

# The Effect of Sample Site, Illness Duration, and the Presence of Pneumonia on the Detection of SARS-CoV-2 by Real-time Reverse Transcription PCR

Stephanie Sutjipto,<sup>1,3</sup> Pei Hua Lee,<sup>1,3</sup> Jun Yang Tay,<sup>1,3</sup> Shehara M. Mendis,<sup>2</sup> Mohammad Yazid Abdad,<sup>4</sup> Kalisvar Marimuthu,<sup>1,3,7</sup> Oon Tek Ng,<sup>1,3,6,7</sup> Lin Cui,<sup>5</sup> Monica Chan,<sup>1,3,6,7</sup> Margaret Soon,<sup>3</sup> Raymond T. P. Lin,<sup>5,7</sup> Yee-Sin Leo,<sup>1,3,6,7,8</sup> Partha P. De,<sup>2</sup> Timothy Barkham,<sup>2,7</sup> and Shawn Vasoo,<sup>1,3,6,7</sup>; on behalf of the National Centre for Infectious Diseases COVID-19 Outbreak Research Team

<sup>1</sup>Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore, <sup>2</sup>Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore, <sup>3</sup>National Centre for Infectious Diseases, Singapore, <sup>4</sup>Infectious Disease Research Laboratory, National Centre for Infectious Diseases, Singapore, <sup>5</sup>National Public Health Laboratory, National Centre for Infectious Diseases, Singapore, <sup>6</sup>Lee Kong Chian School of Medicine, Singapore, <sup>7</sup>Yong Loo Lin School of Medicine, National University of Singapore, Singapore, <sup>8</sup>Saw Swee Hock School of Public Health, National Centre for Infectious Diseases, Singapore

**Background.** The performance of real-time reverse transcription polymerase chain reaction (rRT-PCR) for SARS-CoV-2 varies with sampling site(s), illness stage, and infection site.

**Methods.** Unilateral nasopharyngeal, nasal midturbinate, throat swabs, and saliva were simultaneously sampled for SARS-CoV-2 rRT-PCR from suspected or confirmed cases of COVID-19. True positives were defined as patients with at least 1 SARS-CoV-2 detected by rRT-PCR from any site on the evaluation day or at any time point thereafter, until discharge. Diagnostic performance was assessed and extrapolated for site combinations.

**Results.** We evaluated 105 patients; 73 had active SARS-CoV-2 infection. Overall, nasopharyngeal specimens had the highest clinical sensitivity at 85%, followed by throat, 80%, midturbinate, 62%, and saliva, 38%–52%. Clinical sensitivity for nasopharyngeal, throat, midturbinate, and saliva was 95%, 88%, 72%, and 44%–56%, respectively, if taken  $\leq 7$  days from onset of illness, and 70%, 67%, 47%, 28%–44% if  $> 7$  days of illness. Comparing patients with upper respiratory tract infection (URTI) vs pneumonia, clinical sensitivity for nasopharyngeal, throat, midturbinate, and saliva was 92% vs 70%, 88% vs 61%, 70% vs 44%, 43%–54% vs 26%–45%, respectively. A combination of nasopharyngeal plus throat or midturbinate plus throat specimen afforded overall clinical sensitivities of 89%–92%; this rose to 96% for persons with URTI and 98% for persons  $\leq 7$  days from illness onset.

**Conclusions.** Nasopharyngeal specimens, followed by throat specimens, offer the highest clinical sensitivity for COVID-19 diagnosis in early illness. Clinical sensitivity improves and is similar when either midturbinate or nasopharyngeal specimens are combined with throat specimens. Upper respiratory specimens perform poorly if taken after the first week of illness or if there is pneumonia.

**Keywords.** illness duration; pneumonia; rRT-PCR; sample site; SARS-CoV-2.

Since its emergence in December 2019, Coronavirus disease 2019 (COVID-19) has infected over 14 million people across 213 countries and territories, as of mid-July 2020. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) offers definitive diagnosis for COVID-19, but the diagnostic yield of rRT-PCR for SARS-CoV-2 may vary by sampling site(s), stage of illness, and whether disease involves predominantly the upper or lower respiratory tract. Nasopharyngeal specimens, including flocked swabs, washes, or aspirates, are generally

considered the optimal specimen type for the diagnosis of respiratory virus infections [1], but compared with oropharyngeal or nasal swabs, these are more technically complex to perform and often unpleasant for patients [2].

While SARS-CoV-2 shedding from various sites may be prolonged, it is maximal in the upper respiratory tract in the first week of illness, as evidenced by studies on viral kinetics [3–6]. Besides more conventional respiratory specimens (nasopharyngeal, oropharyngeal, or throat swabs), recent reports also indicate that SARS-CoV-2 can also be detected in saliva, which has been suggested as an alternate specimen for diagnostics [7–12]. The limitations of available data include a lack of simultaneous sampling, smaller study numbers, and variability in collection techniques.

The National Centre for Infectious Diseases (NCID) is a 330-bed facility (able to ramp up to 586 beds) and has admitted the majority of COVID-19 patients in Singapore. From the start of the COVID-19 outbreak, bilateral nasopharyngeal swabs were the primary specimen type used for SARS-CoV-2 diagnostics at

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Correspondence: Shawn Vasoo, MBBS, MRCP, National Centre for Infectious Diseases, 16 Jalan Tan Tock Seng Singapore, 308442 Singapore, Singapore (shawn\_vasoo@ncid.sg).

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**Table 1. Baseline Characteristics of Patients**

	Total (n = 105)	COVID-19			Non-COVID-19 (n = 21)	PValue	PValue
		Total COVID-19 (n = 84)	Active COVID-19 (n = 73)	Recovered COVID-19 (n = 11)		Active vs Recovered COVID-19	Active vs Non-COVID-19
<b>Gender, No. (%)</b>							
Male	57 (54)	45 (53.6)	40 (54.8)	5 (45.5)	12 (57.1)	.56	.85
Female	48 (46)	39 (46.4)	33 (45.2)	6 (54.5)	9 (42.9)		
<b>Age</b>							
Median (IQR), y	44 (20–79)	44 (20–79)	44 (31–56)	44 (26–79)	45 (22–73)	.81	.94
<b>Clinical syndrome, No. (%)</b>							
Pneumonia	28 (26.7)	26 (31.0)	23 (31.5)	3 (27.3)	2 (9.5)	1.00 <sup>a</sup>	.045
URTI	77 (73.3)	58 (69.0)	50 (68.5)	8 (72.7)	19 (90.5)		
<b>Days of illness</b>							
≤7 d, No. (%)	57 (54.3)	46 (54.8)	43 (58.9)	3 (27.3)	11 (52.4)	.21 <sup>a</sup>	.68
>7, No. (%)	48 (45.7)	38 (45.2)	30 (41.1)	8 (72.7)	10 (47.6)		
Median (IQR), d	7 (3–10)	7 (3–10)	6 (3–9)	12 (3–20)	7 (3–10)	.054	.71

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; URTI, upper respiratory tract infection.

<sup>a</sup>Fisher test.

our facility. Given the limited comparative data on simultaneously obtained clinical specimens, we sought to assess the clinical sensitivity of various specimen types for the diagnosis of SARS-CoV-2 in relation to duration of onset of illness and the presence of pneumonia.

## METHODS

### Audit Population

As part of a clinical audit, specimens from multiple sites (unilateral nasopharyngeal, midturbinate, throat swabs, and saliva) were simultaneously taken on a single audit day for rRT-PCR for SARS-CoV-2 prospectively from a convenience sample of suspected or confirmed cases of COVID-19 admitted to the National Centre for Infectious Diseases, Singapore, from February 27 to March 19, 2020. Suspected COVID-19 cases were defined as patients with acute respiratory symptoms and epidemiological risk factors admitted for evaluation of COVID-19. Confirmed COVID-19 infection was defined as suspected cases who tested positive for SARS-CoV-2 by PCR at any point during their admission. Recovered COVID-19 infection was defined as confirmed cases who tested negative on the day of audit and on all subsequent repeat SARS-CoV-2 PCRs until discharge.

Besides the samples taken on the day of the clinical audit, a standard bilateral nasopharyngeal swab was done upon admission, as per protocol, for all patients admitted for suspected COVID-19, and this was repeated 24 hours later if initial testing was negative [13]. If SARS-CoV-2 was detected (confirmed case of COVID-19), rRT-PCR was performed daily upon clinical recovery to ascertain virologic clearance (clinical recovery was defined as being afebrile for at least 24 hours, with improvement of clinical symptoms, and being deemed medically fit for discharge by managing clinicians).

Two negative PCR results 24 hours apart were required as a prerequisite for discharge.

### Clinical Specimens

The nasopharyngeal swab was collected using a flexible minitip flocked swab (220252, Copan Diagnostics, Brescia, Italy), inserted half the distance from the nares to the base of the ear, or to a depth of ~5 cm, and only unilateral swabs were sampled. Both midturbinate and throat swabs were done using a regular flocked swab (502C201, Copan Diagnostics, Brescia, Italy). For midturbinate sampling, the swab was inserted ~1–2.5 cm, rubbed along the septum of the contralateral nostril for 3–5 seconds around the area of the middle turbinate, and withdrawn [14]. For throat (oropharyngeal) swabs, the posterior oropharynx was swabbed under direct vision. Each specimen was collected in an individual universal transport medium (UTM-RT, Copan Diagnostics, Brescia, Italy). To collect saliva samples, patients were asked to rinse their mouth with plain water at least 30 minutes postmeal and 10 minutes precollection to remove residual food debris. Two milliliters of fresh salivary sample was then spit out (drooling method) by the patient into a sterile container containing an equal amount of nucleic acid stabilization formula (SF; Institute of Bioengineering and Nanotechnology, Singapore), and this was mixed after capping by gently inverting the container 5 times. All specimens were obtained by trained nurses and processed within 24 hours.

### Diagnostic Testing

The A\*STAR Fortitude Kit (Accelerate Technologies, Singapore) was used for all samples after extraction (NucliSens EasyMAG, bioMérieux, Marcy L'Etoile, France), as previously described [15]. Additionally, saliva samples were tested with a second rRT-PCR assay targeting the *N* and *ORF1ab* genes, after

extraction (EZ1 virus mini kit, version 2.0, Qiagen, Hilden, Germany) [15].

### Statistical Analysis

Reference standard true positives were defined as patients with at least 1 positive SARS-CoV-2 result detected from any site on the day of the audit or at any time point thereafter (including extrapulmonary sites), until discharge. These include positive rRT-PCR by the Fortitude kit for nasopharyngeal, nasal, or throat samples and/or positive SARS-CoV-2 from either the *N* or *ORF1ab* target genes for saliva specimens. Performers of the rRT-PCR assays were blinded to reference standard results. Index test results on the day of audit and clinical information were assessed by the audit team to determine reference standard positivity.

Diagnostic performance was assessed for each site and compared with true-positive values to determine test sensitivity and specificity. Subanalyses were performed for patients' day of illness on audit day (early, defined as patients with  $\leq 7$  days, and late, defined as  $> 7$  days from onset of illness on audit day) and the presence of pneumonia. Pneumonia was defined as reported pulmonary opacities suggesting infection on chest x-ray and/or computed tomography taken during admission.

Diagnostic accuracy for combinations of multiple sites was extrapolated from the primary performance at each individual site. Data analysis was performed using SPSS, version 26.0 (SPSS), and Vassarstats (<http://vassarstats.net/>). The McNemar test was used for test pairs, and *P* values  $< .05$  were considered statistically significant.

### Patient Consent Statement

This audit was performed as part of clinical operations at the National Centre for Infectious Diseases, and results were used as part of routine clinical care. The reporting of this audit with waiver of written informed consent was approved by the institutional review board (National Healthcare Group Domain Specific Review Board reference number 2020/00338). This audit followed the Standards for Reporting Diagnostic Accuracy study (STARD) guidelines (<https://www.equator-network.org/reporting-guidelines/stard/>) (Supplementary Data).

## RESULTS

### Baseline Characteristics

A total of 581 patients were potentially eligible (171 with COVID-19). We included 105 patients in this evaluation, 32 of whom tested negative for SARS-CoV-2 (11 patients recovered from COVID-19 and 21 had alternate diagnoses) and 73 of whom had active SARS-CoV-2 infection (Table 1; STARD flow diagram in Supplementary Figure 1). Twenty-eight (27%) patients had pneumonia as evidenced by radiological changes, while 77 (73%) had upper respiratory tract infection (URTI) with normal radiological results. Overall, the median duration from onset of illness to the day of audit (interquartile range) was

7 (3–10) days. One saliva sample was unavailable for analysis with the Fortitude kit, and 7 were unavailable after a second assay targeting the *N* and *ORF1ab* genes, leaving 104 and 98 samples, respectively, for analysis for this particular sample type.

### Overall Results

Data analysis of the clinical sensitivity and specificity of individual sample sites and combination testing is summarized in Table 2. Overall, nasopharyngeal specimens were found to have the highest clinical sensitivity, at 85%, followed by throat, 80%, mid-turbinate, 62%, and saliva, 38%–52%. There was no statistically significant difference between the nasopharyngeal and throat site for rRT-PCR overall, but the nasopharyngeal site was found to be more sensitive compared with midturbinate or saliva (either assay) ( $P < .01$ ) (Supplementary Table 1).

The clinical sensitivity of the combined sites was extrapolated from the results of individual sites. While the midturbinate site alone was less sensitive compared with nasopharyngeal, when either was combined with throat swabs, clinical sensitivity was similar, at 89% vs 92%, respectively ( $P = .5$ ); either combination offered the highest yield compared with others.

### Subgroup Analyses

#### Upper Respiratory Tract Infection vs Pneumonia

We performed a subgroup analysis of data based on the presence or absence of radiologic evidence of pneumonia and day of illness (Tables 2 and 3). For patients with upper respiratory tract infection (URTI) who by definition had normal radiological findings ( $n = 77$ ), a similar pattern was found, with nasopharyngeal swabs having the highest clinical sensitivity (92%), followed by throat (88%) and midturbinate (70%). Saliva had poor clinical sensitivity (43–54%). Combined nasopharyngeal and throat swabs or midturbinate and throat swabs showed the best performance, at 96% and 94%, respectively ( $P = 1.00$ ). Nasopharyngeal specimens were found to be more sensitive than both midturbinate or saliva in patients with URTIs ( $P = .003$  and  $P < .001$ , respectively) (Supplementary Table 1).

For patients with radiographic evidence of pneumonia ( $n = 28$ ), performance of upper respiratory tract samples was overall much less sensitive, with nasopharyngeal swabs having the highest clinical sensitivity, but only at 70%, and the other upper respiratory samples having 26%–61%. Testing with a nasopharyngeal or midturbinate swab combined with a throat swab improved clinical sensitivity to 82% and 78%, respectively. Nasopharyngeal specimens were found to be more sensitive than saliva, but not midturbinate specimens, in patients with pneumonia ( $P = .002$  and  $P = .11$ , respectively) (Supplementary Table 1).

#### Day of Illness

In patients with onset of symptoms of  $\leq 7$  days ( $n = 57$ ), unilateral nasopharyngeal swab showed the highest clinical sensitivity for COVID-19 diagnosis (95%), followed by throat swab (88%) and midturbinate swab (72%; nasopharyngeal

**Table 2. Overall Positive Clinical Sensitivities and Clinical Specificities of Sample Sites for SARS-CoV-2**

Sample Site <sup>a</sup>	Clinical Sensitivity, % (95% CI)	Clinical Specificity, % (95% CI)
Single site		
Unilateral nasopharyngeal	84.9 (74.2–91.9)	100 (86.7–100)
Unilateral nasal midturbinate	61.6 (49.4–72.6)	100 (86.7–100)
Throat	79.5 (68.1–87.7)	100 (86.7–100)
Saliva (n = 104)	37.5 (26.6–49.7)	100 (86.7–100)
Saliva (N and <i>ORF1ab</i> assay; n = 98) <sup>b</sup>	51.5 (39.0–63.9)	100 (86.7–100)
Combination of sample sites <sup>c</sup>		
Unilateral nasopharyngeal + throat	91.7 (82.4–96.6)	100 (86.7–100)
Unilateral nasal midturbinate + throat	89.0 (79.0–94.8)	100 (86.7–100)
Unilateral nasal midturbinate + saliva	72.6 (60.7–82.1)	100 (86.7–100)
Throat + saliva	83.6 (72.7–90.9)	100 (86.7–100)

<sup>a</sup>All samples (unless otherwise stated) were tested with the A\*STAR Fortitude Kit (Accelerate Technologies, Singapore).

<sup>b</sup>A second polymerase chain reaction assay targeting the *N* and *ORF1ab* genes was used for saliva samples.

<sup>c</sup>For data for sample site combinations, data from the A\*STAR Fortitude Kit were used for saliva.

vs throat  $P = .25$ ; nasopharyngeal vs midturbinate  $P = .006$ ) (Table 4; Supplementary Table 1). Combination testing from patients in their first week of COVID-19 showed the best performance, with a clinical sensitivity of 98% for either midturbinate or nasopharyngeal swabs combined with throat swabs.

As the illness progresses to day 8 and beyond, the clinical sensitivity of individual sample sites decreased significantly and ranged from 28% to 70%, with nasopharyngeal swabs still showing the best clinical sensitivity among the upper respiratory specimens. Notably, among the true positives in this audit, 3 were detected on postaudit of lower respiratory specimens (2 rRT-PCR positive by sputum, and another via endotracheal aspirate).

**Table 3. Subanalysis Based on Clinical Syndromes**

Test Site(s)	Clinical Sensitivity, % (95% CI)	Clinical Specificity, % (95% CI)
Upper Respiratory Tract Infection (n = 77)		
Single site <sup>a</sup>		
Unilateral nasopharyngeal	92.0 (79.9–97.4)	100 (84.5–100)
Unilateral nasal midturbinate	70.0 (55.2–81.7)	100 (84.5–100)
Throat swab	88.0 (74.9–95.0)	100 (84.5–100)
Saliva (n = 76)	42.9 (29.1–57.7)	100 (84.5–100)
Saliva (N and <i>ORF1ab</i> assay; n = 73) <sup>b</sup>	54.3 (39.2–68.8)	100 (84.5–100)
Combination of sites <sup>c</sup>		
Unilateral nasopharyngeal + throat	96.0 (85.1–99.3)	100 (84.5–100)
Unilateral nasal midturbinate + throat	94.0 (82.5–98.4)	100 (84.5–100)
Unilateral nasal midturbinate + saliva	82.0 (68.1–91.0)	100 (84.5–100)
Throat + saliva	94.0 (82.5–98.4)	100 (84.5–100)
Pneumonia (n = 28)		
Single site <sup>a</sup>		
Unilateral nasopharyngeal	69.5 (47.0–85.9)	100 (46.3–100)
Unilateral nasal midturbinate	43.5 (23.9–65.1)	100 (46.3–100)
Throat	60.9 (38.8–79.5)	100 (46.3–100)
Saliva (n = 28)	26.1 (11.1–48.7)	100 (46.3–100)
Saliva (N and <i>ORF1ab</i> assay; n = 25) <sup>b</sup>	45.0 (23.8–68.0)	100 (46.3–100)
Combination of sites <sup>c</sup>		
Unilateral nasopharyngeal + throat	82.6 (60.5–94.3)	100 (46.3–100)
Unilateral nasal midturbinate + throat	78.2 (55.8–91.7)	100 (46.3–100)
Unilateral nasal midturbinate + saliva	52.2 (31.1–72.6)	100 (46.3–100)
Throat + saliva <sup>a</sup>	60.9 (38.8–79.5)	100 (46.3–100)

Abbreviations: URTI, upper respiratory tract infection.

<sup>a</sup>All samples (unless otherwise stated) were tested with the A\*STAR Fortitude Kit (Accelerate Technologies, Singapore).

<sup>b</sup>A second polymerase chain reaction assay targeting the *N* and *ORF1ab* genes was used for saliva samples.

<sup>c</sup>For data for sample site combinations, data from the A\*STAR Fortitude Kit were used for saliva.

### Cycle Threshold

The performance of each sampled site and their associated rRT-PCR cycle threshold (Ct) values (Fortitude kit) with days of illness are summarized in Figure 1. Nasopharyngeal and throat swabs showed generally good performance results across the first week of illness up to day 10 after onset

of symptoms, but saliva samples did not yield consistent results even in the first week of illness in our study. These findings were corroborated by lower Ct values (a surrogate marker for higher viral loads) in the corresponding sampling sites, demonstrating higher sensitivity in earlier illness ( $\leq 7$  days) as opposed to later illness ( $>7$  days). The median Ct values

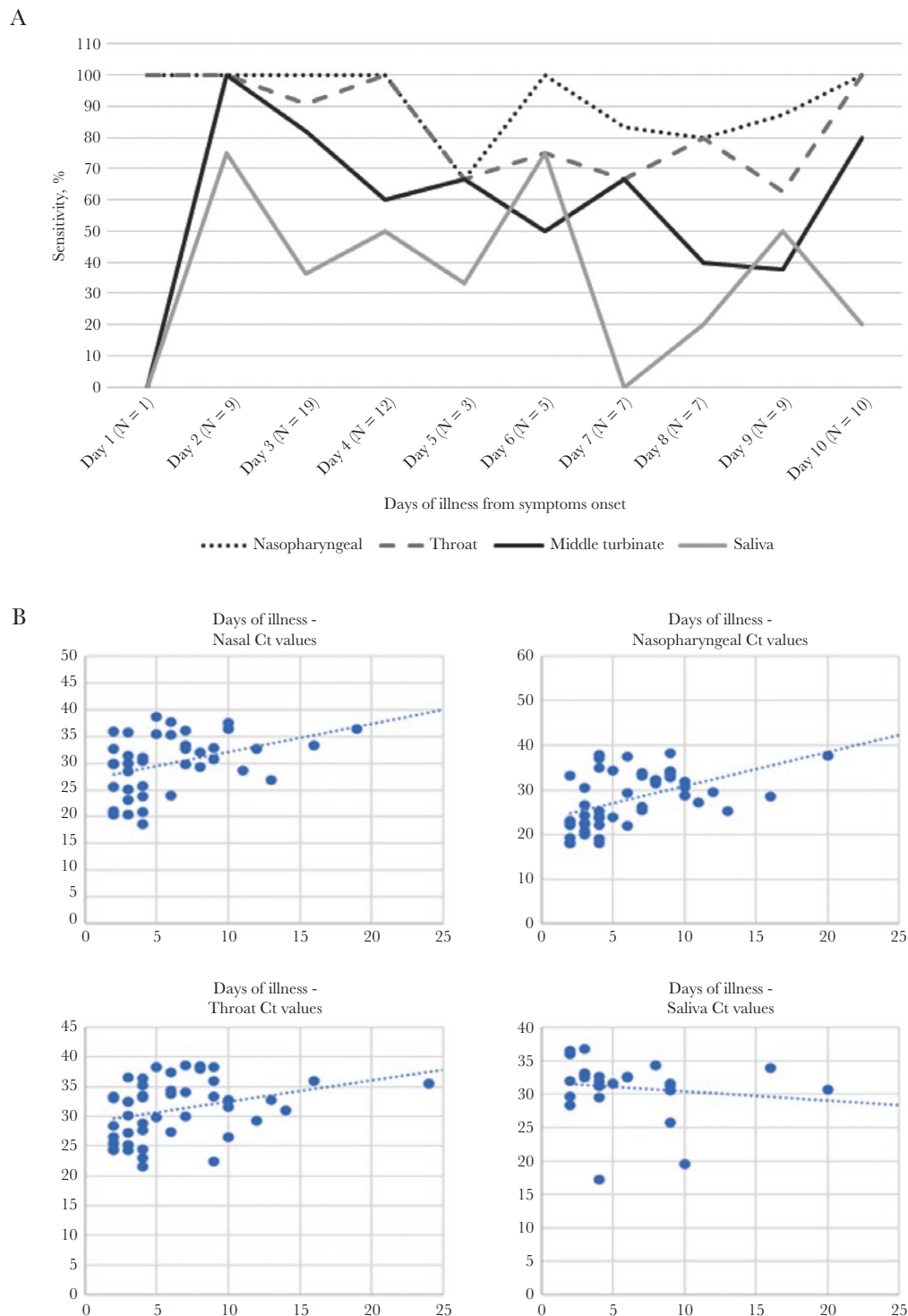


Figure 1. Performance of testing sites and Ct values over days of illness.



**Table 4. Subanalysis Based on Duration of Illness**

Test Site(s)		
Days of Illness		
≤7 d (n = 57)	Clinical Sensitivity, % (95% CI)	Clinical Specificity, % (95% CI)
Single site <sup>a</sup>		
Unilateral nasopharyngeal	95.3 (83.0–99.1)	100 (73.2–100)
Unilateral nasal midturbinate	72.1 (56.1–84.2)	100 (73.2–100)
Throat	88.4 (74.1–95.6)	100 (73.2–100)
Saliva (n = 57)	44.2 (29.4–60.0)	100 (73.2–100)
Saliva (N and <i>ORF1ab</i> assay; n = 53) <sup>5</sup>	56.4 (39.8–71.8)	100 (73.2–100)
Combination of sites <sup>c</sup>		
Unilateral nasopharyngeal + Throat	97.7 (86.2–99.9)	100 (73.2–100)
Unilateral nasal midturbinate + Throat	97.7 (86.2–99.9)	100 (73.2–100)
Unilateral nasal midturbinate swab + saliva <sup>a</sup>	79.1 (63.5–89.4)	100 (73.2–100)
Throat swab + saliva <sup>a</sup>	90.7 (76.9–97.0)	100 (73.2–100)
>7 d (n = 48)		
Single site <sup>a</sup>		
Unilateral nasopharyngeal	70.0 (50.4–84.6)	100 (78.1–100)
Unilateral nasal midturbinate	46.7 (28.8–65.4)	100 (78.1–100)
Throat	66.7 (47.1–82.1)	100 (78.1–100)
Saliva (n = 47)	27.6 (13.4–47.5)	100 (78.1–100)
Saliva (N and <i>ORF1ab</i> assay; n = 45) <sup>b</sup>	44.4 (26.0–64.4)	100 (78.1–100)
Combination of sites <sup>c</sup>		
Unilateral nasopharyngeal + throat	83.3 (64.5–93.7)	100 (78.1–100)
Unilateral nasal midturbinate + throat	76.7 (57.3–89.4)	100 (78.1–100)
Unilateral nasal midturbinate + saliva	63.3 (43.9–79.5)	100 (78.1–100)
Throat + saliva	73.3 (53.8–87.0)	100 (78.1–100)

<sup>a</sup>All samples (unless otherwise stated) were tested with the A\*STAR Fortitude Kit (Accelerate Technologies, Singapore).

<sup>b</sup>A second polymerase chain reaction assay targeting the *N* and *ORF1ab* genes was used for saliva samples.

<sup>c</sup>For data for sample site combinations, data from the A\*STAR Fortitude Kit were used for saliva.

for nasopharyngeal, midturbinate, throat, and saliva samples by the Fortitude assay were 24.05, 29.89, 30.10, and 32.49 (≤7 days) and 32.20, 32.69, 33.03, and 30.98 (>7 days), respectively (Supplementary Table 2).

#### Bilateral vs Unilateral Nasopharyngeal Swabs

Although the audit was not designed to address this primarily, we also examined the performance of bilateral nasopharyngeal swabs done on the pre-audit day (24 hours prior, with likely higher viral loads) as compared with the unilateral nasopharyngeal swabs done on the audit day. Given that viral shedding from the upper respiratory tract is highest in the first week of illness, we limited this subanalysis to patients with ≤7 days from onset of symptoms (n = 57, 54%). We found the clinical sensitivity of bilateral nasopharyngeal swabs to be 98% (95% CI, 87%–100%), vs unilateral nasopharyngeal swabs at 89% (95% CI, 76%–96%); however, this difference was not statistically significant ( $P = .13$ ).

## DISCUSSION

Our study highlights several important findings and provides clinicians with a practical guidance as to optimal sampling sites

for SARS-CoV-2 detection. First, consistent with reports on higher viral shedding in early COVID-19 [4, 9, 16–18], the clinical sensitivity of PCR diagnostics is highest across upper respiratory specimen types in early illness (≤7 days from symptom onset), compared with later illness. Given that pneumonia occurs in later illness, the sensitivity of PCR was also found to be correspondingly diminished when upper respiratory tract specimens were sampled. This phenomenon is not peculiar to SARS-CoV-2 [19], as viral loads in various anatomic sites are related to pathology at the site and degree of viral replication. With regard to SARS-CoV-2, this is likely related to distribution of ACE2 (the receptor for viral entry) in the respiratory tract and the stage of disease [20, 21].

Second, among upper respiratory specimens, nasopharyngeal swabs were found to offer the best clinical sensitivity, followed by oropharyngeal specimens, although statistically significant differences between both sites were not found overall, or in subanalyses for early illness or the presence of pneumonia. Midturbinate swabs were less sensitive than either naso- or oropharyngeal swabs. From clinical sensitivities extrapolated from the performance of individual sites, we determined that a nasopharyngeal or midturbinate swab combined with a throat swab offered the highest sensitivity (and similar clinical sensitivities).

Midturbinate swabs may be less uncomfortable or invasive compared with nasopharyngeal swabs [2] and, if used, should be combined with oropharyngeal sampling.

Besides clinical sensitivity, the choice of sampling site may also depend on other factors including familiarity and training of staff who are obtaining samples, patient preference, comfort, and adequate supply of required materials (such as the appropriate swabs). In the face of increased global demand on available trained staff and in areas with limited supply of personal protective equipment, patient-collected midturbinate samples may be considered as they offer up to 96% clinical sensitivity for the detection of SARS-CoV-2 when compared with health care worker-collected NP samples, as suggested in a study by Tu et al. [22], although based on our findings we suggest that this be combined with a oropharyngeal specimen to increase sensitivity.

Third, although saliva was previously reported as a promising sample and a more convenient diagnostic sample for COVID-19, our data suggest otherwise. Several factors may account for this. Studies reporting higher sensitivities had small numbers, ranging from 12–39 SARS-CoV-2-positive patients studied [7–12]. The method of collection and what constituted “saliva” were also different in various studies. To et al. collected “early morning saliva” from the posterior oropharynx (“coughed up by clearing throat”), which is technically a combination of saliva, nasopharyngeal, and bronchopulmonary secretions [9, 11]. Kojima et al. studied both self-collected and clinician-supervised oral fluid collection, which was comprised of expectorated sputum and secretions and an oral swab of the buccal mucosa, tongue, gums, and the hard palate [7]. Also, the severity of illness differed in some studies. For example, Azzi et al. studied 25 patients who had severe COVID-19, collecting salivary specimens by the “drool technique” or pipetting pooled oral secretions, and found SARS-CoV-2 by PCR in all patients studied. This group also found that higher lactate dehydrogenase (LDH) levels (indicative of tissue damage) were correlated with lower Ct values with salivary samples. Another reason for the differences in our study for saliva was that sensitivity could also have been influenced by prerinsing, as instructed by the developer of the transport medium. Nonetheless, given the findings of the various studies on saliva as a diagnostic for COVID-19 and older supportive data for saliva as a diagnostic specimen in humans and a rhesus macaque infection model [23, 24] for SARS-CoV-1 (which also uses ACE2 as its receptor), further standardization of methods for salivary diagnostics and larger confirmatory studies are needed.

The strengths of our study include its large sample size and simultaneous sampling from multiple sites of each patient. The limitations of our study were that results from a combination of sites were extrapolated from the results of each site. We were also not able to systematically study lower respiratory tract or nonrespiratory samples and are thus not able to assess

the comparative performance of these. It is noted, however, that not all patients with COVID-19 have a productive cough (and thus are unable to produce sputum) or they may be ill enough to warrant more invasive sampling (eg, endotracheal aspirates or bronchoalveolar lavage). Stool shedding, on the other hand, has been reported to persist for as long as 5 weeks in some patients. SARS-CoV-2 may be detected in stool beyond the duration of respiratory shedding and could be an alternate diagnostic specimen if upper respiratory samples are negative, although shedding may be not be detectable in all patients or may be intermittent [25–27]. Anecdotally, in our institution, we have diagnosed several cases of COVID-19 in the pneumonic phase, with stool being the only positive sample by PCR. Stool PCR, along with other adjuncts to diagnosis such as serology or computed tomography (which may have characteristic findings, albeit not specific), may assist clinicians in the diagnosis of COVID-19. Another limitation is that although 1 nasopharyngeal swab is sufficient for the detection of SARS-CoV-2, 2 swabs from each nostril are recommended for midturbinate swabs [28]. This was not performed in our audit so as to minimize discomfort and trauma from repetitive sampling of the same nostrils, as sampling of all 4 sites (nasopharyngeal, midturbinate, throat, and saliva) was done in the same setting during audit day. Lastly, the design of our audit did not allow us to fully compare the relative performance of unilateral vs bilateral nasopharyngeal swabs. Although the clinical sensitivity of bilateral nasopharyngeal swabs was found to be slightly higher than unilateral in early illness, our comparison was biased toward bilateral nasopharyngeal swabs (as these were obtained ~24 hours before the unilateral swab). Reassuringly, these differences were not statistically different.

## CONCLUSIONS

In summary, nasopharyngeal and throat specimens offer the best clinical sensitivity for the molecular diagnosis of COVID-19 in early illness. More data and standardization of collection techniques are required to assess the utility of saliva as a diagnostic specimen. Although the sensitivity of midturbinate specimens was lower than nasopharyngeal and throat specimens, test sensitivities improve and are similar when midturbinate or nasopharyngeal specimens are combined with throat specimens. Midturbinate combined with throat swabs may thus be an alternative to nasopharyngeal swabs, which are more invasive and may cause more patient discomfort. In the pneumonic stage or later disease (>7 days), upper respiratory specimens perform poorly, and clinicians should be aware and seek alternate specimen types and other adjuncts to support a diagnosis of COVID-19.

## Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility

of the authors, so questions or comments should be addressed to the corresponding author.

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National Centre for Infectious Diseases COVID-19 Outbreak Research Team

Sean Wei Xiang Ong, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Brenda Sze Peng Ang, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

David Chien Lye, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Poh Lian Lim, MD, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Cheng Chuan Lee, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Li Min Ling, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Lawrence Lee, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Barnaby Edward Young, MB BChir, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Tau Hong Lee, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Chen Seong Wong, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Sapna Sadarangani, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Ray Lin, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Deborah Hee Ling Ng, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Mucheli Sadasiv, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Po Ying Chia, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

Chiaw Yee Choy, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Glorijoy Shi En Tan, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Frederico Dimatatac, MD, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Isais Florante Santos, MD, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Chi Jong Go, MD, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Yeo Tsin Wen, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Yu Kit Chan, MBBS, National Centre for Infectious Diseases, Singapore, Tan Tock Seng Hospital, Singapore

Pooja Rao, MBBS, Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore

Jonathan WZ Chia, MBBS, Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore

Constance Yuan Yi Chen, MD, Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore

Boon Kiat Toh, MBBS, Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore

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