Universal SARS-CoV-2 testing among obstetrical patients (UNIVERSE-OB) in Ottawa, Canada

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Funding statement: This research was supported by the Canadian Institutes of Health Research [Funding Reference Number: VR4-172760]

Disclosures: No disclosures to declare.

Target Journal: CMAJ

Word limit: 2500 words

Table/Figure limit: no limit listed on website

Author contributions: Authors contributed meaningfully to all aspects of this study including: concept and design (El- Chaâr, Fakhraei, Dingwall-Harvey, Murphy, Rennicks White, Fell, Langlois); acquisition, analysis, or interpretation of data (El- Chaâr, Erwin, Fakhraei, Alibhai, Jia, Liu, Arnold, Galipeau, Shir-Mohammadi, Storey, LaRose, Grattan, Alton, Dy); drafting of the manuscript (Fakhraei, Alibhai, Erwin, Murphy); critical revisions for important intellectual content (El- Chaâr, Fakhraei, Dingwall-Harvey, Murphy, Rennicks White, Fell, Walker); statistical analysis (Erwin, Fakhraei); obtained funding (El- Chaâr, Walker, Fell, Dy, Langlois); administrative, technical, or material support (Murphy, Erwin, Fakhraei, Harvey, Rennicks White, Alton, Alibhai, Storey); or supervision (El- Chaâr, Langlois Harvey, Rennicks White).

Acknowledgments: We would like to acknowledge the nurses, obstetricians, family physician, midwives and clerks at the Birthing Unit and Mother-Baby Unit within The Ottawa Hospital for their support and assistance with the UNIVERSE-OB program. We also gratefully acknowledge the patients who participated in the study. Lastly, we want to acknowledge several individuals for their assistance in study recruitment and coordination including Chantal Horth, Marisa Murray, Kate Lee, Joseph Cyr, Krystina Stutely, Kirstyn Locke and Kathryn Denize.

ABSTRACT

Background
Universal testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within birthing units is an effective strategy to contain infection and estimate community prevalence. Given the high-prevalence of COVID-19 cases in Ontario, we aimed to determine the prevalence of active and recovered SARS-CoV-2 infection among pregnant individuals in Ottawa through universal SARS-CoV-2 and serology testing.

Methods
From October 19th to November 27th, 2020, pregnant individuals admitted to triage assessment units at The Ottawa Hospital (TOH) were consented for SARS-CoV-2 testing. Swab and serology samples were analyzed using digital droplet polymerase chain reaction (ddPCR) and enzyme-linked immunosorbent assays, respectively. SARS-CoV-2 seropositivity was defined as a positive result for immunoglobulin (Ig) G, either alone or in combination with IgM and/or IgA.

Results
From the 395 enrolled participants, 284 swab and 353 serology samples were collected. We found that 18 of 395 (4.6%) participants had evidence of SARS-CoV-2 exposure: 2/284 (0.70%) were positive for SARS-CoV-2 and 16/353 (4.5%) were positive for anti-SARS-CoV-2 IgG. Seropositive participants were similar to seronegative participants in terms of demographics, clinical characteristics, and pregnancy outcomes.

Interpretation
The prevalence of SARS-CoV-2 ddPCR positivity and seropositivity in the obstetrical population at TOH was 0.70% and 4.5%, respectively in the fall of 2020. According to local public health data, the infection rate peaked at 0.6% during the study time period. Universal SARS-CoV-2 testing programs can approximate community prevalence, however, justification of this strategy may depend on testing capabilities and the local context of COVID-19 infection.

Word limit: 250
Word Count: 244

Key words: pregnancy; COVID-19; SARS-CoV-2; universal testing; seroprevalence

INTRODUCTION

Since the emergence of the COVID-19 pandemic, concern for vulnerable populations has prompted widespread adoption of policies aimed at reducing the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The heightened vulnerability of obstetrical patients to SARS-CoV-2 infection and related complications is concerning due to the potential short and long-term impacts on the developing fetus and the pregnant individual.[2] Pregnant individuals represent a unique population as they require regular access to healthcare services and are admitted to the hospital independent of illness and choice.[3] As large-scale universal SARS-CoV-2 testing poses many challenges, many hospitals implemented temporary testing of all obstetrical patients in attempts to minimize in-hospital transmission and extrapolate infection prevalence in the general population.[4–8]

Early pandemic universal testing of obstetric patients (n=215) in New York City revealed a 13.5% prevalence of asymptomatic SARS-CoV-2 infection.[9] Subsequent universal testing initiatives in Tokyo, Boston, Seattle and Los Angeles reported lower prevalence of asymptomatic SARS-CoV-2 prevalence among obstetric patients, ranging from 0% to 4.0%.[4–6,8] Whether universal testing is a justifiable use of resources remains a subject of debate.[5,10] Variability in the estimates across studies may be attributed to differences in population densities, socioeconomic make-up of local areas, laboratory testing technologies, timing and duration of testing programs and dynamics of community infection rates.[5] Furthermore, the majority of these studies were conducted during the first COVID-19 pandemic wave and did not employ serological testing. Flannery et al. performed serological testing for 1,293 parturient individuals in Philadelphia between April and June 2020 and found that 6.2% were seropositive.[11] As the number of recovered cases grows, serological data provide important complementary information to PCR data by providing insight into the prevalence of recovered cases within a population.[12]

SARS-CoV-2 data from obstetrical settings in Canada are limited. One study from Montreal, Quebec (n=803) noted a 5% prevalence of SARS-CoV-2 infection among pregnant individuals during the first pandemic wave.[13] A subsequent study from Toronto, Ontario evaluated the utility of questionnaire-based PCR testing versus universal PCR testing for identifying pregnant individuals with SARS-CoV-2 infection and found no difference.[14] Since the conclusion of the Toronto study on May 25th, 2020, the burden of SARS-CoV-2 infections in Ontario have risen from 25,904 cases (~178 cases per 100,000) to 65,075 confirmed cases (~447 cases per 100,000) on October 19th, 2020.[15] Given continued rises in incidence and the limited Canadian serological data available, we aimed to assess the prevalence of SARS-CoV-2 infection through universal viral and antibody testing of pregnant individuals admitted to triage units within a tertiary care hospital in Ottawa, Canada.

Methods

Study Design & Sample Collection

This was a cross-sectional study of pregnant individuals admitted to obstetrical triage units at The Ottawa Hospital (TOH – General and Civic campuses). Universal testing for SARS-CoV-2 was implemented during the second local wave of the pandemic,[16] between October 19th and November 27th, 2020 (39 days). Since universal testing was not adopted at an institutional level, research staff were on site 7 days a week (07:30 to 23:30) to recruit patients. Pregnant patients who were ≥18 years and English or French speaking were eligible.

Participants were asked to provide a nasopharyngeal (Norgen CM-96000) or oropharyngeal swab sample (Norgen CY-93050), and a blood sample. Swab samples were placed in viral transport media (Norgen's Total Nucleic Acid Preservation Tubes Dx). Blood samples were collected as peripheral whole blood via venipuncture or as dried blood spots via finger prick. To minimize burden to patients and providers, participants who presented to the triage unit more than once during the study timeframe were only asked to provide multiple samples if they were suspected to have COVID-19 at a subsequent visit based on symptomology or contact/travel history, both of which were screened for upon hospital entry (**eAppendix 1**). If a participant was found positive for SARS-CoV-2 infection through the testing program, they were notified by their clinical care team and instructed to follow public health guidelines.[17] This study was approved by the Ottawa Health Science Network Research Ethics Board (20200640-01H).

Data Sources

We individually linked lab results with several other databases. Electronic medical records were used to ascertain the results of in-hospital SARS-CoV-2 testing. The Better Outcomes Registry & Network (BORN) Ontario, the provincial birth registry, provided information on demographics, pre-existing medical conditions, pregnancy characteristics and birth outcomes. Data from the Case and Contact Management System were used to ascertain participant results from community SARS-CoV-2 testing during the study time period. Finally, the TOH data warehouse was used to estimate the total potential population and the number of individuals not screened by research staff. The data sources are detailed in supplement **eAppendix 2**.

SARS-CoV-2 Virus and Antibody Testing

All virus and antibody testing occurred at a Containment Level II+ facility at the University of Ottawa. Swab samples were analyzed for SARS-CoV-2 RNA using digital droplet polymerase chain reaction (ddPCR) assays (Bio-Rad QX 200 Droplet Reader) targeting the ORF1ab region (nsp14), the Nucleocapsid gene (N), and Envelope gene (E).[18]

Serum samples were evaluated for SARS-CoV-2-specific antibodies (IgA, IgG, IgM) using enzyme-linked immunosorbent assays (ELISAs). ELISAs were run on automated high-throughput instruments (Hamilton Microlab STAR Liquid Handling System), to quantify antibody levels against the full-length viral spike protein and nucleoprotein. Assay signals were used as correlates of detected antibody levels in each patient sample. A positive cut-off value was established for each assay plate as a value equal to the mean of negative control optical density (OD) values (manual ELISA) or mean of negative samples (automated ELISA) plus three times the standard deviation (SD) of the OD value distribution from pre-COVID-19 plasma/serum. Antibody levels in positive samples were correlated to SARS-CoV-2 control antibodies for IgA, IgG and IgM (CR3022). A cut-off of 1:50 +100% was used to classify serology results as positive or negative. A positive result for antibody reactivity against both the full-length viral spike protein and the nucleoprotein was required for a sample to be classified as seropositive. A positive serological result was determined to be a positive IgG antibody result alone, or IgG in combination with an IgM and/or IgA positive result. When testing for antibodies against SARS-CoV-2, it is important to note that antibodies develop between 2 and 4 weeks after onset of clinical illness.[12,20] Full laboratory methodology is presented in the supplementary materials (**eAppendix 3**).

Cases were categorized as either active (i.e., measurable SARS-CoV-2 at the time of presentation to the triage unit) or recovered (i.e., lab-confirmed history of infection earlier in pregnancy or positive serology results). The distribution of antibodies among recovered cases is presented.

Statistical analysis

Descriptive summary statistics, including demographic information, obstetrical histories and delivery details were presented for the overall study population and the subgroup of participants with serological data. Data were summarized using frequency distributions for categorical variables and using means and standard deviations (SD) or medians and interquartile ranges (IQR) for continuous variables. Categorical variables were compared using a Chi-Square test with continuity correction or a Fisher Exact test (where small cell counts were present). Normally distributed continuous variables were compared using ANOVA and nonnormally distributed continuous variables were compared using the Kruskal-Wallis test. Standardized Mean Differences (SMD) were also calculated for all variables. We used the table one package in R to produce the tables examining the characteristics of our study cohort and of participants who consented to serological testing.[19] All other analyses were performed using SAS v9.4 (SAS Institute, Cary, NC). Chart reviews were conducted to address missing variables when possible.

RESULTS

Between October 19 and November 27, 2020, 888 pregnant individuals presented to an obstetrical triage unit at TOH. Of these, 650 arrived while research staff were on site and were assessed for eligibility. After excluding those who were ineligible (n=81) or declined (n=174), a total of 395 pregnant people were included in the

analysis (**Figure 1A)**, for a participation rate of 60.7%. A total of 639 maternal biosamples were collected, however, two swab samples were contaminated upon collection and excluded from analysis. Thus, a total of 284 swab samples and 353 blood samples were analysed (**Figure 1B)**.

Participant Characteristics

Participant sociodemographic and clinical characteristics are summarized in **Table 1**. The mean age (±SD) of participants was 32.4 years (±5.07). A total of 350 (88.6%) participants were in their third trimester of pregnancy at the time of their triage visit and 216 (54.7%) were multiparous. Participants were evenly distributed across neighbourhood median family income quintiles. Just over half of participants were White (54,9%) and most neither engaged in substance use during pregnancy (89.1%) nor had pre-existing health conditions (85.3%). Most participants were receiving antenatal care from only an obstetrician (68.9%).

Biosamples and Prevalence of SARS-CoV-2 Infection and Antibodies

Among the 284 participants who provided a swab sample, 2/284 (0.70%) tested positive for SARS-CoV-2 during the study period either by ddPCR testing as part of this study or by PCR testing as part of clinical care.

Among the 353 participants who provided a blood specimen, 16 (4.5%) were positive for IgG. Of these, 3 (19%) were also positive for IgA and 1 (6.3%) was positive for IgM. The proportion of individuals positive with any of the three antibodies is presented in **eAppendix 4**. Neither of the 2 PCR-positive participants were seropositive. None of the seropositive participants were PCR positive during the study timeframe, however three had received a positive PCR result through community testing prior to study initiation. Seropositive and seronegative participants were similar in terms of sociodemographic and clinical characteristics (**Table 2**).

Overall, 18 (4.6%) participants had evidence of current or previous SARS-CoV-2 infection, by PCR or antibody testing. Seven (1.8%) were symptomatic (2/2 PCR-positive participants, and 5/16 seropositive participants) and 11 (2.8%) were asymptomatic at the time of infection. Participant-reported symptoms included dry cough, shortness of breath, congestion, nausea, vomiting and unexplained fatigue.

Pregnancy and perinatal outcomes

The pregnancy characteristics and outcomes are reported in **Table 3.** Median gestational age at delivery was similar between seropositive (38.5 weeks) and seronegative (39.0 weeks) participants. None required admission to intensive care. Among seronegative participants, 7 (2.1%) experienced preterm premature rupture of membranes, 95 (28.2%) had documented atypical or abnormal fetal surveillance and 128 (38.0%) had a cesarean delivery. Corresponding results in seropositive participants were: 0 (0%), 7 (43.8%) and 10 (62.5%), respectively.

DISCUSSION

From October 19 to November 27, 2020, the prevalence of active and recovered SARS-CoV-2 infection among pregnant individuals presenting to obstetrical triage units at a tertiary care facility in Ottawa, Canada was 0.7% and 4.5%, respectively. There were no notable differences between seropositive and seronegative individuals for sociodemographic characteristics, obstetrical and medical histories, or perinatal outcomes. The rate of SARS-CoV-2 infection in our sample was comparable to the local rate of infection (0.6%) reported in Ottawa during the same time period[16] suggesting that universal testing of obstetric patients at the height of the second local pandemic wave would have been an appropriate surrogate measure for disease prevalence in the general population. Furthermore, among the 16 participants who were seropositive, just 3 had PCR-confirmed infections prior to the study. As such, serological testing may be used in combination with PCR testing to better estimate total prevalence of prior and current infection, respectively.

Our findings suggest a lower prevalence of SARS-CoV-2 infection among the obstetrical population in Ottawa compared to similar universal testing studies performed elsewhere, where prevalence rates have ranged from 0.012% to 37%.[6,21] This may be attributable to lower testing positivity and infection rates in Ottawa, as well as

differences in population density.[22] New York City, where universal testing revealed a 37% prevalence of SARS-CoV-2 infection in the obstetric population, has a population density 33 times greater than that of Ottawa [23]. Implementation of universal testing protocols necessitates consideration of several factors. First, the local prevalence of SARS-CoV-2 infection needs to be considered as local public health units may not have the capacity to support contact tracing for positive PCR results. Second, to ensure a truly universal approach, testing policies and procedures need to be adopted at the institutional level and sample collection resources need to be widely available. Third, testing methods should be reliable and ideally available via rapid testing. In our study, ddPCR test results were typically available 24 to 72 hours after collection. As all patients with a pending test were assumed to be infected and faster delivery of test results to patients and clinicians would have enabled implementation of PPE-saving protocols. Rapid testing would have reduced both workload on healthcare providers, minimized unnecessary use of resources and mitigated unwarranted stress for providers and participants.

Our study has several strengths. First, swab samples were analyzed using ddPCR, which is a more sensitive method (sensitivity 87.4-97.6%) [24,25] for clinical detection of SARS-CoV-2 than traditional qPCR methods (sensitivity approximately 70%) [26]. Second, we employed serological testing, a service that was otherwise not available through the Ontario publicly-funded health care system or through private clinics at the time of the study. As this study took place during the second local pandemic wave, serology testing allowed us to identify individuals who had been previously infected with SARS-CoV-2. Finally, although not population-based, our sample was derived from a large patient population accessing the largest hospital network in the region. Our study is not without limitations. This study did not employ a true universal testing model, as protocol were not adopted at the institutional level. As a result, 238 individuals were not screened because they presented to the triage unit when research staff and 174 declined to participate. As such, the reported prevalence of SARS-CoV-2 infection may not be generalizable to the TOH obstetrical population as a whole. Next, the presence or absence of COVID-19 symptoms was self-reported by participants at the time of consent, and we were unable to ascertain symptoms participants experienced outside of their triage visit. This limited our ability to capture information on symptoms that may been experienced by recovered participants to determine if they were symptomatic or asymptomatic at the time of infection. It also limited our ability to capture symptom information from pre-symptomatic cases.

CONCLUSION

The prevalence of active and recovered SARS-CoV-2 among the obstetrical population participating in a SARS-CoV-2 universal testing program during the Fall of 2020 in Ottawa, Ontario was 0.7% and 4.5%, respectively. These rates were in keeping with community prevalence during the second local pandemic wave. Our study highlights the need for investigation into the clinical and health system impacts of universal testing programs and to re-examine optimal testing strategies of high-risk groups for asymptomatic SARS-CoV-2 infection. Future work should explore the factors associated with pregnant individuals' decisions to participate in universal testing programs and determine if those who decline participation are more likely to reside in high-risk areas. This may increase our understanding of patients' behaviours in the context of SARS-CoV-2 testing.

REFERENCES

1. Menezes M de O, Andreucci CB, Nakamura-Pereira M, Knobel R, Magalhães CG, Takemoto MLS. Testagem universal de COVID-19 na população obstétrica: impactos para a saúde pública. *Cad Saúde Pública*. 2020;36(8):e00164820. doi:10.1590/0102-311x00164820

2. Wei SQ, Bilodeau-Bertrand M, Liu S, Auger N. The impact of COVID-19 on pregnancy outcomes: a systematic review and meta-analysis. *Can Med Assoc J*. 2021;193(16):E540-E548. doi:10.1503/cmaj.202604

3. Gagliardi L, Danieli R, Suriano G, et al. Universal severe acute respiratory syndrome coronavirus 2 testing of pregnant women admitted for delivery in 2 Italian regions. *Am J Obstet Gynecol*. 2020;223(2):291-292. doi:10.1016/j.ajog.2020.05.017

4. Goldfarb IT, Diouf K, Barth WH, et al. Universal SARS-CoV-2 testing on admission to the labor and delivery unit: Low prevalence among asymptomatic obstetric patients. *Infect Control Hosp Epidemiol*. 2020;41(9):1095-1096. doi:10.1017/ice.2020.255

5. LaCourse SM, Kachikis A, Blain M, et al. Low Prevalence of Severe Acute Respiratory Syndrome Coronavirus 2 Among Pregnant and Postpartum Patients With Universal Screening in Seattle, Washington. *Clin Infect Dis*. 2021;72(5):869-872. doi:10.1093/cid/ciaa675

6. Naqvi M, Burwick RM, Ozimek JA, Greene NH, Kilpatrick SJ, Wong MS. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Universal Testing Experience on a Los Angeles Labor and Delivery Unit. *Obstet Gynecol*. 2020;136(2):235-236. doi:10.1097/AOG.0000000000003987

7. Dória M, Peixinho C, Laranjo M, Mesquita Varejão A, Silva PT. Covid-19 during pregnancy: A case series from an universally tested population from the north of Portugal. *Eur J Obstet Gynecol Reprod Biol*. 2020;250:261-262. doi:10.1016/j.ejogrb.2020.05.029

8. Ochiai D, Kasuga Y, Iida M, Ikenoue S, Tanaka M. Universal screening for SARS-CoV-2 in asymptomatic obstetric patients in Tokyo, Japan. *Int J Gynecol Obstet*. 2020;150(2):268-269. doi:10.1002/ijgo.13252

9. Breslin N, Baptiste C, Gyamfi-Bannerman C, et al. Coronavirus disease 2019 infection among asymptomatic and symptomatic pregnant women: two weeks of confirmed presentations to an affiliated pair of New York City hospitals. *Am J Obstet Gynecol MFM*. 2020;2(2):100118. doi:10.1016/j.ajogmf.2020.100118

10. Metz TD. Is Universal Testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Needed on All Labor and Delivery Units? *Obstet Gynecol*. 2020;136(2):227-228. doi:10.1097/AOG.0000000000003972

11. Flannery DD, Gouma S, Dhudasia MB, et al. SARS-CoV-2 seroprevalence among parturient women in Philadelphia. *Sci Immunol*. 2020;5(49):eabd5709. doi:10.1126/sciimmunol.abd5709

12. Galipeau Y, Greig M, Liu G, Driedger M, Langlois M-A. Humoral Responses and Serological Assays in SARS-CoV-2 Infections. *Front Immunol*. 2020;11:610688. doi:10.3389/fimmu.2020.610688

13. Trahan M-J, Mitric C, Malhamé I, Abenhaim HA. Screening and Testing Pregnant Patients for SARS-CoV-2: First-Wave Experience of a Designated COVID-19 Hospitalization Centre in Montréal. *J Obstet Gynaecol Can*. 2021;43(5):571-575. doi:10.1016/j.jogc.2020.11.001

14. Mei-Dan E, Satkunaratnam A, Cahan T, Leung M, Katz K, Aviram A. Questionnaire-based vs universal PCR testing for SARS-CoV-2 in women admitted for delivery. *Birth*. 2021;48(1):96-103. doi:10.1111/birt.12520

15. Canada PHA of. COVID-19 daily epidemiology update. aem. Published April 19, 2020. Accessed August 24, 2021. https://health-infobase.canada.ca/covid-19/epidemiological-summary-covid-19-cases.html?stat=num&measure=total&map=pt#a2

16. Ottawa Public Health. Daily COVID-19 Dashboard. COVID-19 Cases and Deaths in Ottawa. Published September 22, 2020. Accessed March 23, 2021. https://www.arcgis.com/home/item.html?id=6bfe7832017546e5b30c5cc6a201091b

17. Self-Isolation Instructions for Novel Coronavirus (COVID-19). Published August 24, 2021. Accessed August 24, 2021. https://www.ottawapublichealth.ca/en/public-health-topics/self-isolation-instructions-for-novel-coronavirus-covid-19.aspx

18. Research CI of H. Government of Canada invests in new research to address COVID-19 variants. Published March 26, 2021. Accessed June 23, 2021. https://www.canada.ca/en/institutes-health-research/news/2021/03/government-of-canada-invests-in-new-research-to-address-covid-19-variants.html

19. Yoshida K. *Tableone: Create "Table 1" to Describe Baseline Characteristics. R Package Version 0.11.1*.; 2020. Accessed February 3, 2021. https://cran.r-project.org/package=tableone

20. Molina LP, Chow S-K, Nickel A, Love JE. Prolonged Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA in an Obstetric Patient With Antibody Seroconversion. *Obstet Gynecol*. 2020;136(4):838-841. doi:10.1097/AOG.0000000000004086

21. Maru S, Patil U, Carroll-Bennett R, et al. *Universal Screening for SARS-CoV-2 Infection among Pregnant Women at Elmhurst Hospital Center, Queens, New York*. Health Systems and Quality Improvement; 2020. doi:10.1101/2020.08.12.20171694

22. Wong DWS, Li Y. Spreading of COVID-19: Density matters. Xue B, ed. *PLOS ONE*. 2020;15(12):e0242398. doi:10.1371/journal.pone.0242398

23. Planning-Population-NYC Population Facts - DCP. Accessed July 26, 2021. https://www1.nyc.gov/site/planning/planning-level/nyc-population/population-facts.page

24. Dong L, Zhou J, Niu C, et al. Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR. *Talanta*. 2021;224:121726. doi:10.1016/j.talanta.2020.121726

25. Suo T, Liu X, Feng J, et al. ddPCR: a more sensitive and accurate tool for SARS-CoV-2 detection in low viral load specimens. *medRxiv*. Published online May 5, 2020:2020.02.29.20029439. doi:10.1101/2020.02.29.20029439

26. Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. *BMJ*. Published online May 12, 2020:m1808. doi:10.1136/bmj.m1808

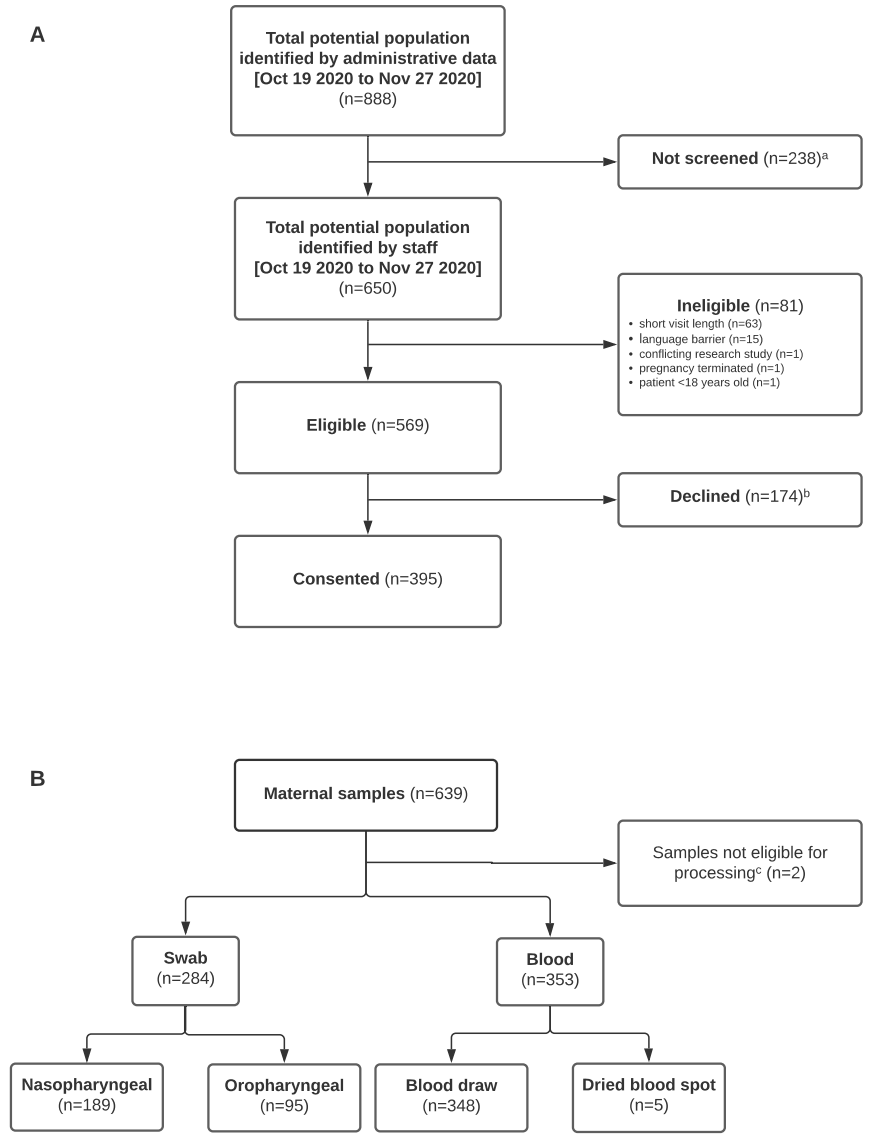


Figure 1. Flow Diagram of Study Recruitment (**Panel A**) and Sample Collection (**Panel B**). [a]These individuals presented to triage units during times when research staff was not on-site to support screening and enrollment. [b]Most commonly reported reasons for declining testing include possible repercussions of testing, including separation from child at birth, being unable to enter daycare and recreational centres due to a "pending COVID test", and fear of receiving a positive test. [c]Samples discarded due to contamination.

Table 1. Sociodemographic and clinical characteristics of all study participants that provided a maternal sample (n=395)

Characteristics	All participants (n=395)
Maternal age, years, mean ± SD	32.4 ± 5.07
Pre-pregnancy BMI, kg/m^2, median [IQR]	25.4 [8.4]
Trimester at triage visit [a], n(%)	
First	<6 (S)
Second	44 (11.1)
Third	350 (88.6)
Parity, n(%)	
Nulliparous	178 (45.1)
Multiparous	216 (54.7)
Missing	<6 (S)
Neighbourhood median family income quintile, n(%)	
Quintile 1 (poorest)	60 (15.2)
Quintile 2	83 (21.0)
Quintile 3	82 (20.8)
Quintile 4	87 (22.0)
Quintile 5 (richest)	81 (20.5)
Missing	<6 (S)
Race, n(%)	
White	217 (54.9)
Asian	29 (7.3)
Black	30 (7.6)
Other	49 (12.4)
Missing	70 (17.7)
Obesity (BMI ≥30 kg/m^2) [b], n(%)	
No	249 (63.0)
Yes	93 (23.5)
Missing	53 (13.4)
Substance use during pregnancy [c], n(%)	
No	352 (89.1)
Yes	38 (9.6)

Missing	<6 (S)
Anxiety, n(%)	
No	305 (77.2)
Yes	90 (22.8)
Depression, n(%)	
No	342 (86.6)
Yes	53 (13.4)
Pre-existing Health Conditions [d], n(%)	
None	337 (85.3)
Asthma	45 (11.4)
Chronic hypertension	7 (1.8)
Diabetes	8 (2.0)
Gestational diabetes, n(%)	
No	353 (89.4)
Yes	42 (10.6)
Hypertensive disorder in pregnancy [e], n(%)	
No	345 (87.3)
Yes	50 (12.7)
Antenatal healthcare provider, n(%)	
Family physician only	27 (6.8)
Obstetrician only	272 (68.9)
Family physician and obstetrician	48 (12.2)
Midwife	34 (8.6)
None	<6 (S)
Other	10 (2.5)
Missing/unknown	<6 (S)

Column statistics are provided. BMI: body mass index; IQR: interquartile range; S: suppressed due to small cell size; SD: standard deviation

[a] Date of triage visit is the date that the patient presented to obstetrical triage and was enrolled in the study.

[b] BMI values below 10.3 and above 79.9 were excluded as outliers and set to missing for BMI grouping and obesity

[c] Substance use during pregnancy is defined as alcohol, drug, or smoking during pregnancy.

[d] Pre-existing conditions include pre-existing asthma, diabetes, and hypertension.

[e] Conditions include eclampsia, gestational hypertension, HELLP, preeclampsia, pre-existing hypertension with superimposed preeclampsia.

Table 2. Sociodemographic and clinical characteristics of study participants who underwent serological testing (n=353)

Characteristics	All Participants with serology (n=353)	Seronegative (n=337)	Seropositive (n=16)	SMD[a]
Maternal age, years, mean ± SD	32.6 ± 4.82	32.6 ± 4.83	31.6 ± 4.53	0.207
Pre-pregnancy BMI (kg/m^2), median [IQR]	25.5 [8.4]	25.4 [8.4]	26.6 [6.6]	0.019
Trimester at triage visit [b], n(%)				
First	<6 (S)	<6 (S)	0 (0.0)	0.095
Second	38 (10.8)	36 (10.7)	<6 (S)	
Third	314 (89.0)	300 (89.0)	14 (87.5)	
Parity, n(%)				
Nulliparous	164 (46.5)	156 (46.3)	8 (50.0)	0.105
Multiparous	188 (53.3)	180 (53.4)	8 (50.0)	
Missing	<6 (S)	<6 (S)	0 (0.0)	
Race, n(%)				
White	198 (56.1)	192 (57.0)	6 (37.5)	0.618
Asian	26 (7.4)	22 (6.5)	<6 (S)	
Black	25 (7.1)	23 (6.8)	<6 (S)	
Other	42 (11.9)	40 (11.9)	<6 (S)	
Missing	62 (17.6)	60 (17.8)	<6 (S)	
Obesity (BMI ≥30 kg/m^2) [c], n(%)				
No	221 (62.6)	212 (62.9)	9 (56.2)	0.163
Yes	84 (23.8)	80 (23.7)	<6 (S)	
Missing	48 (13.6)	45 (13.4)	<6 (S)	
Substance use during pregnancy [d], n(%)				
No	314 (89.0)	298 (88.4)	16 (100)	0.512
Yes	35 (9.9)	35 (10.4)	0 (0.0)	
Missing	<6 (S)	<6 (S)	0 (0.0)	
Anxiety, n(%)				
No	272 (77.1)	257 (76.3)	15 (93.8)	0.505
Yes	81 (22.9)	80 (23.7)	<6 (S)	

Depression, n(%)				
No	304 (86.1)	288 (85.5)	16 (100)	0.583
Yes	49 (13.9)	49 (14.5)	0 (0.0)	
Pre-existing health conditions [e], n(%)				
None	300 (85.0)	285 (84.6)	15 (93.8)	0.299
Asthma	41 (11.6)	40 (11.9)	<6 (S)	0.197
Chronic hypertension	7 (2.0)	7 (2.1)	0 (0.0)	0.206
Diabetes	7 (2.0)	7 (2.1)	0 (0.0)	0.206
Gestational diabetes mellitus, n(%)				
No	318 (90.1)	304 (90.2)	14 (87.5)	0.086
Yes	35 (9.9)	33 (9.8)	<6 (S)	
Hypertensive disease in pregnancy, n(%)				
No	305 (86.4)	292 (86.6)	13 (81.2)	0.147
Yes	48 (13.6)	45 (13.4)	<6 (S)	
Antenatal healthcare provider, n(%)				
Family physician only	24 (6.8)	22 (6.5)	<6 (S)	0.512
Obstetrician only	245 (69.4)	236 (70.0)	9 (56.2)	
Family physician and obstetrician	42 (11.9)	38 (11.3)	<6 (S)	
Midwife	31 (8.8)	30 (8.9)	<6 (S)	
None	<6 (S)	<6 (S)	0 (0.0)	
Other	8 (2.3)	8 (2.4)	0 (0.0)	
Missing/unknown	<6 (S)	<6 (S)	0 (0.0)	

Column statistics are provided. BMI: body mass index; IQR: interquartile range; S: suppressed due to small cell size; SD: standard deviation; SMD: standardized mean difference

[a] Standardized mean difference comparing seronegative and seropositive participants

[b] Date of triage visit is the date that the patient presented to obstetrical triage and was enrolled in the study.

[c] BMI values below 10.3 and above 79.9 were excluded as outliers and set to missing for BMI grouping and obesity

[d] Substance use during pregnancy is defined as alcohol, drug, or smoking during pregnancy.

[e] Pre-existing conditions include pre-existing asthma, diabetes, and hypertension.

Table 3. Pregnancy characteristics and outcomes for all participants and by seropositivity status (n=395)

Characteristics	All Participants (n=395)	Serological testing (n=353)		SMD[a]
		Seronegative (n=337)	Seropositive (n=16)	
Number of fetuses, n(%)				
Singleton	377 (95.4)	321 (95.3)	16 (100.0)	0.32
Multiple	18 (4.6)	16 (4.7)	0 (0.0)	
Gestational age at delivery in weeks, median [IQR]	39.0 [3.0]	39.0 [2.0]	38.5 [2.3]	0.14
Preterm delivery (<37 weeks), n(%)				
No	337 (85.3)	289 (85.8)	13 (81.2)	0.15
Yes	57 (14.4)	47 (13.9)	<6 (S)	
Missing	<6 (S)	<6 (S)	0 (0.0)	
PPROM (<37 weeks), n(%)	7 (1.8)	7 (2.1)	0 (0.0)	0.21
Atypical or abnormal fetal surveillance, n(%)	110 (27.8)	95 (28.2)	7 (43.8)	0.33
Labour type, n(%)				
Spontaneous	184 (46.6)	158 (46.9)	<6 (S)	0.35
Induced	129 (32.7)	109 (32.3)	6 (37.5)	
No labour	81 (20.5)	69 (20.5)	<6 (S)	
Missing	<6 (S)	<6 (S)	0 (0.0)	
Mode of delivery, n(%)				
Vaginal	242 (61.3)	208 (61.7)	6 (37.5)	0.51
Caesarean	152 (38.5)	128 (38.0)	10 (62.5)	
Missing	<6 (S)	<6 (S)	0 (0.0)	
Birth outcome, n(%)	**n=413**	**n=353**	**n=16**	
Live birth	409 (99.0)	350 (99.0)	15 (93.8)	0.39
Intrapartum stillbirth	<6 (S)	0 (0.0)	<6 (S)	
Missing/Not reported	<6 (S)	<6 (S)	0 (0.0)	

Column statistics are provided. IQR = interquartile range; PPROM= preterm premature rupture of membrane; S: suppressed due to small cell size; SMD = standardized mean difference

[a] Standardized mean difference comparing seronegative and seropositive participants

SUPPLEMENTARY MATERIALS

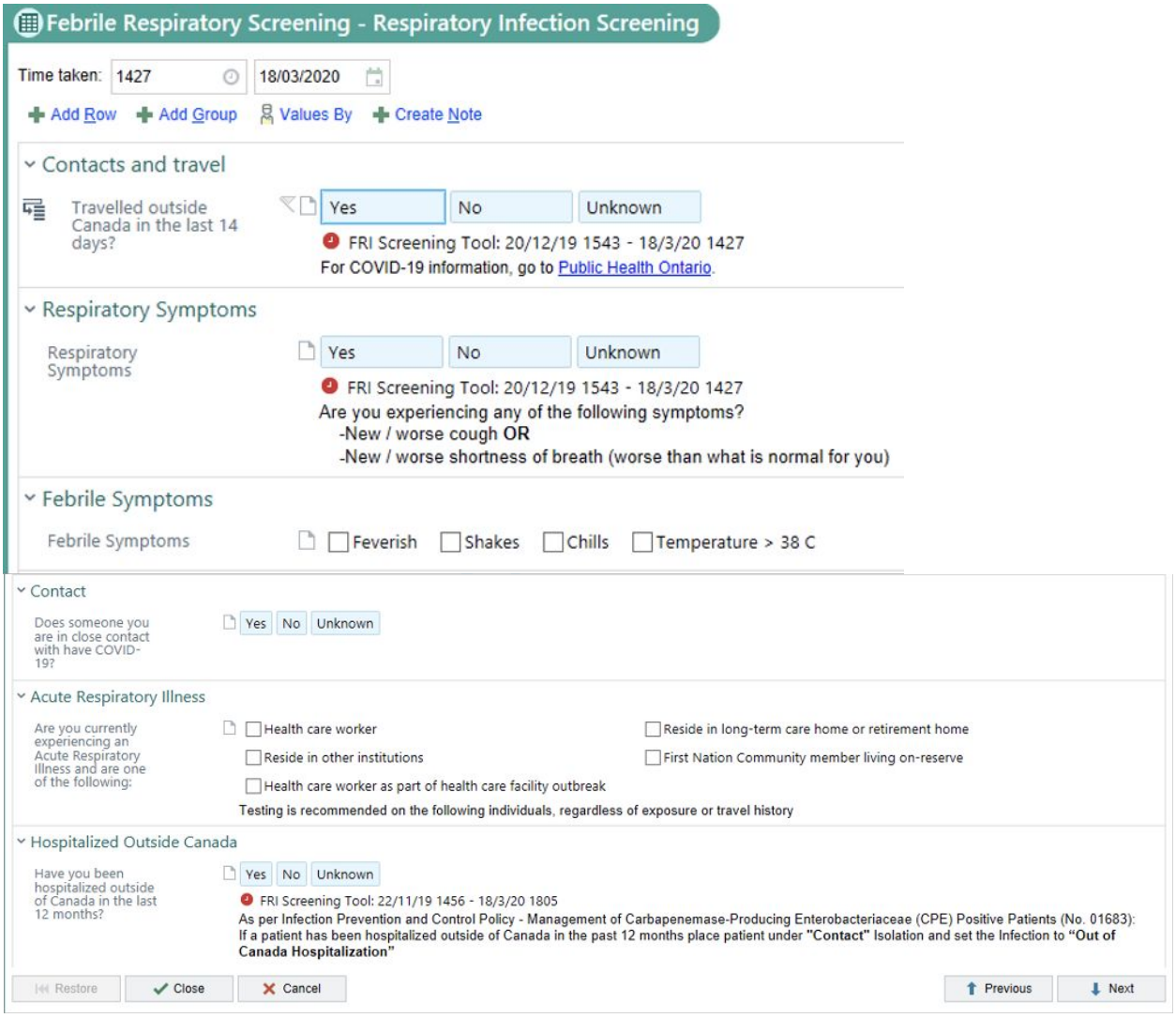
eAppendix 1. Febrile Respiratory Illness (FRI) Screening Tool

eAppendix 2. Data Sources

eAppendix 3. Laboratory Methodology

eAppendix 4. Distribution of all antibodies among participants who provided serological samples (n=353)

eAppendix 1. Febrile Respiratory Illness (FRI) Screening Tool



eAppendix 2. Data Sources

Data Source	Description
Better Outcomes Registry and Network (BORN) Ontario	BORN Ontario is a provincial birth registry housed at the Children's Hospital of Eastern Ontario (CHEO) that captures record-level 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. In response to the COVID-19 outbreak, BORN Ontario now collects data on women admitted to hospital, at any time in pregnancy regardless of the timing of delivery, with confirmed or suspected COVID-19 cases.
Case and Contact Management System	This database is used by public health units in the province of Ontario to report information regarding cases of reportable diseases, including COVID-19, to the Ontario Ministry of Health and Long-Term Care (MOHLTC). Each month, BORN Ontario receives data from CCM+ that includes COVID-19 case information among pregnant women in Ontario.
The Ottawa Hospital Data Warehouse	The Ottawa Hospital Data Warehouse is a relational database containing information from multiple information systems including patient registration, clinical data repository and patient abstracts. The total number of pregnant persons presenting to obstetrical triage units was determined using this database. Patients who were placed in this were had to meet the following criteria: 1. any pregnant person who was admitted to after presenting to the obstetrical triage unit or 2. any pregnant person who had an obstetrical triage visit type of 'Hospital Encounter', 'Procedure Pass', Anesthesia', or 'Anesthesia Event'. We augmented this estimate by an additional 5% in order to account for potential administrative data discrepancies.
Electronic Privacy Information Center (EPIC)	EPIC is the electronic medical record system used at The Ottawa Hospital. The electronic EPIC chart for each participant was consulted and the following information was extracted: ethnicity, date of delivery, result of the Febrile Respiratory Illness (FRI) screening tool, symptoms at triage visit, results from clinical and previous COVID tests.

eAppendix 3. 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)

eAppendix 4. Distribution of all antibodies among participants who provided serological samples (n=353)

Antibody type and distribution	N (%)
IgG alone	12 (3.4%)
IgM alone	40 (11.3%)
IgA alone	4 (1.1%)
IgG + IgM	1 (0.28%)
IgG + IgA	4 (1.1%)
IgM + IgA	4 (1.1%)