SCIENTIFIC APPENDIX

Saliva-based collection as an alternative to nasopharyngeal swab for SARS-CoV-2 testing: Preliminary analysis

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Abstract

Rationale
In the expectation of outbreaks as Australian governments relax lockdown restrictions, the public health response is focusing on identifying cases through widespread testing, and the quarantining of close contacts. Testing procedures that maximise the identification of cases that can be sustained over many months become critical. Saliva-based collection (SBC) is a safer, less invasive alternative to the nasopharyngeal swab (NPS) procedure used to collect a sample for SARS-CoV-2 testing.

Aims
Our aim was to develop a decision aid by quantifying the interplay between the two major variables that determine "effective sensitivity", defined as the product of (1) the proportion of cases detectable given the test sensitivity by (2) the proportion of the at-risk population willing to be tested.

Methods
We conducted a rapid systematic review of research examining the accuracy of saliva-based collection (SBC) for SARS-CoV-2 testing, compared with NPS test results. We estimated the sensitivity of SBC-based testing and modelled the effective sensitivity of SBC versus NPS in terms of the number of "missed cases averted". We produced estimates for a range of acceptability levels for SBC versus NPS, and considered the implications of a range of lower sensitivity values.

Results
Nine eligible studies, from seven countries, yielded 201 patients who were NPS positive of whom 173 (86%) tested positive with a SBC procedure. In a subgroup of three studies conducted in "community/outpatient" populations that employed a straightforwardly saliva-based collection procedure, 57 of the 67 NPS-positive individuals were SBC-positive (85%). Modelling showed that in a notional population of 1,000,000 people, assuming 0.5% prevalence (i.e., 5000 cases) of SARS-CoV-2, where the acceptability of NPS and SBC tests are 60% and 90%, respectively, 1125 (23%) fewer cases would be missed.

Discussion
Even a 5% superiority in SBC acceptability over NPS was sufficient to yield improvement in the number of missed cases averted. SBC's superior effective sensitivity was evident across a range of lesser sensitivity values. Two further variables are worthy of consideration, namely: the effects of a false negative result on a case's post-test behaviour in relation to COVID-19 transmission risk; and the reduction in risk of infecting healthcare workers attributable to substituting SBC for NPS.

The nasopharyngeal swab (NPS) procedure used for SARS-CoV-2 testing involves swabbing the back of the throat and the tonsil area on both sides of the throat, and then inserting the swab horizontally 2-3cm into the nose, rotating it 5 times, and leaving it in position for 5-10 seconds [1]. The procedure typically makes people's eyes water, and can induce sneezing and coughing, placing healthcare workers at risk of infection, and requiring the use of personal protective equipment.

To our knowledge, there is no scientific research concerning people's willingness to undergo the procedure, but media reports suggest that many describe it as highly uncomfortable [2]. There may therefore be resistance to repeat testing which would hamper pandemic control.

Saliva-based collection (SBC) is an alternative that may facilitate testing more of the at-risk population, and greater compliance in high-risk groups and settings where repeat testing is necessary. Here we consider the potential merits of SBC as an alternative to NPS.

The context of this analysis is late June 2020, three months after the peak in the 'first wave' of the COVID-19 epidemic in Australia, on 28 March, when 460 cases were recorded (www.covid19data.com.au), a large proportion of them from inbound flights and cruise ships. Travel restrictions, physical distancing regulations, and public health unit activity reduced daily counts of confirmed cases to <50 by mid-April and they have fluctuated between 2 and 31 per day since then. In mid-June, evidence emerged of community transmission in Victoria, raising concerns about a possible 'second wave'.

In the expectation of outbreaks as governments relax physical distancing and travel restrictions, the focus of the public health response is on containment, involving the identification of cases through widespread testing and the quarantining of close contacts. Testing procedures that maximise the identification of cases and that can be sustained over many months, become critical to managing the pandemic.

We aimed to develop a decision aid by quantifying relations between the two major variables that determine what we term *effective sensitivity*, on the basis that a test with 100% sensitivity has a 0% chance of detecting the disease if people refuse to be tested. We operationalise *effective sensitivity* as the product of (a) the proportion of cases detectable given the test sensitivity and (b) the proportion of the at-risk population willing to undergo (i.e., who 'accept') the sample collection procedure.

We hypothesise two further variables relevant to testing strategy, namely: possible deleterious effects of a false negative result on the subject's behaviour relating to SARS-CoV-2 transmission risk; and the reduction in risk of infecting healthcare workers using SBC instead of NPS. Estimating their effects is beyond the scope of the present investigation; however, the first concern may be mitigated by giving careful instruction to testing subjects concerning the meaning of a negative result. In situations where the pre-test probability of having the virus is high, people may be encouraged also to have an NPS. With regard to the second concern, there may be value in reviewing evidence concerning the risk to healthcare workers associated with each procedure.

We present preliminary results of (1) a rapid review and narrative synthesis of studies estimating the sensitivity of SBC for SARS-CoV-2 testing; and (2) modelling of the effective sensitivity of a SBC procedure under a range of assumptions about the acceptability of SBC- and NPS-based tests.

Part I. Evidence on the accuracy of SBC vs NPS

Design
We conducted a rapid review of research examining the accuracy of saliva-based collection (SBC) for the testing of infection with SARS-CoV-2.

Search
We searched the *Cochrane COVID-19* register and *Google Scholar* for relevant articles on 8 June 2020. We applied the diagnostic/prognostic filter and searched for the term "saliva" in Cochrane COVID-19, and "COVID-19 AND saliva" in Google Scholar.

Eligible studies
Any study reporting primary data on using saliva as a diagnostic test for COVID-19 was eligible for inclusion. We excluded opinion pieces, guidance pieces, and consensus statements.

Types of participant
We included studies using both healthy individuals and confirmed or suspected COVID-19 cases, without restriction by age.

Types of outcome
We extracted estimates of sensitivity, specificity and concordance rates of saliva diagnostics.

Analysis
For computational ease, we made the conservative assumption that the standard NPS-based procedure has 100% sensitivity and specificity, despite evidence that SBC sometimes detects the virus in COVID-19 cases which the NPS-based procedure fails to detect (Wang et al., 2020; Wolfel et al., 2020; Xie et al., 2020, Zhao et al, 2020; Zou et al., 2020).

Results
Table 1 summarises the results of the nine studies we identified. Of 201 people who were NPS positive, 173 (86%) tested positive with a so-called SBC procedure. However, the populations in studies 1, 3, 5, and 9 (Azzi et al., 2020; Chen et al., 2020; Jamal et al., 2020; Wyllie et al., 2020) were inpatients who would typically have more severe COVID-19 symptoms (and would therefore be more easily detected as cases through testing) than the "community/outpatient" populations, we want to generalise to here. Secondly, in several studies, the collection procedure was either not clearly specified or appeared to involve the production of sputum (e.g., "cough from throat"), rather than saliva *per se*, which complicates the internal and external validity of sensitivity estimates.

In a subgroup analysis, we limit our focus to three studies (4, 6, and 8) of "community/outpatient" populations that employed a straightforwardly saliva-based collection procedure. In the 67 NPS-positive individuals in these studies, 57 were SBC-positive, giving a sensitivity of 86%. Notably, the inpatient studies alone (1, 3, 5, and 9) yield a sensitivity of 86% (105/122), indicating low heterogeneity across the studies.

Study number 8, the largest of the three most relevant studies, was conducted in Australia among ambulatory outpatients in a busy screening clinic, using a practical collection procedure in which an instruction is given to "Pool saliva in mouth for 1-2 minutes and then spit 1-2 mL" into a provided container (Williams et al 2020).

Conclusion
We considered 85% a suitably conservative sensitivity estimate for the SBC procedure (given the NPS 100% accuracy assumption), with high external validity, to underpin modelling of effective sensitivity in the Australian community/outpatient setting.

Table 1. Studies of test sensitivity of a saliva-based collection procedure assuming 100% accuracy of nasopharyngeal swab (NPS) procedure

First author, city/region, country where study was conducted	Saliva positive N	NPS positive N	Sensitivity	Population / Setting	Collection method	Comment
1. Azzi, Varese, Italy	25	25	100%	Inpatients; infected patients with severe or very severe condition	'Drooling technique' "saliva collected intraorally by a physician with the use of a pipette"	"This technique allows to collect only oral fluids, thus excluding mucous secretions from oropharynx or lower respiratory tract (i.e., sputum)….
2. Becker, Nevada, USA	unclear	unclear		Outpatients; community members with symptoms	Not specified	E-mailed lead author 19JUN20. To e-mail author again for clarification (26NUN20)
3. Chen, Hong Kong, China	49	55	89%	Inpatients; positive for COVID-19	Cough and spit	
4. Iwasaki, Tokyo, Japan	8	9	89%	Outpatients ?; 66 patients suspected of having COVID-19 and 10 patients with a COVID-19 diagnosis	"Self-collected saliva spit…"	
5. Jamal, Toronto, Canada	31	42	73%	Inpatients; positive for COVID-19	Spit	
6. Pasomsub, Bangkok, Thailand	16	19	84%	Outpatients; persons seeking care at an acute respiratory infection clinic in a university hospital	"provide saliva without couging"	
7. To, Hong Kong, China	11	12	92%	Outpatients; patients with suspected infection based on clinical and epidemiological criteria	"Cough from throat into container"	
8. Williams, Melbourne, Australia	33	39	85%	Outpatients; ambulatory patients in a busy screening clinic	"Pool saliva in mouth for 1-2 min; spit 1-2 ml"	
9. Wyllie, New Haven, USA	unclear			Inpatients, and Asymptomatic health care workers	"Repeatedly spit"	To e-mail corresponding author for clarification (26NUN20)
Pooled estimate (unweighted)	173	201	86%			
Subgroup analysis (Community/outpatients and spit procedure; studies 4, 6, 8)	57	67	85%			

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Part II. Effective sensitivity and number of "missed cases averted" for SBC- versus NPS-based tests

Step 1
We assume a sensitivity of 85% for SBC and 90% for NPS, though we note that some studies suggest considerably lower sensitivity for NPS-based tests, and that SBC-based tests identify some true cases that NPS-based tests miss (e.g., [3]). Accordingly, our assumption of inferior sensitivity may be conservative.

Step 2
To our knowledge, there are no scientific studies examining the acceptability of the NPS or SBC procedures, in any setting. We therefore tested a range of pairs of values, shown in Table 2.

In all pairs, we assumed SBC has higher acceptability, under the assumption that if SBC has inferior sensitivity it will by definition have inferior "effective sensitivity".

Table 2. Plausible pairs of acceptability values for SBC versus NPS procedure

NPS	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
SBC	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95
NPS	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	
SBC	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00	
NPS	0.70	0.70	0.70	0.70	0.70	0.70			
SBC	0.75	0.80	0.85	0.90	0.95	1.00			
NPS	0.80	0.80	0.80	0.80					
SBC	0.85	0.90	0.95	1.00					

Step 3
We assumed an underlying population of 1,000,000 people who should be tested in a given period (4% of the Australian population), and a true SARS-CoV-2 prevalence (p_v) of 0.5% in that period (i.e., 5,000 cases). In Table 3, we show the number of cases being correctly identified (true positives) and the number missed (false negatives) using the NPS, under acceptability estimates (i.e., the proportion of people that should be tested who submit for testing) ranging from 50-80%.

Table 3. Expected number of cases correctly identified and missed using NPS procedure

Acceptability: Proportion who agree to be tested (p_A)	Expected number of cases tested ($N \times p_v \times p_A$)	Expected number correctly identified	Expected number of cases missed	"Effective sensitivity" of NPS
50%	2500	2250	2750	0.45
60%	3000	2700	2300	0.54
70%	3500	3150	1850	0.63
80%	4000	3600	1400	0.72

Step 4
We calculate the equivalent values for the SBC procedure and subtract the equivalent NPS-based value to calculate the number of "missed cases averted" for each of the pairs shown in

Table 2. Table 4 presents these and we highlight cells reflecting plausible comparisons to illustrate the interpretation. An Excel spreadsheet containing values and formulae is available upon request.

In the first example (in green), where the acceptability values for NPS and SBC are 60% and 90%, respectively, 1125 (23%) fewer cases are missed.

In the second example (in blue), where the acceptability values for NPS and SBC are 70% and 95%, respectively, 888 (18%) fewer cases are missed.

Table 4. Number of missed cases averted for a range of acceptability pairs

Number of false negatives averted (Δ = SBC - NPS)							
NPS Acceptability (50%)		NPS Acceptability (60%)		NPS Acceptability (70%)		NPS Acceptability (80%)	
Δ Acceptability of SBC	Δ Missed	Δ Acceptability of SBC	Δ Missed	Δ Acceptability of SBC	Δ Missed	Δ Acceptability of SBC	Δ Missed
0.05	-87.5	0.05	-62.5	0.05	-37.5	0.05	-12.5
0.1	-300	0.1	-275	0.1	-250	0.1	-225
0.15	-512.5	0.15	-487.5	0.15	-462.5	0.15	-437.5
0.2	-725	0.2	-700	0.2	-675		
0.25	-937.5	0.25	-912.5	0.25	-887.5		
0.3	-1150	0.3	-1125				
0.35	-1362.5	0.35	-1337.5				
0.4	-1575						
0.45	-1787.5						

Step 5
In a final step, we perform two sets of robustness analyses:

(a) We modify the basic assumption concerning the sensitivity of SBC downward.

In the examples presented above, the values in the cells highlighted green and blue change to -900 and -650, respectively, when sensitivity is 0.80; and to -400 and -175, when sensitivity is 0.70.

(b) We determine at what sensitivity, under various pairs of acceptability values, the number of missed cases averted becomes 0 (i.e., equal effective sensitivity).

For NPS/SBC pairs 0.50/0.75 and 0.60/0.90, equal effective sensitivity occurs with SBC sensitivity of 0.60.

For the NPS/SBC pair 0.70/0.90, equal effective sensitivity occurs with SBC sensitivity of 0.70.

Conclusion
Our analysis showed that even a 5% superiority in SBC acceptability over NPS is sufficient to yield a reduction in the number of SARS-CoV-2 cases in the population that are missed. The estimated superiority of SBC was shown across a range of lesser sensitivity values.

References

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Contributors

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