**Supplemental Digital Content 1. Alberta Public Health Laboratory (ProvLab) lab developed RT-PCR test**

For the ProvLab test, 200 μL of VTM was extracted on the MagMAX Express-96 or Kingfisher Flex (Thermo Fisher Scientific, Waltham, Mass) using the MagMAX-96 Viral RNA Isolation Kit (Thermo Fisher Scientific) or the PurePrep Pathogen Kit (MolGen, Veenendaal, Netherlands) according to the manufacturer’s instructions, and eluted into a volume of 110 μl. Samples with Ct value <35 were considered positive. If the Ct was ≥35, amplification from the same eluate was repeated in duplicate and considered positive if at least 2/3 results had a Ct <41. VoC screening was performed according to provincial Public Health criteria and included using whole genome sequencing and/or Alpha VoC screening PCR[16] or a variation of the assay detecting the 69/70 deletion and N501Y mutation.

**Supplemental Digital Content 2. ddPCR Testing Procedures**

Briefly, specimens were extracted using the QIAamp Viral RNA Mini Kit (QIAgen, Hilden, Germany). Isolated RNA (9.9 µL) was eluted in AVE buffer and added to the master mix comprised of 1.1 µL of the 2019-nCoV CDC ddPCR triplex assay (made by BioRad using the CDC recommended sequences for N1, N2, and RNAse P), 2.2 µL of reverse transcriptase, 5.5 µL of supermix, 1.1 µL of dithiothreitol, and 6.6 µL of nuclease-free water. Twenty-two microliters from these samples and master mix RT-ddPCR mixtures were loaded into the wells of a 96-well PCR plate (Bio-Rad, Pleasanton, CA). Appropriate positive and negative extraction controls, as specified in the Bio-Rad SARS-CoV-2 ddPCR Kit protocol, were performed alongside each batch of samples, which included a no template control, and a positive quantitation control consisting of a spike of a quantitated EDX SARS-CoV-2 Standard (Bio-Rad) containing 200,000 cp/mL of the E, N, ORF1ab, RdRP and S genes of SARS-CoV-2. The mixtures were then fractionated into 10,000 to 20,000 nanoliter-sized droplets in the form of a water-in-oil emulsion in the Automated Droplet Generator (Bio-Rad). The 96-well RT-ddPCR ready plate containing droplets was sealed with foil using a plate sealer (Bio-Rad) followed by RT of RNA, and finally PCR amplification of cDNA in a C1000 Touch thermocycler (Bio-Rad, Pleasanton, CA, USA). Following PCR, the plate was loaded into the QX200 Droplet Reader (Bio-Rad). The droplets were then singulated and flowed past a two-color fluorescence detector. The fluorescence intensity of each droplet was measured using FAM and HEX, and droplets were determined to be positive or negative for each target within the Bio-Rad SARS-CoV-2 ddPCR Test: the N1 and N2 genes of SARS-CoV-2, and using human RNase P as an amplification control. The fluorescence data was then analyzed by QX Manager 1.2 Standard Edition software to determine the presence of SARS-CoV-2 N1 and N2 in the specimen. Results were reported as N1 and N2 gene copies/µL in the extracted RNA.

**Supplemental Digital Content 3.** Study participants.

RT-PCR: reverse transcription real-time polymerase chain reaction; ddPCR, droplet digital polymerase chain reaction.

**Supplemental Digital Content 4**. Demographics and clinical characteristics of participants categorized based on concordance of ddPCR and RT-PCR test results.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | ddPCR and RT-PCR positive  n=52 | ddPCR and RT-PCR negative  n=15 | | ddPCR positive and RT-PCR negative  n=10 | | ddPCR negative and RT-PCR positive  n=2 | |
|  |  |  | P Value\* |  | P Value\* |  | P Value\* |
| Age, year, median (IQR) | 2.0 (1.0, 11.0) | 2.0 (1.0, 4.0) | 0.77 | 2.5 (0.9, 8.3) | 0.80 | 4.5 (min 1.0, max 8.0) | 0.91 |
| Sex, female, n (%) | 25 (48.1) | 7 (46.7) | >0.99 | 6 (60.0) | 0.73 | 2 (100) | 0.49 |
| Past history of pneumonia, n (%) | 2 (3.8) | 0 (0) | >0.99 | 0 (0) | >0.99 | 0 (0) | >0.99 |
| Chronic condition, n (%) | 14 (26.9) | 3 (20.0) | 0.74 | 3 (30.0) | >0.99 | 1 (50.0) | >0.99 |
| Duration of illness at index ED visit, days, median (IQR) | 1.0 (0, 3.0) | 2.0 (0, 4.0) | 0.32 | 2.0 (0, 3.25) | 0.45 | 1.0 (min 0, max 2.0) | 0.84 |
| Fever, n (%) | 31 (59.6) | 9 (60.0) | >0.99 | 9 (90.0) | 0.08 | 2 (100) | 0.52 |
| Cough, n (%) | 27 (51.9) | 3 (20.0) | 0.04 | 2 (20.0) | 0.09 | 1 (50.0) | >0.99 |
| Vomiting, n (%) | 18 (34.6) | 3 (20.0) | 0.36 | 3 (30.0) | >0.99 | 1 (50.0) | >0.99 |
| Diarrhea, n (%) | 15 (28.8) | 3 (20.0) | 0.54 | 1 (10.0) | 0.27 | 0 (0) | 0.59 |
| Admitted at the index ED visit, n (%) | 1 (1.9) | 0 (0) | >0.99 | 0 (0) | >0.99 | 0 (0) | >0.99 |
| Hospitalized During the 14 Days Follow-up, n (%) | 2/48 (4.2) | 0 (0) | >0.99 | 1 (10.0) | >0.99 | 0 (0) | >0.99 |
| Persistent Symptoms at 90-Days, n (%) | 5/30 (16.7) | 0 (0) | 0.15 | 0/9 (0) | 0.32 | 1 (50.0) | 0.35 |

\*P values were obtained from Chi square tests, Fisher’s exact tests, or Mann-Whitney U test as appropriate. All comparison made relative to the ddPCR/RT-PCR concordant positive group.

**Supplemental Digital Content 5.** Comparing nucleocapsid (N) gene copy number by droplet digital polymerase chain reaction from patients who tested negative (n=10) or positive (n=52) on reverse transcription polymerase chain reaction; A) N1; B) N2.

A) N1 gene

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B) N2 gene

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**Supplemental Digital Content 6.** Scatter plot of SARS-CoV-2 nucleocapsid (N) gene copy number quantified by droplet digital polymerase chain reaction in relation to reverse transcription polymerase chain reaction cycle threshold (Ct) values.\* The solid lines represent LOESS scatterplot smoothers.

A) N1 gene; Pearson correlation coefficient =-0.91, P<0.001

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B) N2 gene; Pearson correlation coefficient ==-0.91, P<0.001 P:\PERN COVID REDCap\PERN COVID viral load data\data Aug 9 2021\ggplot\Ct plot\ddpcrN2 Ct value x rev.tiff

**\***N1 target of BD max assay (n=27); E gene target of PLSA MT assay (n=19); S gene of Simplexa assay (n=3); E gene target of Xpert assay (n=2); N2 gene target of Xpert assay (n=1).

**Supplemental Digital Content 7.** Scatter plot of SARS-CoV-2 nucleocapsid (N) gene copy number quantified by droplet digital polymerase chain reaction in relation in A) Age and B) day of illness. Each dot represents an individual specimen’s N gene copy number; the solid lines are the LOESS scatterplot smoothers.

1. Age

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1. Day of illness

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**Supplemental Digital Content 8.** Regression analysis on association between select clinical features and SARS-CoV-2 nucleocapsid (N1 and N2) gene copy numbers (log10 copies/μL).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **SARS-CoV-2 nucleocapsid (N1) gene copy number (log10 copies/μL)** | | | **SARS-CoV-2 nucleocapsid (N2) gene copy number (log10 copies/μL)** | | |
|  | **adjusted mean difference (95%CI)** | **adjusted geometric mean ratio (95%CI)** | **P Value** | **adjusted mean difference (95%CI)** | **adjusted geometric mean ratio (95%CI)** | **P Value** |
| **Age**  < 1 year  1 - 2 years  2 to 10 years  > 10 years | 1.60 (-0.04, 3.24)  reference  1.36 (-0.08, 2.80)  2.19 (0.83, 3.55) | 40.04 (0.91, 1756.62)  reference  22.81 (0.83, 624.33)  155.46 (6.74, 3586.64) | 0.06  N/A  0.06  0.002 | 1.57 (-0.04, 3.19)  reference  1.36 (-0.05, 2.76)  2.19 (0.85, 3.52) | 37.54 (0.92, 1539.8)  reference  22.73 (0.90, 576.08)  153.3 (7.12, 3300.24) | 0.06  N/A  0.06  0.002 |
| **Baseline illness duration**  < 1 day  1 day  2 days  3 days  > 3days | reference  -1.38 (-3.10, 0.33)  -0.15 (-1.61, 1.30)  -1.66 (-3.24, -0.07)  -0.53 (-1.78, 0.73) | reference  0.04 (0.000797, 2.16)  0.70 (0.02, 19.85)  0.02 (0.000572, 0.84)  0.30 (0.02, 5.31) | N/A  0.11  0.83  0.04  0.40 | reference  -1.36 (-3.06, 0.34)  -0.10 (-1.51, 1.31)  -1.67 (-3.26, -0.08)  -0.52 (-1.77, 0.74) | reference  0.04 (0.00088, 2.21)  0.80 (0.03, 20.33)  0.02 (0.00055, 0.82)  0.31 (0.02, 5.47) | N/A  0.12  0.89  0.04  0.41 |
| **Cough**  Yes  No | 2.55 (1.48, 3.62)  reference | 353.96 (29.87, 4194.56)  reference | <0.001  N/A | 2.52 (1.45, 3.59)  reference | 331.9 (28.23, 3902.56)  reference | <0.001  N/A |

N/A, not applicable.