Supplementary Online Content

Chambers C, Krogstad P, Bertrand K, et al. Evaluation of SARS-CoV-2 in breast milk from 18 infected women. *JAMA*. doi:10.1001/jama.2020.15580

eAppendix. Recruitment and Sample Collection

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Recruitment and sample collection

Women residing in the U.S. were invited to enroll in the Human Milk Research Biorepository at the University of California San Diego. Women were interviewed about COVID-19 symptoms and SARS-CoV-2 test results. Sample collection kits were mailed to participants. Women followed instructions for collecting breastmilk using a personal breast pump and sterile milk collection bags. Instructions included hand washing before and after milk expression and cleansing of the nipple and areola with an alcohol wipe. Women who had recovered from their illness at the time of enrollment were asked to ship frozen samples from the peak of their symptoms in addition to a fresh milk sample. Samples were shipped to the Biorepository on ice within 24 hours of collection. Samples were aliquoted and stored at -80ºC prior to shipment on dry ice to the University of California Los Angeles.

Detection of SARS-CoV-2 RNA in breastmilk

We established and validated a quantitative RT-PCR assay using the U.S. Food and Drug Administration Emergency Use Authorization approved Abbott m2000sp/rt platform and the Abbott RealTime SARS-CoV-2 Assay. We spiked known amounts of viral RNA into breastmilk samples collected from 30 healthy, uninfected women participating in our studies prior to 2017. We established a limit of detection of 250 copies of SARS-CoV-2 RNA per ml of breastmilk.

Detection of replication-competent SARS-CoV-2 in breastmilk

We established tissue culture methods to detect replication-competent SARS-CoV-2 in breastmilk and to determine if factors present in human milk interfere with its infectivity. A stock of SARS-CoV-2 (USA-WA1/2020, BEI Resources, Manassus, VA) was propagated in Vero-E6 green monkey kidney cells and quantified by limiting dilution with viral titers expressed as $TCID_{50}$ (Tissue Culture Infectious Doses). A 20-fold range of SARS-CoV-2 was added to two samples of breastmilk donated by different women before 2017. After four days in Vero-E6 culture, SARS-CoV-2 was detected even at the lowest amount of virus added (100 $TCID_{50}$). This was confirmed in additional samples from eleven different women spiked with 200 $TCID_{50}$. In each case, replication was demonstrated by cytopathic effects in culture and SARS-CoV-2 replication was confirmed by RT-PCR.