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Diagnostic Accuracy of Saliva for SARS-CoV-2 Detection in State-sponsored Quarantine in Thailand

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Abstract

The aim of this study was to assess the diagnostic accuracy of saliva for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes among people in state-sponsored quarantine in Thailand. A cohort of 233 Thais in state-sponsored quarantine in Bangkok was enrolled into the study. Baseline demographic characteristics, presence of underlying diseases, and symptoms related to COVID-19 were collected on day 1 of the quarantine. Saliva specimens and nasopharyngeal (NP) swabs collected on day 7 at the quarantine premises were tested for SARS-CoV-2 RNA by real-time reverse transcription polymerase chain reaction. Overall, the viral RNA was detected in 32 (13.7%) NP swab samples, but only in 12 (5.2%) of the saliva samples. No person had NP negative but saliva positive result. Among the SARS-CoV-2 infected cases, nearly 20% had COVID-19-like illness and around 80% were asymptomatic. Sensitivity and specificity of saliva specimen were found to be 37.5% (95% confidence interval (CI)=21.1-56.3%) and 100% (95% CI=98.2-100%), respectively compared to the NP swab specimens. The area under the receiver operating characteristic curve was found to be 0.7 (95% CI=0.6-0.8). Our findings indicate that despite no false-positives, a high false-negative rate can occur with saliva specimen due to its low sensitivity, which limits its application in ruling out SARS-CoV-2 infection in quarantine settings.

Keywords: nasopharyngeal swab, saliva, SARS-CoV-2, state quarantine, Thailand

Introduction

Thailand was the first country outside of China to report cases of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] In response, the Thai government activated the Emergency Operation Center in January 2020 to mitigate the impact of the disease.[2] Since then, COVID-19 has gone on to become a global pandemic with over 13 million cases and 570,000 deaths, while the disease burden of COVID-19 in Thailand has stalled at 3,844 cases and 60 deaths as of 11 Nov 2020.[3,4]

Following the "Communicable Diseases Act B.E. 2558", all people returning to Thailand are subjected to enter a state quarantine (SQ) as part of the public health response in which they undergo surveillance and monitoring for COVID-19-like illness (CLI) for 14 days.[2] Thai nationals are provided with individual hotel rooms and three daily meals in the SQ sponsored by the government. Laboratory confirmation of the presence or absence of the virus by real-time reverse transcription polymerase chain reaction (rRT-PCR) tests using nasopharyngeal (NP) swabs are conducted twice in the SQ: the first test during initial 3-5 days and the second test during day 10-12 of SQ. However, NP swab collection can be invasive and uncomfortable to many people, and can also pose a risk of infection to the healthcare workers who perform specimen collection due to close contact with the infected cases.[5]

Saliva has been presented as a potential alternative to NP swabs as a non-invasive sample with considerably high sensitivity and specificity for detecting SARS-CoV-2 RNA in recent studies-[6,9] Saliva has many advantages over NP swab as it can be self-collected by individuals with relative precision, reducing the demand for specialized healthcare personnel and personal protective equipment.[5] This can be particularly beneficial in resource-limited settings outside hospitals such as the quarantine facilities. Using data from people in SQ at Bangkok, we tested the feasibility and the diagnostic accuracy of saliva specimens for SARS-CoV-2 detection in field settings.

Methods

Study Setting and Population

SQ measure has been implemented by the Thai government for travelers entering Thailand since 4 Apr 2020. There are 12 hotels designated as SQ in Bangkok which have quarantined 8,541 people till 31 May 2020. This study was conducted among a cohort of Thai nationals in SQ in Bangkok from 22 May to 8 Jun 2020. Three SQ hotels were chosen purposively as they housed Thai returnees from high prevalence areas of COVID-19, such as the USA, European, and the Middle Eastern countries. All individuals aged more than 18 years in the quarantine at the time of this study were included, but those unwilling or unable to provide saliva specimen were excluded.

Study Design and Data Collection

This was a prospective cohort study that entailed a one-time collection of saliva (index test) and NP swabs from the participants. Baseline demographic characteristics, presence of underlying diseases, and symptoms related to COVID-19 were collected from the participants on day 1 of the quarantine. Saliva specimens and NP swabs were collected on day 7 at the quarantine premises. Participants testing positive were sent to designated hospitals for isolation and treatment as per the national guidelines.

Sample Size and Sampling Technique

In a recently conducted study at Ramathibodi Hospital, Mahidol University, Bangkok, the sensitivity of saliva specimens was found to be 84.2% compared to NP and throat swabs.[9] We calculated the minimum sample size for our study using this equation in the general formula:[10]

$$n = \frac{\left[Z_{\alpha/2}\sqrt{(P_0(1-P_0)} + Z_{\beta}\sqrt{P_1(1-P_1)}\right]^2}{(P_1-P_0)^2}$$

Where, α and β are type I and II errors; $Z_{\alpha/2}$ and Z_{β} denote the upper $\alpha/2$ and β percentiles of standard normal distribution (1.96 and 0.84, respectively); P_0 is the sensitivity of null hypothesis, and P_1 is the sensitivity of alternate hypothesis.

The sample size needed to have 95% confidence interval (CI) and 80% power to detect a difference of 7% from 84.2% sensitivity is 232. We enrolled study participants using consecutive sampling until the required sample size was obtained.

Definition

COVID-19-like illness (CLI) was defined as any symptom of fever, cough, shortness of breath, chills, myalgia, sore throat, or loss of taste or smell.

Specimen Collection and Transport

The participants were asked to collect saliva (at least 2 ml) by spitting into a plastic device containing 2 ml of viral transport medium (VTM), after at least one hour of waking up in the morning on an empty stomach (before brushing teeth, eating or drinking anything). The container was covered with a lid, placed in a zip-lock bag, and packed into a foam cooler box. In the afternoon of the same day, NP swab was collected from the participant's posterior nasopharynx using flexible tip swabs by trained healthcare staff donned with standard personal protective equipment (PPE), following universal precautions of infection control. The NP swab was placed in a sterile tube containing 2 ml of VTM and securely covered. Both saliva specimen and NP swabs were labeled with different laboratory numbers and sent in foamed boxes, maintained at 4-8°C, to the Thai National Institute of Health (NIH), Nonthaburi, for rRT-PCR testing.

Specimen Processing and RNA Extraction[11]

The average time from specimen collection to specimen processing was four hours. Laboratory staffs were blinded to the names and participant numbers. Total RNA was extracted from a 200 µl volume of the NP swab solution or saliva sample using the GenTi™ 32 Ultimate Flexible Automatic Extraction System (GeneAll Biotechnology) according to the manufacturer's instructions, and a 50 µl final volume of total RNA was eluted. A negative extraction control was included in each test run to monitor the extraction process, in which the RNaseP RNA must be detected.

Real Time Reverse Transcription-polymerase Chain Reaction[12-13]

The SARS-CoV-2 genomes in the RNA extracts were detected by TaqMan real-time quantitative RT-PCR (qRT-PCR) targeting the RNA dependent RNA

polymerase (RdRp) gene, nucleoprotein (N gene), and Ribonuclease P (RNaseP RNA) using the COVID 19 RT-PCR reagent kit from the Department of Medical Sciences, and the Bio-Rad, CFX96 Real-time PCR Detection System (USA). The duplex reaction targeted RdRp gene and RNaseP RNA, while the uniplex reaction targeted N gene as described previously.[14,15] The sequences of primers and probes are shown in Table 1. A 20 µl volume of each reaction was composed of 5 µl of RNA template, 5 µl of 4X CAPITAL qPCR Probe Mix, primers and probe, 6.3 µl of enhancer mix (30% Tween 20 + 50% glycerol), and 1 µl of 20X RTase with RNase inhibitor. The reaction consisted the step of reverse transcription at 50°C for 30 minutes, polymerase enzyme activation at 95°C for 2 minutes, and followed by 45 cycles of DNA denaturation at 95°C for 15 seconds, annealing and extension at 55°C for 45 seconds.

Table 1. The primer and probe sequences for SARS-CoV-2 qRT-PCR diagnostic assays

Target gene	Primer/Probe	Sequence (5' -> 3')	Reference
RdRp	WH-NIC IN-F	CTCACCTTATGGGTTGGGATTATC	Okada[11]
	WH-NIC IN-R	AGTGAGGCCATAATTCTAAGCATGT	
	WH-NIC IN-P	FAM-TAAATGTGATAGAGCCATGCC-BHQ1	
N	WH-NIC N-F	CGTTTGGTGGACCCTCAGAT	Okada[11]
	WH-NIC N-R	CCCCACTGCGTTCTCCATT	
	WH-NIC N-P	FAM-CAACTGGCAGTAACCA-BHQ1	
RNaseP	RNaseP-F	AGATTTGGACCTGCGAGCG	WHO[12]
	RNaseP-R	GAGCGGCTGTCTCCACAAGT	
	RNaseP-P	HEX-TTCTGACCTGAAGGCTCTGCGCG-BHQ1	

Interpretation

The cycle threshold (Ct) values of ≤40 was considered as positive "genome detected", and those >40 were considered negative or "genome not detected". In case the Ct value for the test or control probe was undetermined or greater than the threshold, the experimental result was considered "invalid".

Data Analysis

Descriptive statistics were carried out and presented in terms of frequency and percentage for categorical variables; and mean and standard deviation for continuous variables. Differences in baseline characteristics between participants testing positive for SARS-CoV-2 on the reference test were assessed using Fisher's exact test (for categorical variables) and t-test (for continuous variables). Sensitivity, specificity, positive predictive value, negative predictive value, area under receiver operating characteristic (ROC), and their 95% confidence intervals were calculated to assess the diagnostic performance of saliva specimens in comparison with NP swabs. Statistical significance was set at p <0.05 and all data analyses were conducted using STATA software version 14.2 (StataCorp LP, College Station, TX, USA).

Ethical Considerations

Verbal consent was obtained from all participants and this study followed the principles of the Declaration of Helsinki. Ethical clearance was not required as this study was considered as a part of the routine investigation in the national public health response to the emergency situation of COVID-19.

Results

Participant Characteristics

In total, 235 eligible participants were approached and two persons declined to provide saliva specimen (Figure 1). Therefore, 233 participants were enrolled in the study with a mean age of 37.2 years (standard deviation, 11.2 years). A majority of the participants were male (180; 77.2%), with no underlying disease (216; 92.7%), and had no CLI symptoms (225; 96.6%) (Table 2). The prevalence of SARS-CoV-2 infection was 13.7% (32/233) based on the rRT-PCR with the NP swab samples, and 5.2% with the saliva samples. Among the positive cases with NP swab samples, 18.8% (6/32) had CLI and 81.2% (26/32) were asymptomatic. The participants who tested positive for SARS-CoV-2 in the reference test using NP swab were comparatively older and had more CLI symptoms than those who tested negative (Table 2).

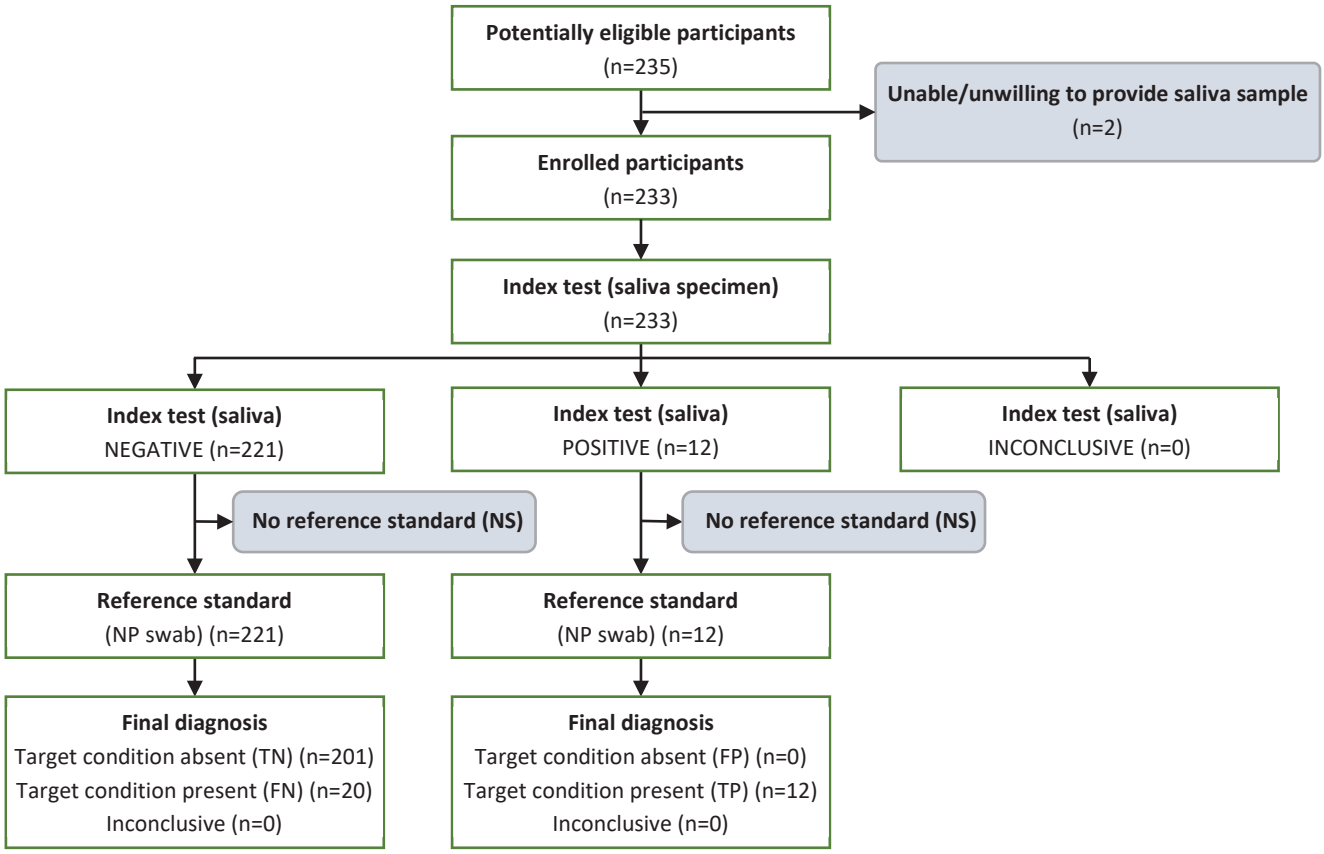

Figure 1. Study flow-diagram

Table 2. Characteristics of participants providing specimens for SARS-CoV-2 detection in state-sponsored quarantine, 22 May to 8 Jun 2020, Bangkok, Thailand (n=233)

Characteristics	Total N=233 n (%)		SARS-CoV-2 infection (Nasopharyngeal swab result)				*p*-value
			Positive (n=32)		Negative (n=201)		
			n	%	n	%	
Age (year) [mean (SD)]	37.2 (11.2)		41.4 (8.1)	-	36.5 (11)	-	0.023[a]
Gender							
Male	180	(77.2)	29	90.6	151	75.1	0.068
Female	53	(22.8)	3	9.4	50	24.9	
State quarantine site							
N Hotel	204	(87.5)	19	59.4	185	92.0	<0.001
P Hotel	9	(3.9)	5	15.6	4	2.0	
B Hotel	20	(8.6)	8	25.0	12	6.0	
Underlying disease							
No	216	(92.7)	30	93.8	186	92.5	0.679
Yes	17	(7.3)	2	6.3	15	7.5	
Diabetes Mellitus	2	(11.8)	0	0.0	2	1.0	
Hypertension	2	(11.8)	1	4.5	1	0.5	
Dyslipidemia	1	(5.9)	0	0.0	1	0.5	
Allergy	5	(29.4)	0	0.0	5	2.5	
Sinusitis	1	(5.9)	1	4.5	0	0.0	
Asthma	2	(11.8)	0	0.0	2	1.0	
Pulmonary Tuberculosis	1	(5.9)	0	0.0	1	0.5	
Others	3	(17.6)	0	0.0	3	1.5	
CLI symptoms							
Yes	8	(3.4)	6	18.8	2	1.0	<0.001
No	225	(96.6)	26	81.2	199	99.0	

Note: [a]p-value from t-test, other p-values from Fisher's exact tests.

Diagnostic Performance of rRT-PCR of Saliva

Saliva sample were rRT-PCR detected for SARS-CoV-2 in 12 cases, whereas the NP swab found 32 positive cases among the participants (Figure 1 and Table 3). Using NP swab as the reference standard, the sensitivity and specificity of saliva samples were 37.5% (95% CI=21.1-56.3%) and 100% (95% CI=98.2-100%), respectively (Table 4). Similarly, positive predictive value was 100% (95% CI=73.5-100%) and negative predictive value was 91% (95% CI=86.4-94.4%). The area under the ROC curve was found to be 0.7 (95% CI=0.6-0.8).

Table 3. Comparison of saliva samples with nasopharyngeal swabs for SARS-CoV-2 detection in rRT-PCR

	Positive n (%)	Negative n (%)
Saliva sample result	12 (5.2)	221 (94.8)
Nasopharyngeal swab result	32 (13.7)	201 (86.3)

Table 4. Diagnostic performance of saliva specimens for SARS-CoV-2 detection compared to NP swab specimens

Diagnostic characteristic	Percentage (%)	95% confidence interval	
		Lower bound	Upper bound
Sensitivity	37.5	21.1	56.3
Specificity	100.0	98.2	100.0
Positive predictive value	100.0	73.5	100.0
Negative predictive value	91.0	86.4	94.4
Area under ROC curve	68.8	60.2	77.3
Likelihood ratio (positive)	-	-	-
Likelihood ratio (negative)	62.5	47.8	81.7

Discussion

We tested the diagnostic accuracy of self-collected saliva for SARS-CoV-2 rRT-PCR among Thai nationals in state quarantine at Bangkok and found that while its specificity was high, the sensitivity was very low. The findings of this study provide important implications to exercise caution in using saliva in place of NP swabs for the detection of SARS-CoV-2 in quarantine settings.

High specificity and positive predictive value in our study mean that positively identified cases by saliva are most certainly infected with SARS-CoV-2. However, low sensitivity produces a high rate of false-negatives which renders saliva samples inept to rule out the virus infection. False-negative outcomes are more dangerous in SQ as people returning from endemic areas can have high pre-test probability of infection, and when infected persons test negative (especially asymptomatic), they may infect another in the SQ if they do not remain isolated in their own room.[16] Low sensitivity in the final test at the end of SQ period can lead to the release of people with undetected infection, who may go on to infect others in the community.

Our finding of a low sensitivity of saliva for SARS-CoV-2 genome detection is in stark discordance with most prior studies that report the sensitivity of saliva to be near or even better than NP swab.[6,9,17-19] This study showed the sensitivity and specificity of 37.5% (95% CI=21.1-56.3%) and 100% (95% CI=98.2-100%), respectively of the saliva samples; while the other group of Thai investigators showed the sensitivity and specificity of 84.2% (95% CI=60.4-96.6%) and 98.9% (95% CI=96.1-99.9%), respectively compared to the NP swab samples as the reference standard.[9] One likely reason for the difference in the results could be the participant selection. A relatively higher sensitivity of saliva has been found in studies recruiting patients in hospitals, especially in-patients and intensive care units, possibly since hospitalized patients have more acute and severe symptoms, hence, higher viral load in their saliva specimen.[20] In comparison, people in community settings, including quarantine in our study, may have milder symptoms and low viral load in saliva for detection of SARS-CoV-2.[21,22] Given that all Thai returnees require a “fit to fly” certificate before flying back to Thailand, people in SQ are less likely to have severe symptoms, as evident by less than 4% of participants having CLI in our study. Similar to our study, few other studies have also reported a less than optimal sensitivity of saliva in the community for people with mild symptoms.[22,23]

Other factors for the discrepancy of findings may be the quality of saliva collection and the transport medium used.[22] Although we used a standard VTM in our study, prior studies have used different preservation solutions which may have conferred

them with higher protection against degradation of SARS-CoV-2 RNA. Furthermore, people can have psychological stress in quarantine,[24] and prior research indicate that stress can impact the quality and quantity of saliva produced.[25] Stress in SQ could have also affected the participants' capacity to adhere to the instructions for proper specimen collection. Therefore, the compliance of subjects to spit the saliva specimens may have also contributed to the low sensitivity. However, this may be reduced as there was no financial burden for the people in SQ for their food and lodging, and clear instructions were provided in the Thai language.

The strengths of this study lie in the high proportion of asymptomatic cases, less time lag between saliva and NP swab collection, and reduced risk of bias as the index test and reference tests were analyzed without prior knowledge of each's results. Nevertheless, our findings may be limited by lack of clinical correlations and imprecision due to some human errors which might have crept in during sample collection as they were not strictly under ideal research conditions.

Conclusion and Recommendations

In conclusion, despite high specificity, saliva was not sensitive compared to nasopharyngeal swabs in state quarantine (SQ) at Bangkok. As the local transmission of SARS-CoV-2 has ceased in Thailand, the source of infection is limited to imported cases from people returning from high prevalence areas. A high rate of false-negatives in detection of SARS-CoV-2 from saliva specimens in rRT-PCR due to its low sensitivity in our study restricts its probable applicability for large-scale implementation.

While saliva is a potential non-invasive sample for laboratory detection of SARS-CoV-2 due to its practical advantages and may even be essential for cases when NP or throat swabs are contraindicated, larger studies with higher precision are needed for full validation and further confirmation. Until such evidence is available, nasopharyngeal swabs remain the standard, particularly in quarantine or other community settings with low prevalence. Saliva specimen is not recommended for use in the diagnosis of COVID-19 in an individual. It may be used in the field epidemiology for the purposes of investigating and controlling an outbreak that occurs in a big community where the prevalence of the SARS-CoV-2 infection is high.

Conflict of Interest

The authors have no conflicts of interest associated with the material presented in this paper.

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Author Contributions

Conceptualization: SS, AM. Data curation: SS, PAO, PP, KP, SI. Formal analysis: KP, MS. Funding acquisition: None. Methodology: SS, AM. Project administration: AM, SS, PP. Visualization: KP, MS. Writing - original draft: MS. Writing - review & editing: SS, MS, PAO, KP, PP, SI, AM.

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