APPENDIX 1

Section 1. Febrile Respiratory Illness Screening Tool—Questions and Answer Options

Section 2. Laboratory Methodology

Section 3. Data Sources

Section 4. Common Reasons for Declining Participation in SARS-CoV-2 Screening Study (n=206)

Appendix 1, as supplied by the authors. Appendix to: Fakhraei R, Erwin E, Alibhai KM, et al. Prevalence of SARS-CoV-2 infection among obstetric patients in Ottawa, Canada: a descriptive study. *CMAJ Open* 2022. DOI:10.9778/cmajo.20210228.

Section 1. Febrile Respiratory Illness Screening Tool—Questions and Answer Options*

Question	Answer Options
Contacts and Travel: Travelled outside Canada in the last 14 days?	□ Yes □ No □ Unknown
Respiratory Symptoms: Are you experiencing any of the following symptoms – new/worse cough or new/worse shortness of breath (worse than what is normal for you)?	□ Yes □ No □ Unknown
Febrile Symptoms: Do you have any of the following symptoms (select all that apply)?	□ Feverish □ Shakes □ Chills □ Temperature >38.0°C
Contact: Does someone you are close contact with have COVID-19?	□ Yes □ No □ Unknown
Acute Respiratory Illness: Are you currently experiencing an Acute Respiratory Illness and are one of the following?	□ Health care worker □ Reside in other institutions □ Health care worker as part of the health care facility outbreak □ Reside in long-term care home or retirement home □ First Nation community member living on-reserve
Hospitalized Outside Canada: Have you been hospitalized outside of Canada in the last 12 months?	□ Yes □ No □ Unknown

*Adapted from The Ottawa Hospital Febrile Respiratory Illness Screening tool

Section 2. Laboratory Methodology

SARS-CoV-2 testing

All samples of viral RNA were extracted using QIAamp viral RNA mini kit (Qiagen). Infection was determined by droplet digital PCR (ddPCR), a method that is more sensitive and precise for the clinical detection of SARS-CoV-2 than traditional qPCR methods. Primer sequences targeted the ORF1ab (nsp14), nucleocapsid protein (N), and envelope protein (E) genes of SARS-CoV-2, as well as the human RPP30 gene (internal control) in a 2-channel probe mix based triplex assay. A ddPCR reaction mix, containing purified RNA, target primers, probes, and one-step RT-ddPCR Supermix Advanced Kit for Probes (Bio-Rad), was partitioned into droplets using an automated Droplet Generator (Bio-Rad), and transferred into 96-well ddPCR plates. PCR was carried out on a C1000 touchscreen thermal cycler (Bio-Rad) under published cycling conditions: reverse transcription at 50°C for 60min, heat activation at 95°C for 10min, 40 cycles of denaturation at 95°C for 30 sec, and annealing/extension at 55°C for 1 min. A final step of enzyme deactivation was performed at 98°C for 10min, followed by a 4°C 'infinite' hold. Each run contained a positive control and no-template control (water). Cycled plates were analyzed for amplified viral products on a QX200 Droplet Reader (Bio-Rad) for the absence or presence of the targeted genes, along with the number of SARS-CoV-2 genome copies.

Antibody Testing

Overview

Serological assays were conducted using automated ELISAs to quantify antibody titers against the full-length viral spike protein and nucleoprotein. Automated ELISAs were run using a Hamilton Microlab STAR Liquid Handling System.

Plate preparation

Flat-bottom, immune nonsterile 384-well assay plates (Thermo #12-565-345) were coated with 50ng of target antigen (Spike: SmT1 (NRC PRO1-429), N: COVID19-NFSH6G (NRC PRO47-3), diluted in sterile 1X PBS (Multicell #311-010-CL). Plates were covered with adherent seal (Plate Seal #PS-PET-100) and incubated while rocking overnight at 4°C.

Prior to use, coated plates were washed 3 times with 200µL of PBS-T using a BioTek plate washer (model ELX405). After the final wash, a blocking step was performed by adding 80µL of PBS-T + 3% milk powder to each well. The plate was placed on a shaker at room temperature for one hour, then the blocking solution was removed.

Calibration Curve

A calibration curve was generated for each plate according to the antigen and antibody being assayed, as per Table 1. Control antibodies (anti-SARS-CoV-2 Spike (clone CR3022 from Absolute Antibody, clone # CR3022 (anti-Spike) and CR3018 (anti-NP)) were diluted in 1% skim milk powder in PBS-T. IgG (Absolute antibody Ab01680-10.0) was initially diluted to 1/5000. IgM (Absolute antibody Ab01680-15.0) and IgA (Absolute antibody Ab01680-16.0) were diluted to 1/4000. Subsequent ½ serial dilutions spread over 10 wells were used to generate the calibration curve.

Primary Antibody/Samples

Patient serum was diluted 1:50 with 1% skim milk in PBS-T. Dried blood spots were punched into 3.2mm diameter discs using a DBS puncher (Perkin Elmer) and eluted in 100uL PBS/disc overnight at RT. Samples were spun down, eluates were transferred to a new plate and diluted 1:2 in 1% skim milk/PBS-T. 10uL of diluted sample was added to appropriate wells. 10uL of standard curves and controls were added in quadruplicate to columns 1, 2, 23, and 24. Assay plates were incubated shaking at RT for 2 h.

Secondary Antibody

Secondary antibodies used were α-human IgG (NRC HRP-Fusion anti-IgG #5), α-human IgM (Jackson Immuno Laboratories #109-035-129), and α-human IgA (Jackson Immuno Laboratories #109-035-011).

Following plate wash in PBS-T, 10uL of HRP-conjugated secondary antibodies against IgG, IgM and IgA were added at a dilution of 1:5400, 1:9600, and 1:8000, respectively, in 1% skim milk/PBS-T. Plates were incubated 1 h shaking at RT.

Plate development

Plates were washed 4 x with PBS-T, then 10uL of luminescent HRP substrate (SuperSignal™ ELISA Pico Chemiluminescent Substrate, Thermo # 37069) was added to each well. Plates were incubated for 5 min at room temperature prior to reading on a BioTek NEO2 plate reader (20 ms/well at a read height of 1.0 mm).

Table 1. Calibration curve antibodies and concentrations

Antigen	Ab Isotype	Primary Ab Control	Range - final/well (concentrations)
Spike SmT1 & RBD	IgG	Absolute Antibody – CR3022 Ab01680-10.0 anti-spike IgG	10, 2.5, 0.625, 0.156, 0.039, 0.0098, 0.0024, 0.0006 ng (1 ug/mL to 0.00006 ug/mL)
	IgM	Absolute Antibody – CR3022 Ab01680-15.0 anti-spike IgM	20, 10, 2.5, 0.625, 0.156, 0.039, 0.0098, 0.0024 (2 ug/mL to 0.00024 ug/mL)
	IgA	Absolute Antibody – CR3022 Ab01680-16.0 anti-spike IgA	10, 2.5, 0.625, 0.156, 0.039, 0.0098, 0.0024, 0.0006 ng (1 ug/ml to 0.00006 ug/ml)
N protein	IgG	Genscript – HC2003 A02039 Human anti-Nucleocapsid IgG	20, 10, 2.5, 0.625, 0.156, 0.039, 0.0098, 0.0024 ng (2 ug/ml to 0.00024 ug/ml)
	IgM	Absolute Antibody – CR3018 (03-018) Ab01690-15.0 – anti-N IgM	20, 10, 2.5, 0.625, 0.156, 0.039, 0.0098, 0.0024 ng (2 ug/ml to 0.00024 ug/ml)
	IgA	Absolute Antibody - CR3018 (03-018) Ab01690-16.0- anti-N IgA	20, 10, 2.5, 0.625, 0.156, 0.039, 0.0098, 0.0024 ng (2 ug/ml to 0.00024 ug/ml)

Section 3. Data Sources

Data Source	Description
Better Outcomes Registry and Network (BORN) Ontario	BORN Ontario is a prescribed registry routinely captures record-level information on all pregnancies and births in Ontario, including information on maternal demographics and health behaviors, pregnancy complications, intrapartum events, and outcomes for all in-hospital births where the infant is born >500 grams or >20 weeks' gestation. BORN Ontario upholds a comprehensive data quality framework to ensure that records maintained by the registry are accurate and reliable. Regular data quality checks, internal audits and validation exercises make BORN Ontario an authoritative resource for pregnancy and birth information in Ontario. During the COVID-19 pandemic, BORN Ontario has been collecting detailed case histories on pregnant individuals admitted to hospital with confirmed or suspected COVID-19 . For this study, BORN Ontario was used to ascertain participant sociodemographic information, medical and obstetrical history, and pregnancy, delivery and newborn outcomes.
Case and Contact Management System	Case and Contact Management System is a data repository used by Ontario public health units to report cases of reportable diseases, including COVID-19, to the Ontario Ministry of Health and Long-Term Care (MOHLTC). This information is used to support public health surveillance. Each month, the Case and Contact Management System data on pregnant individuals are transferred to BORN Ontario. For this study, Case and Contact Management System data were used to identify participants with positive COVID-19 test results obtained via out-of-hospital clinical assessment centres.
The Ottawa Hospital (TOH) Data Warehouse	TOH Data Warehouse is a relational database containing information from multiple information systems including patient registration, clinical data repository and patient abstracts. Data extracts that enter the TOH Data Warehouse from administrative systems undergo quality assurance checks to ensure the data accurately represent the source system data. Additional quality assurance checks are conducted address issues including data missingness, extreme or unexpected values, and consistency with other data sources. Data quality reports are fed back to source system administrators for data corrections. Data corrections are completed in the source systems at the point of origin, and updates flow back into the Data Warehouse. For this study, TOH Data Warehouse was used to ascertain the total number of pregnant persons presenting to TOH obstetrical triage units during the study period. Identification of this population was based on the following criteria: 1. any pregnant person who was admitted to TOH after presenting to the obstetrical triage unit; OR

	2. any pregnant person who had an obstetrical triage visit type labelled ‘Hospital Encounter', 'Procedure Pass’, Anesthesia’, or ‘Anesthesia Event’. The derived estimate was increased by 5% in order to account for potential administrative data discrepancies.
Electronic Privacy Information Center	Electronic Privacy Information Center is the electronic medical record system used at The Ottawa Hospital. For this study, the Electronic Center was used to verify or ascertain the following participant information: maternal ethnicity, date of delivery, results from the Febrile Respiratory Illness screening tool administered at hospital entry, patient reported symptoms at their triage visit, in-hospital COVID-19 laboratory test results

Section 4. Common Reasons for Declining Participation in SARS-CoV-2 Screening Study (n=206)

– Feeling too overwhelmed or anxious already
– Unwilling to participate due to pain/discomfort associated with collecting the samples
– Did not want the burden of additional testing outside what was required as part of standard of care
– Worried about the possible repercussions of waiting for test results/results of the tests, including: ▪ Potential separation from newborn at birth ▪ Isolation from family ▪ Denial of childcare services or access to recreational activities ▪ Requirement to use virtual prenatal care visits instead of in-person services ▪ Having to answer "yes" to common screening questions that ask whether you are awaiting a COVID-19 test result
– Did not want to speak to research team to learn about the study