Surveillance testing using salivary RT-PCR for SARS-CoV-2 in managed quarantine facilities in Australia: A laboratory validation and implementation study

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Summary

Background Regular repeat surveillance testing is a strategy to identify asymptomatic individuals with SARS-CoV-2 infections in high-risk work settings to prevent onward community transmission. Saliva sampling is less invasive compared to nasal/oropharyngeal sampling, thus making it suitable for regular testing. In this multi-centre evaluation, we aimed to validate RT-PCR using salivary swab testing of SARS-CoV-2 for large-scale surveillance testing and assess implementation amongst staff working in the hotel quarantine system in Victoria, Australia.

Methods A multi-centre laboratory evaluation study was conducted to systematically validate the *in vitro* and clinical performance of salivary swab RT-PCR for implementation of SARS-CoV-2 surveillance testing. Analytical sensitivity for multiple RT-PCR platforms was assessed using a dilution series of known SARS-CoV-2 viral loads, and assay specificity was examined using a panel of viral pathogens other than SARS-CoV-2. In addition, we tested capacity for large-scale saliva testing using a four-sample pooling approach, where positive pools were subsequently decoupled and retested. Regular, frequent self-collected saliva swab RT-PCR testing was implemented for staff across fourteen quarantine hotels. Samples were tested at three diagnostic laboratories validated in this study, and results were provided back to staff in real-time.

Findings The agreement of self-collected saliva swabs for RT-PCR was 84.5% (95% CI 68.6 to 93.8) compared to RT-PCR using nasal/oropharyngeal swab samples collected by a healthcare practitioner, when saliva samples were collected within seven days of symptom onset. Between 7th December 2020 and 17th December 2021, almost 500,000 RT-PCR tests were performed on saliva swabs self-collected by 102 staff working in quarantine hotels in Melbourne. Of these, 20 positive saliva swabs were produced by 13 staff (0.004%). The majority of staff that tested positive occurred during periods of community transmission of the SARS-CoV-2 Delta variant.

Interpretation Salivary RT-PCR had an acceptable level of agreement compared to standard nasal/oropharyngeal swab RT-PCR within early symptom onset. The scalability, tolerability and ease of self-collection highlights utility

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for frequent or repeated testing in high-risk settings, such as quarantine or healthcare environments where regular monitoring of staff is critical for public health, and protection of vulnerable populations.

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Keywords: Surveillance testing; Saliva; SARS-CoV-2; COVID-19; RT-PCR

Research in context

Evidence before this study

We conducted a literature search of peer-reviewed articles published between January 2020 and August 2021 focusing on the utility of saliva for SARS-CoV-2 RT-PCR testing for large-scale surveillance. Most studies focused on the usefulness of saliva as an alternative specimen to nasal/oropharyngeal swabbing for patient testing in primary healthcare, mainly in high prevalence settings. To date, there are limited data available on the large-scale utilisation of saliva RT-PCR for routine surveillance testing in high-risk settings, such as staff working in quarantine facilities, particularly during low-prevalence community transmission.

Added value of this study

Here, we provide an overview of salivary swab RT-PCR performance against combined nasal/oropharyngeal swab RT-PCR testing and demonstrate validation of this method across multiple laboratories. In addition, we describe the implementation of a pooled testing method for mass surveillance testing of staff working in managed quarantine facilities. To our knowledge, this is the first study to describe the implementation of mass salivary RT-PCR surveillance in Australia.

Implications of all the available evidence

In the context of new SARS-CoV-2 variants emerging, there may be an ongoing need for SARS-CoV-2 surveillance testing of high-risk frontline staff (e.g., staff working at border interfaces; healthcare workers). Our data demonstrate the feasibility of widespread pooled salivary swab RT-PCR surveillance and applicability of this approach across a range of laboratory testing platforms.

Introduction

For centuries, quarantine, case isolation and border controls have been used as strategies to contain and mitigate the spread of infectious diseases.[1] Most recently, these strategies have been employed globally to limit the spread of SARS-CoV-2 during the COVID-19 pandemic. For countries such as Australia and New Zealand, these measures helped to maintain a comparatively low prevalence of COVID-19 compared to countries such as the United States and the United Kingdom.[2] Between 20th of March 2020 and 1st of November 2021, the Australian international border was only open to travellers with a government-approved exemption, and all returning travellers were required to complete at least 14 days of quarantine in a hotel or another supervised facility.

In Australia, the first wave of COVID-19 was characterised by multiple incursions of SARS-CoV-2 from returning travellers with limited onward transmission.[3] Conversely, until widespread community transmission of the Delta and Omicron variants in late 2021 and early 2022, outbreaks of COVID-19 were largely attributed to transmission from residents within quarantine to hotel staff or fellow residents. Most notably, infection of hotel quarantine staff in the state of Victoria, Australia in mid-2020 led to over 10,000 COVID-19 infections in a large second wave in Victoria.[4] Following this outbreak, major changes were made to the hotel quarantine system; these included limiting staff employed in the hotel quarantine system from working across other settings, and improved infection control practices.[5] In addition, systematic surveillance testing of staff working in the hotel quarantine system was proposed as a means of early detection of possible transmission of SARS-CoV-2 from hotel quarantine residents to staff.[6]

Saliva has been used as an alternative specimen for diagnostic and screening purposes in the COVID-19 pandemic.[7] Compared to nasopharyngeal sampling, saliva collection is less invasive, and a meta-analysis of studies assessing the use of saliva suggested a similar agreement to nasopharyngeal swabs for detection of SARS-CoV-2, although with heterogeneity across studies regarding the populations sampled and the method of saliva collection.[8] Previous work in our setting has demonstrated the feasibility of using drooled neat saliva as an alternative to nasopharyngeal swabs.[9] However, neat saliva specimens can be complex to handle in the laboratory, with challenges relating to sample volume and viscosity.[7] Saliva sampling using standardised collection devices, although impacted by sample dilution, may help reduce variability between samples; in

particular, sampling of oral saliva using a flocked swab ('saliva swabs') offers a simple and consistent way of self-collection of saliva for SARS-CoV-2 RT-PCR and enables integration into standard RT-PCR laboratory workflows. Further, pooling of saliva samples has been demonstrated as a feasible and scalable way of testing large numbers of self-collected saliva samples.[10–12]

Here, we describe the development and validation of salivary RT-PCR testing for SARS-CoV-2 using self-collected saliva swabs from a widespread implementation study amongst staff working in the hotel quarantine system in Victoria, Australia during a period of increasing COVID-19 prevalence in the community. Further, to facilitate large-scale testing we analysed the efficiency of a sample pooling strategy for salivary RT-PCR testing.

Methods

Setting and study population

We performed a retrospective observational study assessing the implementation of salivary RT-PCR testing in hotel quarantine workers in the Victorian capital city of Melbourne (population 4.97 million), Australia. Between March 2020 and November 2021, returning international travellers to Victoria were required to undertake fourteen days of supervised quarantine in designated quarantine hotels. Following the second wave of COVID-19, in Victoria in mid-2020 the hotel quarantine system was managed by COVID-19 Quarantine Victoria (CQV), an agency responsible for overseeing the quarantine programme. As part of this programme, there was a dedicated workforce and daily testing of on-site staff on each day they attended work, with additional voluntary paid testing through community sites on days not attending work. On-site testing was a mix of saliva swabs and combined nasal/oropharyngeal swabs, with the majority of samples being saliva swabs, but at least one sample per week being a nasal/oropharyngeal swab. There was also voluntary regular testing of staff family members. International arrivals who tested positive for SARS-CoV-2 were transferred to a dedicated quarantine hotel ("health hotel") managed by Alfred Health, a public healthcare service in Melbourne. To assess the performance of saliva swab RT-PCR for surveillance testing in this programme, we conducted a multi-centre *in vitro* and clinical validation study, led by the Victorian Infectious Diseases Reference Laboratory (VIDRL) at the Doherty Institute for Infection and Immunity (Melbourne, Australia). VIDRL is the public health virology laboratory for the state of Victoria.

Saliva swab validation and microbiological testing

To assess the analytical sensitivity of saliva swab testing, a panel of 36 simulated positive samples (Supplementary Table 1) was assembled from de-identified healthy donor saliva spiked with gamma-irradiated (50Gy) SARS-CoV-2 (VIC/01), obtained from a previous study.[13] Stock irradiated virus was quantified by digital droplet RT-PCR (ddRT-PCR) (Supplementary Methods) and adjusted from 1×10^9 RNA copies/mL to a working concentration of 1×10^6 RNA copies/mL in saliva matrix (25% donor saliva in saline). Nine four-fold serial dilutions from 3.9×10^3 RNA copies/mL to 0.06 RNA copies/mL were prepared and each dilution was dispensed in quadruplicate 1mL aliquots into 2mL tubes (SSIbio, USA). The end point limit of detection (LoD) was defined as the last dilution for which all four replicates returned a positive result for both Target 1 (SARS-CoV-2 ORF1 gene) and Target 2 (SARS Beta-coronavirus E gene) using the cobas® SARS-CoV-2 assay (Roche Diagnostics) (Supplementary Methods).

Cross-reactivity was examined against a panel of non-SARS-CoV-2 viruses, obtained from stored isolates in VIDRL (Supplementary Table 2). Duplicate 1mL samples were prepared from eight viral isolates harvested from various cell lines (Supplementary Table 2) and spiked into saliva matrix at a 1:20 dilution.

To test for possible inhibitory effects of using different transport media for saliva swab collection on RT-PCR testing, duplicate saliva matrix samples were prepared 1:4 in 0.9% w/v saline; Viral Transport Media (VTM; Life Technologies); Universal Transport Media (UTM, Copan Italy) and liquid Amies (Copan Italy) (Supplementary Table 3). For each saliva/media matrix, irradiated SARS-CoV-2 (VIC/01) was spiked to a final viral load of 1×10^6, 1×10^5 and 2.5×10^4 RNA copies/mL, quantified by ddRT-PCR. Saliva matrices were also tested without SARS-CoV-2 virus as negative controls (Supplementary Table 3).

Clinical sensitivity was assessed by parallel testing on matched nasal/oropharyngeal swabs and saliva swabs (Copan flocked Eswab) obtained from individuals with RT-PCR confirmed SARS-CoV-2, collected at a range of time intervals post-symptom onset and grouped as >7 days post-symptom onset and ≤7 days post-symptom onset (Table 1). Nasal/oropharyngeal swabs were collected in 1mL of liquid Amies (Copan Eswab, 480 CE Interpath Services) as per the Australian Public Health Laboratory Network (PHLN) guidelines[14] and tested using the cobas® SARS-CoV-2 assay (Roche Diagnostics). Saliva swab samples were self-collected using an instructional sheet and short video. Briefly, a sterile flocked swab was inserted into the mouth for 30 seconds with the mouth closed. The swab was removed and collected into a tube containing 1mL of liquid Amies (480 CE Interpath Services) and tested using the same assay.

Pooling and decoupling algorithm for large-scale testing

To enable large-scale RT-PCR testing, we established a sample pooling and decoupling algorithm. Batched

Days post-symptom onset	Cohort size	Average Ct Target 1 (95% CI)		Average Ct Target 2 (95% CI)		Sensitivity	Specificity	PPA	NPA
		Nasal/OP	Saliva	Nasal/OP	Saliva				
≤7 days P.S.O	40	25.0	31.5	25.7	33.1	84.8	50.0	96.6	16.7
		(23.5-26.5)	(30.1-32.9)	(24.1-27.2)	(31.7-34.6)	(68.8-93.8)	(9.5-90.6)	(81.4-100)	(1.1-58.2)
>7 days P.S.O[a]	13	28.4	30.39	29.41	31.79	9.1	100	100	9.1
		(26.3-30.55)	(28.0-32.7)	(27.0-31.8)	(29.1-34.51)	(<0.01-39.3)	(16.8-100)	(16.8-100)	(<0.01-39.9)
Total	53	26.18	31.45	27.20	33.43	59.2	66.7	96.7	9.1
		(24.8-27.5)	(30.1-32.8)	(25.7-28.7)	(32.0-34.3)	(45.2-71.8)	(20.2-94.4)	(81.8-100)	(1.3-29)
Wilcoxon matched-pairs signed test				Exact *p* value <0.0001					
Target 1 nasal/oropharyngeal vs saliva (total)									
Wilcoxon matched-pairs signed test				Exact *p* value <0.0001					
Target 1 nasal/oropharyngeal vs saliva (≤7 days P.S.O)									

Table 1: **Assessment of salivary swab RT-PCR performance using paired nasal/oropharyngeal and salivary swab specimens.**
P.S.O – Post-symptom onset.
PPA (Percentage Positive Agreement) was calculated as percentage of positive saliva swabs against nasal/oropharyngeal swabs. Values are displayed with 95% confidence intervals (95% CI).
NPA (Negative Percentage Agreement) was calculated as percentage of negative saliva samples against nasal/oropharyngeal swabs. Values are displayed with 95% confidence intervals (95% CI).
[a] Days P.S.O collected >7 days encompasses *n* = 13 samples between 8 and 33 days.
OP - Oropharyngeal.

samples were prepared as four-sample pools (Figure 1) equivalent to a 1:4 dilution based on our previous experience with saliva pooling.[15] A panel of twenty 1mL pooled samples (Supplementary Table 4) was assembled, each consisting of 250µL of blank VTM combined from 4 individual samples (i.e. pool 1 consisted of samples 1-4, pool 2; samples 5-8 etc.). Four of these pools were prepared as simulated infectious samples using VTM spiked with SARS-CoV-2 (VIC/01) to different concentrations (1×10^3, 4×10^3, 2.5×10^2 and 1.25×10^2 RNA copies/mL). Pooled panels were tested using the cobas® SARS-CoV-2 assay (Roche Diagnostics) by staff blinded to sample content. Positive pools were subsequently retested to identify individual positive specimens.

Validation of salivary swab testing across diagnostic laboratories

To facilitate implementation of salivary swab RT-PCR testing across other laboratories, we conducted a validation exercise assessing RT-PCR platforms in two community and five public hospital laboratories in Melbourne. Sample panels were prepared to assess the analytical sensitivity and specificity of different RT-PCR assays and to challenge each laboratories capacity to pool, decouple and retest. Briefly, analytical sensitivity and specificity panels were assembled in de-identified healthy donor saliva, diluted 1:4 with 0.9% w/v saline and spiked with 1×10^6 RNA copies/mL of gamma-irradiated (50Gy) SARS-CoV-2 (VIC/01) and prepared as 1mL quadruplicate serial dilutions from 3.9×10^3 RNA copies/mL to 0.06 RNA copies/mL as shown in Supplementary Table 1. The end point LoD was defined as the last dilution where all assay targets were detected in all four replicates. Specificity of each platform was evaluated using a panel of eight duplicate samples of non-SARS-CoV-2 virus isolates (described above) diluted 1:20 in VTM. To test for possible inhibitory effects of using different transport media for saliva swab analysis, duplicates samples of transport media (0.9% w/v saline, VTM, UTM and Liquid Amies) were spiked with irradiated SARS-CoV-2 to final concentrations of 1×10^6, 1×10^5 and 2.5×10^4 RNA copies/mL and tested by each workflow. De-identified healthy donor saliva samples diluted 1:4 in each transport media were also included as negative controls.

To evaluate pooling for batch testing at multiple laboratories, twenty 1mL four-sample pools were prepared by combining 250µL of blank VTM from each sample, as described above (Figure 1). Four of these pools were prepared as simulated infectious samples using VTM spiked with SARS-CoV-2 (VIC/01) to 1×10^3, 4×10^3, 2.5×10^2 and 1.25×10^2 RNA copies/mL. Each laboratory performed the sample pooling and retesting and were evaluated on the correct reporting of identified positive samples. Panels were distributed to each

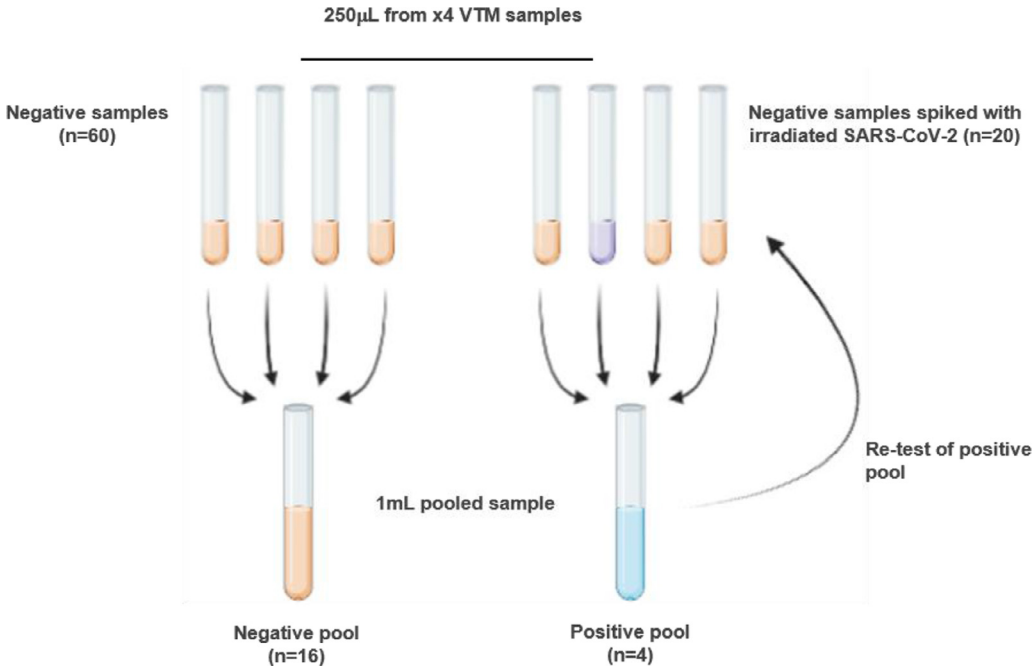

Figure 1. Four-sample pooling for batch testing algorithm. A total of 250µL of each sample was combined into a single pool and tested by RT-PCR. Four positive pools (blue) were prepared from 20 samples consisting of 15 negative donor saliva samples and 5 samples spiked with infectious samples (purple) and tested blind; one pool contained 2 infectious samples and three contained only a single infectious sample. Subsequent re-testing was performed on positive pools to identify the individual samples.

laboratory and tested by staff blinded to the sample contents using the testing platform in their specific laboratory (Supplementary Table 5). Results from testing were provided back to VIDRL for collation, and only laboratories who provided cycle threshold (Ct) values were included for analysis in each exercise.

Implementation of testing programme

Implementation of a salivary surveillance testing programme for staff working in the hotel quarantine system commenced on the 7th December 2020 in Melbourne. In total, fourteen hotels were utilised, of which twelve hotels housed returning international and interstate travellers, along with local Victorian residents requiring hotel quarantine facilities (e.g. unable to self-isolate at home), and two hotels accommodated individuals known to be SARS-CoV-2 RT-PCR positive (Supplementary Figure 1). Saliva swab implementation was supported by staff training in saliva swab self-collection using an instructional sheet and a short training video, similar to methods described above. In some instances, this was supervised self-collection. In addition to clinical risk assessment for COVID-19 (daily temperature checks and daily attestations regarding COVID-19 symptoms and potential exposure), weekly nasal/oropharyngeal RT-PCR testing plus salivary swab PCR testing on all other days of onsite work was implemented. Salivary swab testing was performed on site, on days employees were at work. From February 2021, this was supplemented with offsite voluntary paid PCR testing in the workplace or through community testing sites, that did not offer salivary sampling. Staff with symptoms consistent with COVID-19, or identified in a potential infection control breach in a hotel, were not allowed on site and PCR testing was performed with a nasal/oropharyngeal swab. Employees included staff working as floor monitors, drivers, stewards, on-site cleaners and caterers, specimen collection staff, healthcare workers and other authorised staff. Samples were couriered to three diagnostic laboratories undertaking high-throughput salivary swab RT-PCR testing, and results were provided back to individuals (via text message, usually within 12 hours, as these tests were prioritised in the testing system) and to the Victorian Department of Health via electronic laboratory notification. Positive results were called through to the staff member. Between December 2020 and October 2021 an algorithm was developed to plan for the event of a positive saliva swab test; in that event, an additional sample for nasal/oropharyngeal swab testing would be collected from the staff member and repeat RT-PCR testing expedited at the local laboratory. The purpose of this algorithm was to provide rapid confirmatory RT-PCR results to enable timely clinical and public health action. From October 2021, a positive saliva sample was treated as a true result.

Statistical analysis

Statistical analysis and data visualisation was performed using GraphPad Prism, version 9.0 (San Diego, CA, USA). Positive and negative performance agreements (PPA and NPA) as well as sensitivity and specificity were calculated by comparing the results of the saliva swab RT-PCR with nasal/oropharyngeal swab RT-PCR testing. Where appropriate, results were reported with 95% confidence intervals. Non-parametric Wilcoxon matched-pairs analysis of mean Ct values between saliva and nasal/oropharyngeal samples was performed to determine significance at a p-value ≤0.05 (mean ± SD) and Spearman's linear correlations were performed to identify trend between sample types.

Role of the funding source

The funder was not involved in data collection, analysis or manuscript preparation.

Ethics

This study was approved by the Alfred Health Human Research Ethics Committee (Local HREC number 209/21) and the Monash Health Human Research and Ethics Committee (RES-20-0000-678A).

Results

Salivary swab RT-PCR is a sensitive assay for detection of SARS-CoV-2

Analytical sensitivity for the cobas® SARS-CoV-2 assay against a 4-fold dilution series of simulated infectious saliva samples was 62.5 RNA copies/mL (Supplementary Table 1). No cross-reactivity was observed to non-SARS-CoV-2 viruses, and salivary RT-PCR performance was not significantly different between media types at 1×10^6, 1×10^5 and 2.5×10^4 RNA copies/mL (Supplementary Tables 2 & 3). All external laboratories detected spiked saliva swab specimens at 62 RNA copies/mL (Supplementary Table 5) and reported no cross-reactivity to any of the distractor viruses. Ct values for spiked saliva diluted in different transport media types were similar (Supplementary Figure 2) and no inhibition was observed.

Qualitative evaluation and validation of a sample pooling algorithm

To increase saliva swab RT-PCR testing throughput capacity, we evaluated an algorithm to pool samples for batch testing (Figure 1). From the 20 pooled samples tested, the four simulated infectious pools were correctly identified by the cobas® SARS-CoV-2 assay (Supplementary Table 4). Subsequent re-testing of the individual samples from each positive pool correctly identified the five individual SARS-CoV-2 spiked samples. Similarly, blind evaluation and validation of this testing algorithm by external laboratories resulted in correct identification of infectious pools and subsequent retesting of the individual positive samples (Supplementary Table 4). No false positives or false negatives were observed.

Clinical salivary swab RT-PCR testing

In total, 62 saliva swab specimens were obtained from individuals known to be positive for SARS-CoV-2 from nasal/oropharyngeal swabs, 53 of which had associated symptom onset data (Table 1). Of the 53 samples with symptom onset data, 45 were positive using the saliva swab giving a sensitivity of 59.2% (95% CI 45.2 to 71.8) to nasal/oropharyngeal swabs (excluding single target positives, which were called 'indeterminate'). Of the 41 samples collected within seven days of symptom onset, 28 were positive, whereby the sensitivity increased to 84.5% (95% CI 68.6 to 93.8). A single saliva swab returned a positive result in the absence of a positive nasal/OP test. The Ct values for this sample were 34.9 (Target 1) and 36.2 (Target 2). For individuals with symptom onset greater than seven days, Target 1 Ct values ranged from 16.6 to 34 for nasal/oropharyngeal swabs, and 26.6 to 35.2 for saliva swabs. The Ct values on both targets of the cobas® SARS-CoV-2 assay were significantly lower for nasal/oropharyngeal samples than for saliva swab samples (p-value <0.001) (Supplementary Figure 3).

Saliva swab RT-PCR testing in hotel quarantine workers

From the beginning of the testing programme in Hotel Quarantine staff, from December 2020 to December 2021, 102 staff members tested positive for COVID-19. Six of these were in February 2021, while the remaining 96 were from September to December 2021, when community rates markedly increased, and these later infections were deemed to be community-acquired. Of the 102 staff, 66 (64.7%) were detected using nasal/oropharyngeal PCR, 13 (12.7%) were detected using salivary swab PCR, and the swab type for the remainder was not recorded. Of note, the bias for detection of cases using nasal/oropharyngeal PCR was likely due to the preference for this swab type in symptomatic staff members or following any infection control breach. Between 7th December 2020 and 17th December 2021, a total of 494,770 saliva swabs were tested, with repeat nasal/oropharyngeal tests of staff who returned a positive saliva sample sent to local laboratories for confirmation. The 13 staff (above) produced a total of 20 positive saliva swabs (0.004%). For these individuals the protocol required retesting by nasal/oropharyngeal swab RT-PCR, and, subsequently, 18 were deemed negative. Five of the 18 saliva samples were tested at VIDRL and

returned a high Ct Target 1 (only) positive, and 13 were tested at the Alfred and returned a high Ct Target 2 positive (only) result. For the remaining two positive saliva results (one, Target 1 and Target 2 positive, the other, just Target 2 positive), neither had a follow up confirmatory nasal/oropharyngeal swab available to test.

Discussion

Here, we describe the development and implementation of large-scale salivary RT-PCR testing for SARS-CoV-2 in Melbourne, Australia. Following the second wave of COVID-19 in Melbourne, the hotel quarantine worker testing programme was implemented in December 2020 to coincide with the return of international travel into Melbourne.[6] Specifically, the purpose of the testing programme was to prevent incursions of SARS-CoV-2 from returning travellers, via hotel quarantine staff, into the Australian community. In order for widespread surveillance testing to be successful, both the sampling and laboratory testing aspects needed to be scalable, tolerable and accessible. To address this requirement, we developed and implemented a self-collected salivary swab RT-PCR testing method for use across a range of testing platforms and in multiple laboratories.

In our study, the clinical sensitivity of saliva swab testing compared to nasal/oropharyngeal swabs on individuals within seven days of symptom onset was 84.5% (95% CI 68.6 to 93.8), in keeping with other published work.[16,17] Although the sensitivity of saliva testing using swab-based collection is less than that of nasal/oropharyngeal swabs, likely due to factors such as sample dilution and differential viral tropism, previous studies have suggested that increasing the frequency of testing using less sensitive testing approaches may offset the reduction in sensitivity compared to nasal/oropharyngeal RT-PCR testing.[18,19] As such, high-frequency testing was useful in low disease prevalence settings.[19,20] Salivary swab RT-PCR is ideally suited to frequent sampling as it is non-invasive and is amenable to self-collection. Further, because most laboratory workflows using commercial testing platforms are adapted for handling swabs, swab-based saliva testing is suitable for scaling up across diagnostic laboratories, compared to handling neat saliva specimens. Moreover, we found that using pools of four saliva swab samples demonstrated minimal loss of sensitivity and was an effective strategy to increase testing capacity. Other recent studies have used larger pool sizes for salivary RT-PCR surveillance; for example, Mendoza et al. utilised pools of up to 24 neat saliva samples for surveillance testing in schools in New York, allowing testing of over 250,000 saliva specimens over a 20-week period.[11] Similarly, Joachim et al. used pooled salivary swab samples from school children in Germany, employing one or two pooled testing groups per class.[21] However, one of the key aims of testing hotel quarantine staff is early identification of possible incursions from returning travellers into the community; as such, in our setting, we chose to use pool sizes of four to allow a balance between scale and sensitivity.

Since the commencement of the hotel quarantine saliva RT-PCR surveillance programme, approximately 500,000 saliva swabs have been tested in Victoria, with salivary RT-PCR testing validated and deployed at multiple laboratories. Further, salivary swab surveillance testing has subsequently been utilised in other settings in Australia. For example, salivary swab testing was used in the management of the Australian Open international tennis tournament in January 2021 and was extended to surveillance testing of healthcare workers who provide direct care for patients with COVID-19.[22] In the healthcare setting, early detection of spread from patients to healthcare workers may prevent onward transmission in both the hospital and community.[23,24]

Our study has several limitations. We were unable to collect information on potential false positive and false negative results from all laboratories. However, as part of risk management for the salivary RT-PCR programme, we developed an algorithm to facilitate rapid confirmatory RT-PCR testing by repeat nasal/oropharyngeal swab testing. This algorithm helped to ensure that all positive results obtained using salivary swab testing were confirmed using nasal/oropharyngeal swab RT-PCR testing, thus helping mitigate the risk of false positives, and allowing rapid confirmation of true positive results. In addition, we did not conduct a specific cost-benefit analysis of the salivary swab RT-PCR testing, although work is ongoing in our setting to assess the overall cost-effectiveness of the programme.

For most of 2020 and 2021, Australia relied heavily on strict public health measures (including localised lockdowns), and closure of the international border to maintain comparatively low morbidity and mortality rates due to COVID-19.[2] Until the emergence and spread of the Delta, and subsequently Omicron variant in Victoria and New South Wales (the country's most populous states), Australia had several periods of successful elimination in 2020 and 2021.[4,5] However, to meet the testing demand required for re-opening, scalable and accessible testing approaches became necessary to complement conventional nasal/oropharyngeal RT-PCR, including accessible rapid antigen testing and pooled salivary RT-PCR testing.

Conclusion

In this study, we describe the use of mass salivary swab RT-PCR testing in hotel quarantine staff. Along with stringent infection control practices, our data embedded salivary swab testing as an important component of border security measures to help reduce incursions of COVID-19 into Australia. We demonstrate that saliva swab samples can be used at scale for SARS-CoV-2

surveillance testing; these findings will help inform the ongoing use of salivary RT-PCR surveillance, in conjunction with other testing modalities such as antigen testing.

Contributors

AJ, MB, JD, RM, AS, MC, TT and DAW contributed to preparation and writing of the manuscript. AJ, MB, JD, AD, CM, PK, JB and JR contributed to data collection. CM, TT, JD, MG and DC were involved in sample testing. Data interpretation, verification and depiction was performed by JD, MB, CM, MC and DAW. Decision to submit the manuscript was made by MC and DAW.

Data sharing statement

All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication. De-identified data is available from the time of publication and available for five years following article publication. Requests should be directed to the corresponding author.

Declaration of interests

All authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lanwpc.2022.100533.

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