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Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

The impact of COVID-19 epidemic phase and changes in mean viral loads: implications for SARS-CoV-2 testing strategies

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ARTICLE INFO

Article history:

Received 27 July 2021

Revised in revised form 7 November 2021

Accepted 14 November 2021

Available online 19 November 2021

Keywords:

SARS-CoV-2

viral load

phase

diagnosis

ABSTRACT

The sensitivity of SARS-CoV-2 diagnostic tests is inherently linked to viral load. We explored whether average viral loads changed at a population level in Queensland, Australia during the early phase of the pandemic. RT-PCR threshold cycle (C_T) values, a crude marker for viral load, were compared for samples collected in February/March-2020 to those collected in April/May-2020, noting that the major public health interventions began in late-March 2020. Positive detections peaked mid-March, which coincided with the highest detection numbers and lowest C_T values. However, this changed from April where the later C_T samples ($C_T > 30$) predominated. Overall, in February/March 29% (267/922) of samples had C_T values >30 cycles compared to 88% (559/636) in April/May. Our study shows that SARS-CoV-2 viral loads in patients may vary at a population level over time. This needs considering when assessing suitability of diagnostic methods, particularly when methods in question are known to have reduced sensitivity.

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly since being described in China in December 2019, with the World Health Organization (WHO) declaring a pandemic on March 11, 2020 (World Health Organization WHO 2020). In Australia, like elsewhere coronavirus disease 2019 (COVID-19) cases began to escalate from late January 2020 (Australian Broadcasting Corporation 2020). Unlike most other countries impacted by early pandemic spread, Australia's strict border controls and stringent physical distancing measures corresponded with rapid case reduction and then suppressed community transmission. Notably in Queensland, new daily cases peaked at 78 on March 24, 2020, rapidly declining to 14 or fewer cases by April 6 through to September 23, 2020. Similar trends were observed for hospitalization and ICU admission numbers. (see Supplementary figure 1).

Throughout the pandemic, Pathology Queensland (the major public pathology provider servicing much of Queensland) has solely utilised laboratory based RT-PCR for SARS-CoV-2 detection, initially using an in-house PCR assay based on the widely-used E-gene RT-PCR protocol published by Corman et al., Landt (Corman et al., 2020) up until March 21, 2020, before moving to the commercial cobas[®]

SARS-CoV-2 (Roche Diagnostics Australia) for the majority of our screening. The move from the in-house E-gene RT-PCR to cobas[®] SARS-CoV-2 occurred at the peak of infections in March 2020, and evaluation of the cobas[®] SARS-CoV-2 test in our hands showed good correlation with the in-house RT-PCR using available samples at the time. However, when we later sought to evaluate a second commercial test (name withheld) during late April/early May 2020, we found quite poor correlation between methods using available samples at the time. Upon closer examination of testing data, we observed that the majority of samples collected during this period had very high cycle threshold (C_T) values in the cobas method and is indicative of low viral loads. We therefore suspected that the low correlation was more due to samples being at the edge of detection limits of the PCR methods, and therefore producing hit and miss results in the various assays.

Studies elsewhere have indicated that sensitivity issues can become more noticeable as a local epidemic passes its peak and may be related to the proportion of recovering cases increasing compared to newly acquired cases with higher viral loads. For example, Clementi et al. (2020) reported lower nasopharyngeal viral load during the later phase of a COVID-19 pandemic wave in a Northern Italy University Hospital (Clementi et al., 2020). Suspecting we had a similar trend in Queensland, we analyzed data from the initial months of the pandemic to investigate changes in patient specimens SARS-CoV-2 viral RNA levels, using RT-PCR threshold cycle (C_T) as a crude proxy for viral load. Specifically, we sought to examine whether SARS-CoV-

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2 RT-PCR C_T values differed during the peak and recovery phase of the Queensland epidemic.

2. Materials and methods

We extracted all available C_T value data for samples testing positive by RT-PCR (either in-house or commercial methods) at Pathology Queensland during the period February to May 2020 (n = 1558; Supplementary Table 1). In brief, COVID-19 testing was performed initially by in-house PCR (Corman et al., 2020, Wang et al., 2020) before commercial COVID-19 tests became available on systems such as the cobas® 6800 System (Roche, Australia). During this time period, the bulk of the positive samples (1004/1558; 64%) were identified by the cobas® 6800 System. The RT-PCR testing was performed prospectively but data were retrieved and analysed retrospectively.

The 1,558 samples were from 820 patients and comprised 820 “first samples” (being the first positive sample identified from each patient), and 738 “subsequent samples” (being any additional samples testing positive following the first positive sample from each patient). C_T values were compared over time, particularly before and after April which was when the major public health interventions were implemented, as well as between first and subsequent samples. Statistical analysis (Pearson’s Chi-squared test) was performed to compare the differences in C_T values before and after April, 2020.

Mobility data in Queensland Australia were extracted from Google COVID-19 Community Mobility Reports (<https://www.google.com/covid19/mobility/>) on September 6, 2020 to evidence the impact of the interventions on movement trends and COVID-19 transmission. Mobility data were categorized in 6 places including grocery & pharmacy, parks, transit stations, retail & recreation, residential and workplaces. The dataset showed how visits and length of stay in each place category changed compared to a baseline which was calculated using similar algorithms showing “popular times” on Google Maps (Google LLC 2020). Baseline was defined as the median value for each corresponding day of the week during the 5-week period from January 3 to February 6, 2020 (Google LLC 2020). A chart showing changes in mobility relative to baseline by week between February 15, 2020 and May 31, 2020 were illustrated using Microsoft Excel.

Data for notified SARS-CoV-2 infection cases, hospitalisation, ICU admission and deaths across Queensland between January 25, and July 31, 2020 were retrieved from COVID Live (<https://covidlive.com.au/>) which were verified against state and federal health departments. Number of cases reported per day were plotted on a bar graph. C_T values from during March 18 to 24, 2020 were retrieved from both cobas®6800 System (n = 128) and in-house test (n = 239).

3. Results

3.1. Changing trends of RNA levels among COVID-19 cases

C_T values for all samples tested (n = 1558) were plotted by week (Fig. 1). Positive detections peaked during the week of March 18 (Supplementary Table 1), which coincided with the highest numbers of samples with the lowest C_T values. However, this quickly changed after 2 weeks (April 1) where the later C_T samples ($C_T > 30$) predominated amongst the positive detections. In total, before April 29% (267/922) and 20% (131/659) of total and ‘first’ samples respectively had C_T values > 30 cycles compared to 88% (559/636) and 73% (118/161) of total and ‘first’ samples from April onwards.

A similar pattern was observed in the distribution of collection types (i.e., “first” or “subsequent”; Fig. 2). Notably, the majority of positive detections during peak week (March 18) were from the first samples. The subsequent samples made up the majority 2 weeks later while the first sample numbers were in decline. Overall, high load ($C_T < 30$) and first samples account for the majority of positive detections before April 1. Similar trend of C_T value distributions with significantly higher C_T values ($C_T > 30$) after April 1 was observed after censoring the subsequent samples ($P < 0.001$, chi-squared test, n = 820 first samples; Fig. 3). Upon closer inspection of subsequent samples, C_T values were predominantly higher (median 5 [IQR 0–11] higher in subsequent samples comparing to first samples; n = 223), suggesting a lower RNA load and by extrapolation, a lower viral load (Fig. 4).

3.2. Local public health interventions and Google mobility data

Australian borders were closed to all non-citizens and nonresidents from March 20, 2020. Social distancing measures were implemented in Queensland towards the latter part of March 2020. Legislation specifically incorporating these new measures were introduced into Queensland starting March 19, 2020 (Chief Health Officer 2020a). This included person limits on both outdoor and indoor gatherings (Chief Health Officer 2020b, Chief Health Officer 2020c), along with occupied space density of no more than 1 person per 4 square meters. Further legislation on March 23 directed the closure of non-essential businesses (Chief Health Officer 2020d). Border restrictions were then implemented on March 26 so that anyone arriving from another state or territory were required to self-quarantine for 14 days (Chief Health Officer 2020e). A home confinement direction was introduced on March 29, whereby residents were only allowed to leave their homes for essential shopping,

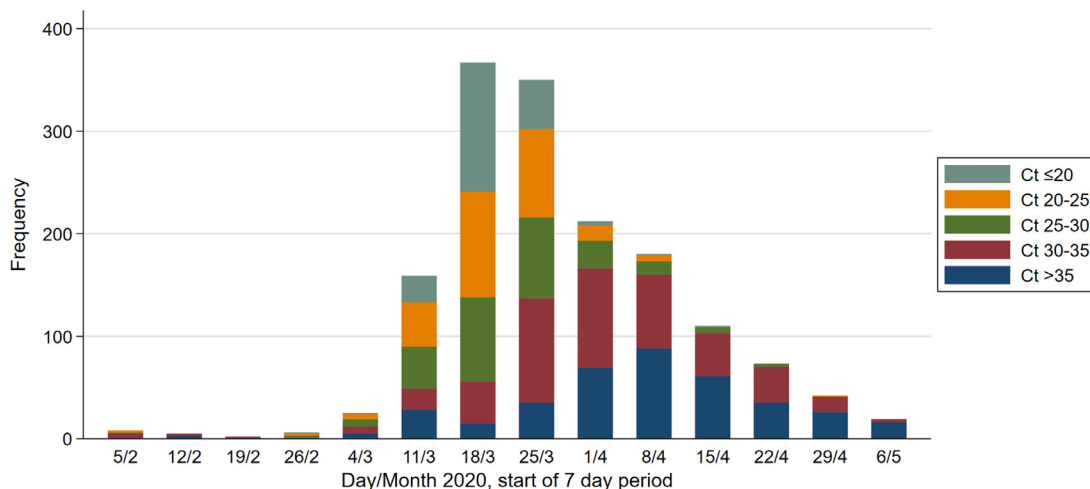


Fig. 1. The distribution of C_T values for all samples by week (n = 1558).

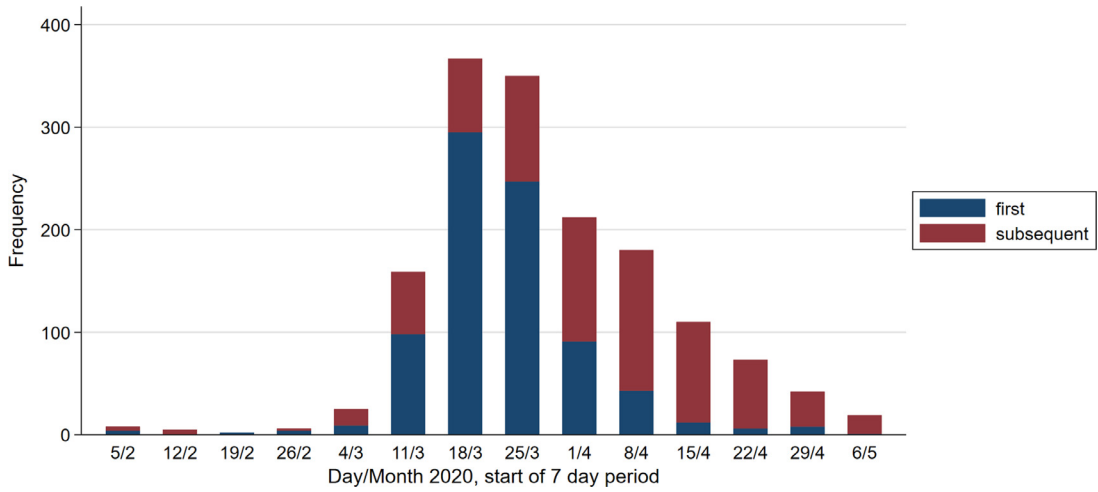


Fig. 2. The distribution of “first” and “subsequent” samples by week (n = 1558). first, first detection; subsequent, any positive samples after first detection.

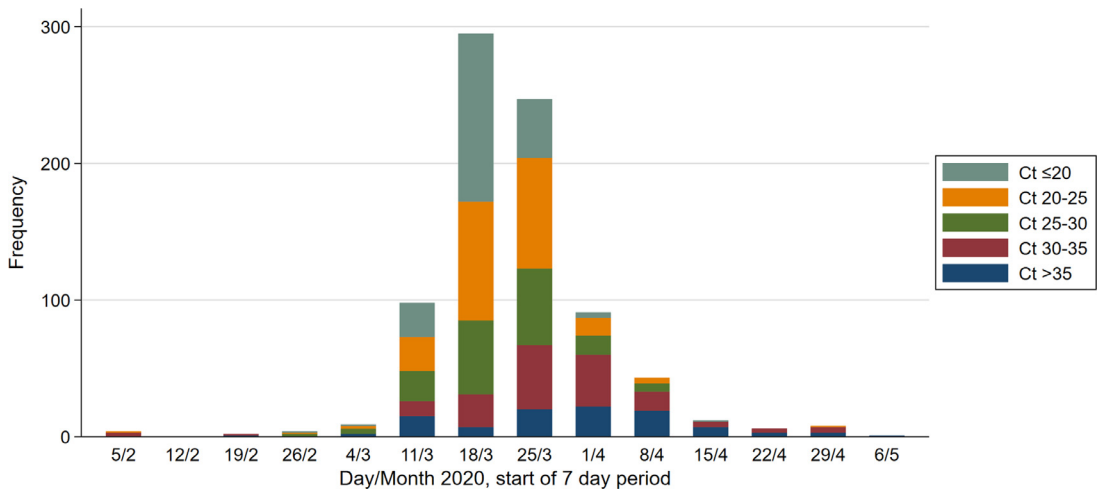


Fig. 3. C_T values for “first” samples, with subsequent samples removed (n = 820).

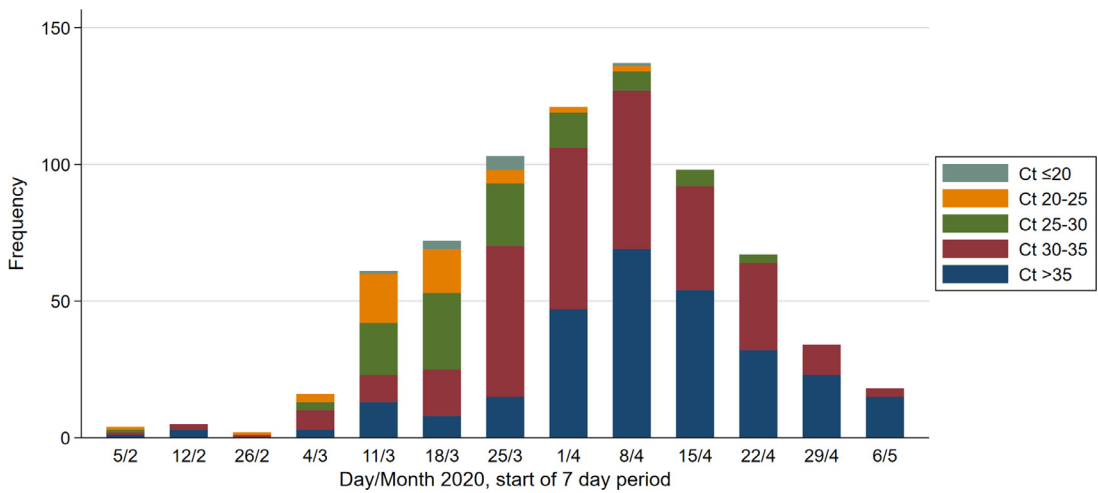


Fig. 4. C_T values for subsequent samples (n = 738).

medical needs, exercise or work and education (Chief Health Officer 2020f). Overall, this severely restricted movement for Queensland residents, and is reflected in the Google mobility data (Google LLC 2020) that identified an average of 29%, 52% and 27% decreases from

baseline associated with (1) retail and recreation, (2) transit stations and (3) workplaces, respectively since March 20, 2020 (Fig. 5). Furthermore, there was concurrently an average of 12% increase from the baseline in the residential category.

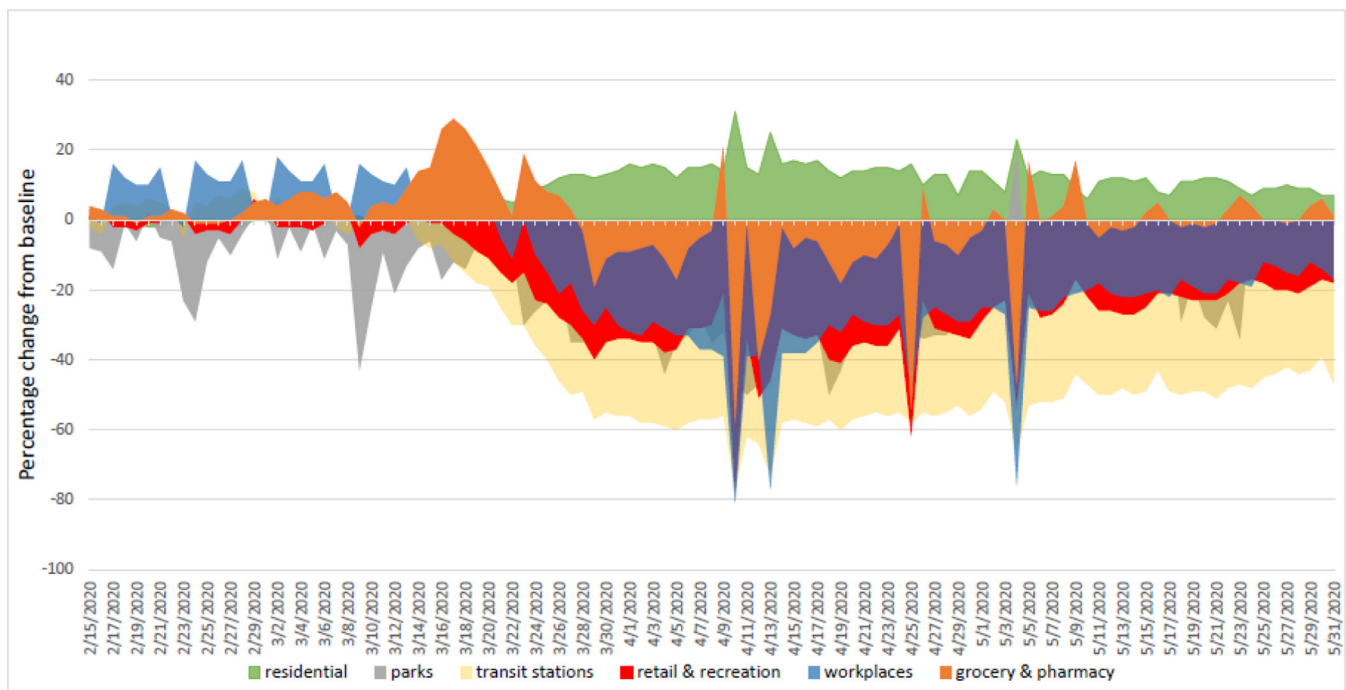


Fig. 5. Changes in community mobility for Queensland; February – May 2020. Legend – Baseline was defined as the median value for each corresponding day of the week during the 5-week period from January 3 to February 6, 2020 (Google LLC 2020). Source: Google mobility data.

4. Discussion

Overall our data clearly showed that SARS-CoV-2 viral loads were significantly lower in local patients following the public health interventions of late March 2020, and helps explain why we experienced problems with our RT-PCR validations during this period. While it is clear that many of these low loads were due to retesting of known positive patients, presumably to show clearance as at the time PCR testing was used to help determine release from isolation, which was the reason why a large number of subsequent swabs were in our study. Our data show that the trend held even when subsequently tested samples were removed. Hence these data reinforce similar observations from Italy (Clementi et al., 2020) and subsequently Switzerland (Jacot et al., 2020) that viral loads may generally be lower after a local epidemic has peaked. There are various reasons that could explain this phenomenon. For example, Kawasuji et al., 2020 has shown estimated viral loads in index patients were higher than those in non-index patients. Alternatively, the shift towards lower viral loads could otherwise be attributable to changes in COVID-19 testing guidelines. During the period of February to May 2020, there were relevant changes in testing guidelines in Queensland. Initially, the testing was limited to anyone with fever or respiratory symptoms within 14 days of travel to China. This was then revised to include anyone returned from overseas and close contacts with known positive cases at the end of February. Due to the increase in cases and suspecting community transmission, the eligibility for testing was broadened on April 5 to include workers in vulnerable settings (such as healthcare, aged care and correction facilities), anyone living in the area where an outbreak has occurred or First Nations community with symptoms. Testing criteria were further expanded on April 30 to include any one living in Queensland with any respiratory symptoms (regardless of severity) when the total state cases reached 1033 (Department of Health media release 2020). The potential net effect of the above was that eligibility for testing in our region initially only included persons with symptoms suggestive of COVID-19 who returned from overseas or in close contact with a confirmed case and thus likely resulting in a bias towards higher viral loads (lower C_T values), whereas the testing guidelines changes after March

2020 to include those with milder symptoms or otherwise with epidemiological links but not necessarily with symptoms which would potentially attribute to the lower viral loads (higher C_T values).

Regardless of the above reasoning, these findings potentially have significant implications for choice of appropriate SARS-CoV-2 diagnostic methods. This is because assay sensitivity for most methods is inherently linked to viral loads, and if viral loads change then performance will equally be affected. For example, less-sensitive rapid antigen detection (RAD) tests are widely marketed for routine SARS-CoV-2 testing. While the specificity is typically very high for most RAD kits, the overall sensitivity can vary (22.9% to 98.3%) (Albert et al., 2020, Chaimayo et al., 2020, Hirotsu et al., 2020, Linares et al., 2020, Mak et al., 2020, Young et al., 2020) between different kits when comparing to RT-PCR as the reference standard. Most studies have highlighted that these RAD kits were most sensitive and accurate during the early phase of the diseases (<5–7 days from symptom onset) (Linares et al., 2020, Mak et al., 2020, Young et al., 2020) which correlated to higher viral loads ($C_t < 20$) and sensitivity can reduce drastically as viral load decreases ($C_t > 20$ –25) (Albert et al., 2020, Linares et al., 2020, Mak et al., 2020). Hence, while RAD kits are cheaper and can be useful in rapid diagnosis of diseases particularly in resource limited areas, they are generally recommended for use in targeted patient groups (i.e., early phase of diseases or symptomatic cases) (Mak et al., 2020, Young et al., 2020, Linares et al., 2020). The variable findings in sensitivity of RAD kits are compounded by various factors including patient age, collection time (i.e., days since symptom onset), specimen type, severity of the symptoms (Albert et al., 2020, Mak et al., 2020, Nagura-Ikeda et al., 2020, Young et al., 2020). Our data now suggest that the phase of pandemic also needs to be considered. Notably, assuming most RAD methods will generally fail to detect positive samples with C_T values in excess of 30 cycles then in our population, based on our ‘first’ sample data, such methods would *at best* have had sensitivities of 82% (542/659) before April 2020, but only 28% (45/161) if used in our population during April and May 2020. Moreover, all of the above may also need to be considered as we move into a SARS-CoV-2 vaccine era. Notably, as initial studies indicate that vaccination is associated with lower SARS-CoV-2 viral

loads (Levine-Tiefenbrun et al., 2021, McEllistrem et al., 2021), vaccination therefore will potentially impact upon the suitability of certain testing platforms for identifying infection.

A limitation of our study was that we were combining C_T values from different PCR platforms. However, we compared C_T s during the peak period and found no difference in C_T s of any note between cobas® 6800 System and in-house RT-PCR method for the 7-day period starting March 18, 2020 (Supplementary Table 2) and likewise similar trends to above were observed when just analyzing Cobas® 6800 T2 data (see Supplementary figures 2 and 3).

5. Conclusion

In summary, our study provides further evidence of how SARS-CoV-2 viral loads in positive patients may vary at a population level over time. This needs to be considered when assessing the suitability of diagnostic methods, particularly where the methods in question are known to have reduced sensitivity.

Acknowledgments

We thank Pathology Queensland staff for their assistance with the provision of samples for this study. This study was approved by the Children's Health Queensland human research ethics committee (HREC/LNR/19/QCHQ/49476).

Funding

This study was funded internally.

Declaration of competing interest

DW is supported by a Queensland Advancing Clinical Research Fellowship from the Queensland Government and reports financial support from SpeedX Pty Ltd outside the submitted work. All other authors declared no conflict of interest.

Authors' contributions

DW: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing. CBU: Methodology, Validation, Writing – original draft, review & editing. CYTW: Investigation, writing. MDC: writing, statistical analysis. CBI: Resources, Writing – review & editing. PH: Supervision, Resources, Writing – review & editing.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.diagmicrobio.2021.115598](https://doi.org/10.1016/j.diagmicrobio.2021.115598).

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