>	Mother and assistant (if present) should again thoroughly clean, rinse, and dry hands for a full 20 seconds.	Ideally this should be done with warm soapy water for cleaning, warm free-flowing water for rinsing, and single-use, clean paper towels for drying.