CMAJ-21-0263 Appendix

Methods

SARS-CoV-2 case and case contact definitions

During the period of the study, a case was defined as an individual with laboratory confirmation of infection with SARS-CoV-2 performed by the public health laboratory or its designate running a validated assay or performed in a community facility with an approved point of care testing. This consists of detection of at least one specific gene target by a nucleic acid amplification test. Close contacts are anyone who, within the period of communicability: 1) provided care for the case, including healthcare workers, family members or other caregivers, or had other similar close physical contact without consistent and appropriate use of personal protective equipment, OR 2) lived with or otherwise had close prolonged contact (within 2 metres) with a probable or confirmed case while the case was infectious, OR 3) had direct contact with infectious body fluids of a probable or confirmed case (for example, was coughed or sneezed on) while not wearing recommended personal protective equipment. Prolonged exposure is defined as lasting for more than 10-15 minutes, cumulative over 24 hours. Period of communicability is defined as 48 hours prior to symptom onset (or if asymptomatic, 48 hours prior to specimen collection for testing) until the case is no longer considered infectious. This is a minimum of 10 days after symptom onset (or from date of specimen collection if asymptomatic) and that the case has been asymptomatic for 24 hours.

Symptoms to test time

For asymptomatic infections, we used 5 days as the median time to infection, based on previously published epidemiologic data.[1] A symptoms to test time of 0 days indicates testing within 24 hours of symptom onset (i.e. the day of symptom onset).

Nasopharyngeal (NP) samples characteristics

Samples were from all regions within Manitoba that had cases of COVID-19. These swabs represented the overall population of Manitoba as COVID-19 outbreaks were in all rural, urban and northern regions during the study period. There were approximately 360,000 NP swab tests performed in Manitoba during the study dates, we therefore included 0.08% in our study. There were approximately 20,000 positive NP swabs. We included 1.5% of positive samples in this current study and 7.2% (175 of 2440) of cases ≤17 years of age.

Tissue Culture Infectious Dose 50% (TCID50) cell culture assay

Vero cells (ATCC: CCL-81) were grown in 25 cm^2 or 50 cm^2 sterile tissue culture flasks with vented caps in modified Eagle's medium (MEM) supplemented with 5% fetal bovine serum (FBS), 1% penicillin/streptomycin, 0.5 µg/mL amphotericin B, and 1% L-glutamine and maintained in a 37°C incubator with 5% CO2. For TCID50 testing, cells were seeded into 96-well plates (Thermo Scientific, 167008) at ~70% confluency. Using dilution blocks, patient samples were serially diluted 10-fold from 10^{-1} to 10^{-8} in MEM supplemented with 2% FBS, 1% penicillin/streptomycin, 0.5 µg/mL amphotericin B, and 1% L-glutamine. Dilutions were placed onto the Vero cells in triplicate and incubated at 37°C with 5% CO2 for 96 to 120 hours for

subsequent assessment of cytopathic effects and TCID50 reading. Mock infected controls served as comparator[2].

Generation of log copies per milliliter SARS-CoV-2 RNA standard curve

All experiments with live SARS-CoV-2 were performed in a BSL4 laboratory. SARS-CoV-2 stock was serially diluted 3-fold from 3.33^{-1} to 3.18^{-11} in VTM. To remove samples from BSL4 for further analysis, 140 µl of sample was inactivated in 560 µl Buffer AVL for 10 minutes and then the contents were transferred to a tube containing 560 µl 100% ethanol for an additional 10 minutes. RNA was extracted from samples using QIAmp viral RNA Minikit (QIAGEN, Valencia, CA) following manufacturer's instructions. MS2 phage was spiked into the AVL as an exogenous PCR control such that 560 µL of AVL contains 500 pfu of MS2 phage (~50,000 RNA copies – data not shown).

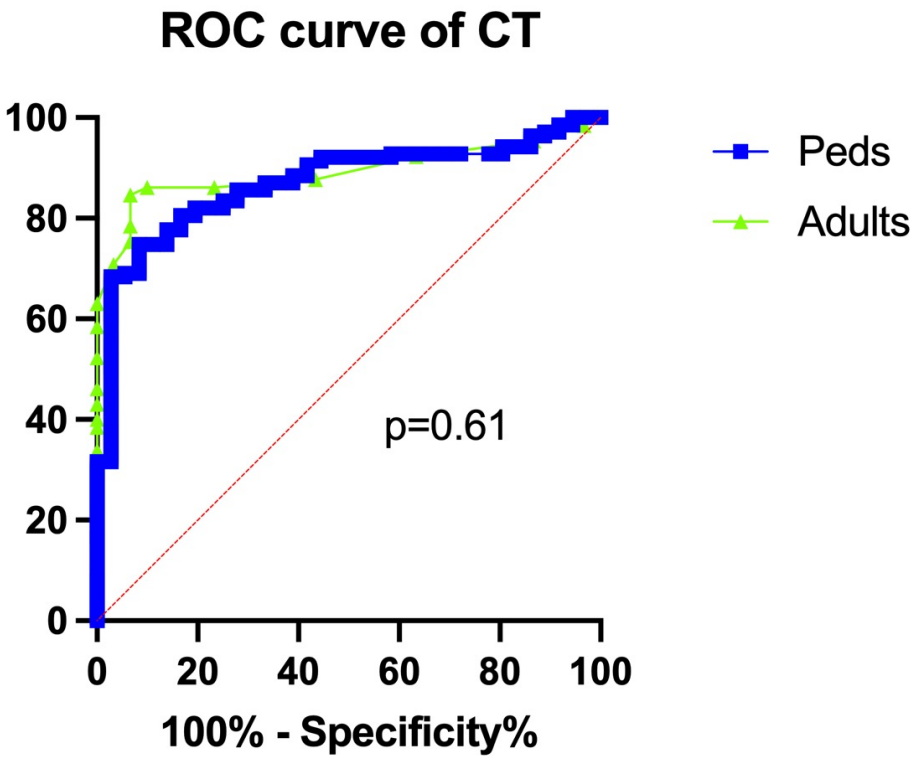
Additionally, to calculate genome copies from a standard curve, SARS-CoV-2 stock virus was inactivated, and RNA was extracted viaQIAmp viral RNA Minikit (QIAGEN, Valencia, CA) following manufacturer's instructions. Extracted RNA was then serially diluted 10-fold in TE buffer to produce the standard curve (data not shown) and quantified against synthetic RNA (BEI Resources) samples that were similarly serially diluted from the original 4.82E7 genome equivalents/mL. The viral stock standard curve was completed with all RT-qPCR runs in order to equivocate Ct value(s) at any given quantity of dilution to genome copies/mL.

The National Microbiology Laboratory (NML) completed RT-PCR using primers and probes to detect regions of the N and E gene as previously described by the US CDC and

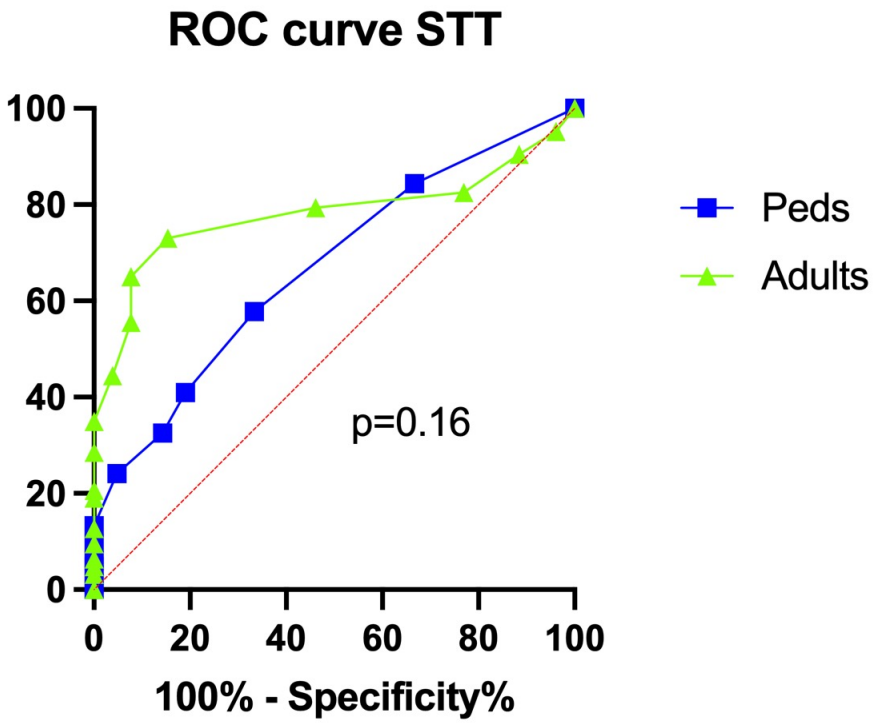
Corman *et al* , respectively[3,4]. Primer sequences were: E_Sarbeco_F1: 5'-ACAGGTACGTTAATAGTTAATAGCGT-3', E_Sarbeco_R2: 5'-ATATTGCAGCAGTACGCACACA-3', E_Sarbeco_P1: 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ-3', 2019-nCoV_N1-F1: 5'-GACCCCAAAATCAGCGAAAT-3', 2019-nCoV_N1-R1: 5'-TCTGGTTACTGCCAGTTGAATCTG-3', 2019-nCoV_N1-P1: 5'-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ-3'. RT-qPCR was performed using the TaqPath qPCR master mix and run on a QuantStudio 5 RT-qPCR system to measure the Ct.

References:

1. Lauer SA, Grantz KH, Bi Q, et al. The incubation period of Coronavirus Disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Ann Intern Med 2020;172:577-82.
2. Reed LJ and Muench H. A simple method of estimating fifty per cent endpoints. *Am J of Epi*. 1938; 27(3): 493-497.
3. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020;25(3).
4. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html.



Supplementary Figure 1: Receiver operating characteristic (ROC) curves for SARS-CoV-2 RT-PCR cycle threshold (Ct) values.



Supplementary Figure 2: Receiver operating characteristic (ROC) curves for Symptom test time (STT).

Supplementary Table 1: Sensitivity, Specificity, 95% Confidence Intervals and Likelihood Ratios for Cycle threshold (Ct) predicting SARS-CoV-2 cell culture negativity in samples from pediatric patients.

Ct	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 11.50	100	97.31% to 100.0%	2.778	0.1425% to 14.17%	1.029
> 12.50	100	97.31% to 100.0%	5.556	0.9871% to 18.14%	1.059
> 13.50	97.12	92.83% to 98.88%	8.333	2.875% to 21.83%	1.06
> 14.50	95.68	90.90% to 98.01%	13.89	6.082% to 28.66%	1.111
> 15.50	93.53	88.15% to 96.56%	19.44	9.753% to 35.03%	1.161
> 16.50	92.81	87.26% to 96.05%	36.11	22.48% to 52.42%	1.453
> 17.50	87.77	81.29% to 92.22%	61.11	44.86% to 75.22%	2.257
> 18.50	83.45	76.39% to 88.71%	72.22	56.01% to 84.15%	3.004
> 19.50	80.58	73.21% to 86.29%	80.56	64.97% to 90.25%	4.144
> 20.50	77.7	70.09% to 83.82%	86.11	71.34% to 93.92%	5.594
> 21.50	72.66	64.72% to 79.39%	91.67	78.17% to 97.13%	8.719
> 22.50	68.35	60.21% to 75.50%	97.22	85.83% to 99.86%	24.6
> 23.50	63.31	55.04% to 70.86%	97.22	85.83% to 99.86%	22.79
> 24.50	58.27	49.96% to 66.14%	97.22	85.83% to 99.86%	20.98
> 25.50	51.8	43.56% to 59.94%	97.22	85.83% to 99.86%	18.65
> 26.50	46.76	38.67% to 55.03%	97.22	85.83% to 99.86%	16.83
> 27.50	41.01	33.18% to 49.32%	97.22	85.83% to 99.86%	14.76
> 28.50	38.13	30.48% to 46.42%	97.22	85.83% to 99.86%	13.73
> 29.50	34.53	27.14% to 42.76%	97.22	85.83% to 99.86%	12.43
> 30.50	30.94	23.85% to 39.05%	100	90.36% to 100.0%	
> 31.50	26.62	19.97% to 34.52%	100	90.36% to 100.0%	
> 32.50	21.58	15.56% to 29.14%	100	90.36% to 100.0%	
> 33.50	14.39	9.511% to 21.18%	100	90.36% to 100.0%	
> 34.50	7.194	3.954% to 12.74%	100	90.36% to 100.0%	
> 35.50	3.597	1.546% to 8.144%	100	90.36% to 100.0%	
> 36.50	2.158	0.5883% to 6.153%	100	90.36% to 100.0%	
> 37.50	1.439	0.2557% to 5.094%	100	90.36% to 100.0%	

Appendix 1, as submitted by the authors. Appendix to: Bullard J, Funk D, Dust K. Infectivity of severe acute respiratory syndrome coronavirus 2 in children versus adults from nasopharyngeal swab samples. *CMAJ* 2021. doi: 10.1503/cmaj.210263.

Supplementary Table 2: Sensitivity, Specificity, 95% Confidence Intervals and Likelihood Ratios for Cycle threshold (Ct) predicting SARS-CoV-2 cell culture negativity in samples from adult patients.

Cycle Threshold	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 14.50	98.46	91.79% to 99.92%	3.333	0.1710% to 16.67%	1.019
> 15.50	95.38	87.29% to 98.74%	13.33	5.310% to 29.68%	1.101
> 16.50	92.31	83.22% to 96.67%	36.67	21.87% to 54.49%	1.457
> 17.50	87.69	77.55% to 93.63%	56.67	39.20% to 72.62%	2.024
> 18.50	86.15	75.73% to 92.54%	76.67	59.07% to 88.21%	3.692
> 19.50	86.15	75.73% to 92.54%	90	74.38% to 96.54%	8.615
> 20.50	84.62	73.94% to 91.42%	93.33	78.68% to 98.82%	12.69
> 21.50	78.46	67.03% to 86.71%	93.33	78.68% to 98.82%	11.77
> 22.50	75.38	63.69% to 84.24%	93.33	78.68% to 98.82%	11.31
> 23.50	70.77	58.80% to 80.42%	96.67	83.33% to 99.83%	21.23
> 24.50	63.08	50.92% to 73.77%	100	88.65% to 100.0%	
> 25.50	58.46	46.34% to 69.64%	100	88.65% to 100.0%	
> 26.50	52.31	40.38% to 63.98%	100	88.65% to 100.0%	
> 28.00	46.15	34.59% to 58.15%	100	88.65% to 100.0%	
> 29.50	43.08	31.76% to 55.17%	100	88.65% to 100.0%	
> 30.50	40	28.97% to 52.14%	100	88.65% to 100.0%	
> 31.50	38.46	27.60% to 50.62%	100	88.65% to 100.0%	
> 32.50	33.85	23.53% to 45.96%	100	88.65% to 100.0%	
> 33.50	24.62	15.76% to 36.31%	100	88.65% to 100.0%	
> 35.00	18.46	10.89% to 29.55%	100	88.65% to 100.0%	
> 36.50	10.77	5.315% to 20.60%	100	88.65% to 100.0%	
> 37.50	7.692	3.330% to 16.78%	100	88.65% to 100.0%	
> 39.00	4.615	1.258% to 12.71%	100	88.65% to 100.0%	