Can SARS-CoV-2 be detected in breastmilk?

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Summer Research Opportunity Project 2020

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UNIVERSITY OF LOUISVILLE
INFECTIOUS DISEASES LABORTORY

INTRODUCTION

- The Summer Research Opportunity Project is directed by the Office of the Executive Vice President for Research and Innovation and the Office of the Provost. SROP allows students to explore a 10-week research experience to provide insight into graduate level research possibilities.
- I conducted my SROP in the Infectious Diseases Laboratory (IDLab) under the guidance of Director Dr. Leslie Wolf-Parrish, and working closely with the Research Lab Manager, Dr. Subathra Marimuthu. The Infectious Diseases Laboratory is a CLIA-certified, high complexity laboratory that completes diagnostic testing for local hospitals, clinics, and private physicians as well as research testing for clinical and applied research for the University.
- The SARS-CoV-2 virus causes the coronavirus disease 2019 (COVID-19). Since SARS-CoV-2 has been declared a world pandemic, there has been much needed research into the spread of the disease. The IDLab started diagnostic testing for SARS-CoV-2 on March 11th, 2020. In true interdisciplinary public health practice, the IDLab has conducted various research projects, working in conjunction with a myriad of professionals throughout the University's departments.
- Research projects worked included the testing of various samples from COVID-19 positive patients for genetic markers of SARS-CoV-2. Samples included upper and lower respiratory specimens; testicular tissue; amniotic fluid; cerebrospinal fluid; stool; blood; urine; and breast milk. The following is the research summary for breast milk samples collected by Dr. Forest Arnold from the UofL Hospital that we tested using RT-PCR testing methods.

HYPOTHESIS

We hypothesize that:

- The SARS-CoV-2 virus could spread from COVID-19 positive parent to infant through breast milk ingestion.
- Breast milk could have SARS-CoV-2 inhibitory properties.

The purpose of testing breast milk for SARS-CoV-2 genetic markers are to find out if breast milk from COVID-19 positive patients can be safely given to infants.

METHODS

- SARS-CoV-2 is an encapsulated RNA virus (Figure 1).
- Reverse Transcription real-time Polymerase Chain Reactions (RT-PCR) (Figure 2) coverts the viral RNA to DNA.
- The ARIES® instruments (Figure 3) first extract RNA from the sample. RNA is converted into complementary DNA by reverse transcription. The instruments then cycle through temperature phases that trigger chemical reactions to create identical copies of the primer specific to nucleocapsid gene N1 and N3 (Figure 4) and amplify this sequence using DNA as a template. Each cycle doubles the amount of viral DNA copies. Instruments complete 45 cycles. Selected fluorescent tagged N1 and N3 probes identify the amplified N1 and N3 DNA. The Luminex ARIES® instrument quantifies the amount of light emitted after processing samples.
  - CDC requirements call for N1 or N2 genome targets because of a higher sensitivity rate.

METHODS (cont'd)

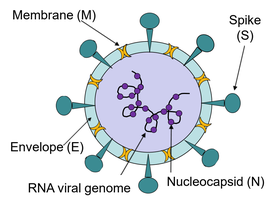

Figure 1. SARS-CoV-2 basic physical structure

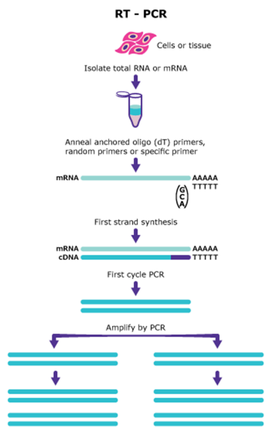

Figure 2. RT-PCR process of converting RNA to DNA copies Specific N1 and N3 primers are used at the IDLab in conjunction with CDC guidelines.

Figure 3. Luminex ARIES® instrument

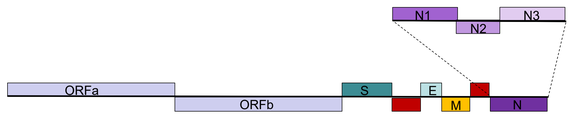

Figure 4. Target location for N1 and N3 genes on SARS-CoV-2 viral RNA

METHODS (cont'd)

- Ct (Cycle Threshold) values tell us when the virus was first detected. A lower Ct value means a higher amount of viral RNA was present in the original sample and was therefore detectable sooner through the cycling process.
- RT-PCR tests take ~2 hours after sample cartridges are primed and loaded, so this provides real-time results to physicians and their patients.
  - Qualitative analysis of test results are determined and reported where appropriate.
- Control samples and internal controls were included to assure quality of testing procedures.
  - Human RNase P is an internal control for ARIES® cartridges to show that the sample was correctly processed and there was no inhibition.

RESULTS

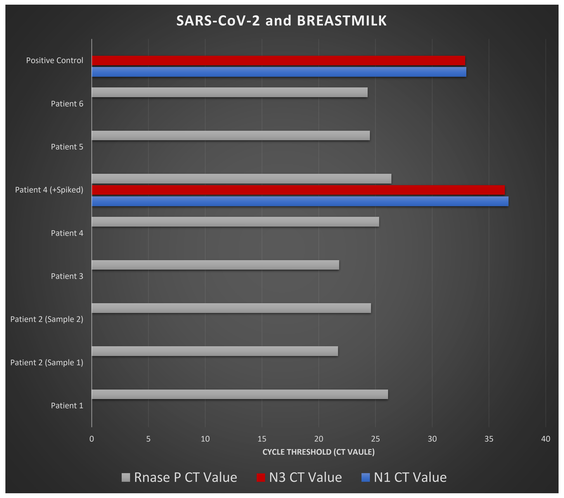

Figure 5. Data for SARS-CoV-2 and Breastmilk

- Figure 5 shows the data collected in bar graph form.
- 7 breastmilk samples were collected from 6 COVID-19 respiratory positive patients.
- The positive control sample is made up of the virus only and has no human cells within the sample. No RNase P was detected within the positive control, as expected.
- The samples from patients 1-4 were tested and were determined to be negative for the viral RNA.
  - This prompted us to ask if breastmilk had inhibitory properties for SARS-CoV-2 so we spiked patient 4's remaining sample with positive control to see if the virus would be detected.
  - The positive spiked sample was confirmed to be positive through ARIES® testing, thus showing there were no inhibitory properties masking the viral RNA from being transcribed and detected in the ARIES® SARS-CoV-2 assay.
- All 7 samples were negative for SARS-CoV-2 RNA.

DISCUSSION

- Because COVID-19 is a very recent disease, there is a lot of research that needs to done for the SARS-CoV-2 virus, its effects on the human body, and how it is transmitted from person to person.
- The results for this limited study suggest that SARS-CoV-2 is not passed from parent to infant through ingestion of breastmilk.
- Whether SARS-CoV-2 can be transmitted through breastmilk is important to know because it can help guide physicians and parent decisions on current and future breastfeeding protocol following the delivery of an infant in a hospital and when a parent returns homes after recovery.
- Many small studies have been conducted on breastmilk of COVID-19 positive patients. Small samples sizes have made conclusive findings difficult to pronounce.
- Only one patient in Germany has tested positive for the SARS-CoV-2 virus through breastmilk samples. Findings were stressed to be anecdotal in this study (Rüdiger Groß, et al., 2020) as it is the only case known so far that shows viral material being present in breastmilk.
- Antibody testing of breastmilk (Alisa Fox, et al., 2020) has shown a significant rise in IgA and IgG antibody types, suggesting breastmilk samples from previously infected, yet recovered, individuals could pass on protective properties to infants. It was also suggested that purified breastmilk antibodies could be used as a COVID-19 therapeutic.
- CDC recommends breastmilk for consumption by infants, in a case by case situation, for COVID-19 positive patients.
- Difficulties for this study were brought on by:
  - Few participants with a low number in the population, COVID-19 positive post-birth lactating individuals.
  - Giant learning curve for the modes of transmission of the noval virus.
  - Regulation changes requiring new and time-consuming validation procedures.
  - Working around other studies being done concurrently.
- The IDLab is in a constant state of starting new research, finishing projects, and continuing diagnostic testing for different pathogens. I learned many assays and procedures throughout the summer.

REFERENCES

- Fox, Alisa et al (2020). Evidence of a significant secretory-IgA-dominant SARS-CoV-2 immune response in human milk following recovery from COVID-19. Preprint. doi: https://doi.org/10.1101/2020.05.04.20089995
- Groß, Rüdiger et al (2020). Detection of SARS-CoV-2 in human breastmilk. *Lancet*. Volume 395, Issue 10239, p.1757-1758. doi: https://doi.org/10.1016/S0140-6736(20)31181-8

ACKNOWLEDGMENTS

- Dr. Leslie Wolf, Laboratory Director
- Dr. Subathra Marimuthu, Research Lab Manager
- Dr. Forest Arnold, Hospital Epidemiologist, UofL Hospital
- The Executive Vice President/University Provost and Executive Vice President for Research and Innovation Offices