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Direct Saliva versus Conventional Nasopharyngeal Swab qRT-PCR to Diagnose SARS – CoV2: Validity Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author MLT did the conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, supervision, validation, visualization, writing and review editing the original draft. Author PJRU did the conceptualization, formal analysis, investigation, methodology, project administration, resources, supervision, validation, visualization, writing and review & editing. Conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing - review & editing done by author DRER. Author CAT data curation, formal analysis, investigation, methodology, validation, visualization, writing and review & editing the original draft. Author AAA did the data curation, formal analysis, investigation, validation, visualization, writing and review & editing. Data curation, investigation, methodology, project administration, resources, supervision, validation, visualization, writing and review & editing done by author LJPLT. Author CMC did the conceptualization, methodology, project administration, supervision, validation, visualization, writing and review & editing. Author RPB did the conceptualization, investigation, project administration, resources, supervision, writing review & editing. Conceptualization, formal analysis, methodology, validation, visualization, writing - review & editing done by author RJDC. Author MCFL did the investigation, project administration, supervision, validation, writing and review & editing. Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, supervision, validation, visualization, writing - review & editing the original draft done by author LMMD. All authors read and approved the final manuscript.

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ABSTRACT

Background: Saliva has been demonstrated as a feasible alternative specimen to nasopharyngeal swab for the detection of SARS-CoV-2 using real-time or quantitative reverse transcription polymerase chain reaction (qRT-PCR) method that bypasses the need for explicit viral ribonucleic acid (RNA) extraction.
Aim: To assess the diagnostic validity of direct saliva-to-qRT-PCR in the detection of SARS-CoV-2 compared to conventional nasopharyngeal swab qRT-PCR.
Methodology: Self-collected saliva samples were processed by heating at 95^{o}C for 30 minutes followed by addition of buffer and detergent while viral RNA from nasopharyngeal swabs were extracted using the Sansure Biotech sample release reagent. Paired samples were used as templates for qRT-PCR using the Sansure Novel Coronavirus (COVID-19) Nucleic Acid Diagnostic Kit and Sansure Biotech MA6000 Real-Time Quantitative PCR System. Direct saliva-to-qRT-PCR was compared to nasopharyngeal swab qRT-PCR in terms of diagnostic validity and agreement parameters, and both platforms were compared separately in terms of similar parameters with a composite reference standard (CRS) wherein the criteria for a positive result is SARS-CoV-2 detection in at least either nasopharyngeal swab or saliva.
Results: Of the 238 nasopharyngeal swab-saliva pairs tested, 20 (8.4%) nasopharyngeal swab and 24 (10.1%) saliva specimens tested positive. We documented a sensitivity of 85.0% (95% CI: 62.1%, 96.8%), specificity of 96.8% (95% CI: 93.5%, 98.7%), accuracy of 95.8% (95% CI: 92.4%, 98.0%) and Cohen Kappa of 0.75 (95% CI: 0.60, 0.90) when direct saliva-to-qRT-PCR was compared to the conventional platform. When the two platforms were individually compared to the CRS, numerically higher but not statistically significant sensitivity and accuracy were noted for direct saliva-to-qRT-PCR than for nasopharyngeal swab qRT-PCR.
Conclusion: Direct saliva-to-qRT-PCR is non-inferior to nasopharyngeal swab qRT-PCR for detecting SARS-CoV-2 using the Sansure Novel Coronavirus Nucleic Acid Diagnostic Kit.

Keywords: Direct saliva; conventional; nasopharyngeal; swab; PCR; SARS-COV-2.

1. INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-sense, single-stranded, enveloped RNA virus, is the etiologic agent of 2019 novel coronavirus disease (COVID-19). As of February 7, 2021, more than 105 million people have been infected with the virus, resulting in 2.2 million deaths. In the Philippines, more than 533,000 people have been infected with a case fatality rate of 2.1% [1]. Several efforts are employed to contain further viral transmission which include personal hygiene measures, social distancing and infection control together with identification of asymptomatic and presymptomatic carriers [2,3]. Ramping up testing capability will then be helpful in identifying and isolating carriers and infected individuals [2].

The US Centers for Disease Control and Prevention (CDC) Interim Guidelines for Clinical Specimens for COVID-19 includes both nasopharyngeal (NP) specimen collected by trained healthcare personnel and saliva obtained through supervised self-collection [4]. Several studies, have established that saliva can be used as a specimen with similar sensitivity as that from a nasopharyngeal swab [5-7].

The United States Food and Drug Administration (FDA) has issued an Emergency Use Authorization (EUA) to the Yale School of Public Health, Department of Epidemiology of Microbial Diseases for the qualitative detection of nucleic acid from SARS-CoV-2 in saliva collected without preservatives in a sterile container. The saliva is treated with proteinase K, heat-inactivated, and then directly used as input in authorized qRT-PCR instrument [8].

Similarly, the University of Illinois at Urbana-Champaign (UIUC) validated its laboratory-developed saliva-to-direct qRT-PCR test under a Clinical Laboratory Improvement Amendments (CLIA)-certified facility by successfully comparing its performance to Yale's FDA EUA protocol. The UIUC protocol uses saliva sample collected in a sterile tube heated to 95^{o}C for 30 minutes, diluted 1:1 with 2x Tris-Borate-EDTA (TBE) with 1% Tween-20 and used directly in qRT-PCR using the ThermoFisher TaqPath COVID-19

Combo Assay Kit, a multiplex-based kit that detects three SARS-CoV-2 genes (*ORF1ab, S* and *N*) and a MS2 phage internal control [9].

This study evaluated whether saliva can be used as an alternative sample to NP swabs in detecting SARS-CoV-2 genes, specifically *ORF1ab* (FAM) and *N-gene* (ROX) with the human *RNAse P-gene* serving as internal control, using the Sansure Biotech MA-6000 Real-Time Fluorescence Quantitative PCR System in combination with UIUC's upstream saliva processing protocol.

2. METHODOLOGY

2.1 Study Population

A prospective observational cross-sectional study was conducted among adult subjects at the Philippine Red Cross (PRC) and the Philippine General Hospital of the University of the Philippines Manila (UPM) who availed of the SARS-CoV-2 nasopharyngeal swab qRT-PCR test and are able to drool saliva on their own. The participants were instructed not to eat, drink, use toothbrush or mouthwash, and smoke for at least 30 minutes prior to providing a saliva sample. Age, sex, reason for swab testing, and time since exposure and/or onset of relevant signs and symptoms, if present, were recorded. De-identified case report forms and coded specimens were processed blindly. The interpretation of results was done by two independent single-blind observers. The subjects were asked to first provide saliva samples, void of coughing, in a plain 5mL sterile container through an individually-wrapped straw. After which, the subjects proceeded with the standard nasopharyngeal swab procedure. The swabs were placed in 2.0 mL sterile tubes containing Sample Storage Reagent (Sansure Biotech; Ref. No. X1002E). The paired nasopharyngeal swab and saliva samples collected from the subjects underwent simultaneous testing using manufacturer-specific RT-qPCR protocol.

2.2 Sample Size Calculation

The sample size was computed using the Kappa statistic or agreement between two tests for a 2x2 analysis [10]. Assuming a power of 80%, alpha of 0.05 and prevalence of less than 20% among those undergoing tests, the sample size is 194 subjects. At 3% withdrawal rate, the minimum target sample size is adjusted to 200.

2.3 Sample Processing

Direct Saliva-to-qRT-PCR approach was conducted following the saliva preparation method described by Ranoa et al. [9] and applied to the Sansure Novel Coronavirus (COVID-19) Nucleic Acid Diagnostic Kit. Collected saliva samples were heated at 95°C for 30 minutes and cooled to room temperature. A 100 uL aliquot was transferred to designated wells containing 100uL 2xTBE+1% Tween-20, for a final 1:1 dilution of sample and buffer [9]. From this mixture, 20uL was used as input in the qRT-PCR reaction. NP swab samples were collected and processed using Sansure Novel Coronavirus (COVID-19) Nucleic Acid Diagnostic Kit manufacturer's prescribed procedure duly approved by the Philippine FDA [11].

2.4 qRT-PCR Workflow

The detection of SARS-CoV-2 in the specimens was performed by qRT-PCR amplification of the SARS-CoV-2 *ORF1ab* and *N* gene fragments, using a SARS-CoV-2 Nucleic Acid Diagnostic Kit (Sansure Biotech Inc., Changsha, China), which was approved for the detection of SARS-CoV-2 by the Philippine FDA. The limit of detection of the test, using the manufacturer-prescribed procedure that includes RNA extraction steps is 200 copies/mL. The detection of the human *RNase P* gene was included in the kit as an internal control. RT-PCR was performed using the MA6000 Real-time quantitative PCR System (Sansure Biotech, Changsha, China) following the manufacturer's program specifications. Provided that the internal control is detectable and shows a cycle threshold (CT) value of $\leq$ 40.00, a sample was considered SARS-CoV-2 positive if the CT value of both *ORF1ab* and *N* genes is ≤ 40.00, and negative when either (1) the CT values of both *ORF1ab* and *N gene* targets were > 40.00 or (2) only at most one gene is detectable regardless of the CT value. Re-testing of a sample was performed if the internal control shows a CT value of > 40.00 [11].

2.5 Statistical Analysis

Data were analysed for normality and descriptive statistics were presented as a number (%) for categorical variables and median (interquartile range; IQR) for continuous variables. Chi-square tests and Kruskal-Wallis median tests were performed to compare subgroups. Diagnostic validity parameters (sensitivity, specificity, positive predictive value [PPV], negative

predictive value [NPV] and accuracy) comparing (1) direct saliva-to-qRT-PCR to NP swab qRT-PCR and (2) direct saliva-to-qRT-PCR or NP swab qRT-PCR to a composite reference standard (CRS), wherein a patient with at least one SARS-CoV-2-positive sample is considered truly positive, were calculated with 95% confidence interval (CI) verified with the MedCalc diagnostic test evaluation calculator (https://www.medcalc.org/calc/diagnostic_test.php). The Cohen Kappa (κ) coefficient for agreement with 95% CI was also estimated between the direct saliva-to-qRT-PCR and NP swab qRT-PCR results. All statistical analyses were performed using Stata statistical software version 14.2 (Stata, College Station, TX, USA).

3. RESULTS

A total of 238 adults provided paired nasopharyngeal swab and saliva samples for SARS-CoV-2 testing. The median (IQR) age of the subject was 34 (range, 27-45) years, and 145 (60.9%) were male (Table 1). Fifty one subjects (21.4%) indicated that they were exposed to a confirmed or suspected COVID-19 case, with a median (IQR) duration from exposure to sample collection of 8.5 (range, 6-12) days. While 35 (14.7%) reported at least one symptom, with median (IQR) duration from symptom onset to sample collection of 5 (range, 3-7) days and the most common reported symptom being cough (7.6%), the rest claimed to be asymptomatic.

Twenty (8.4%) subjects were positive for SARS-CoV-2. They were more likely to report exposure to a suspected or confirmed COVID-19 patient and to being symptomatic than those with SARS-CoV-2-negative NP swab ($P < .05$ in all cases). On the other hand, saliva samples of 24 (10.1%) subjects tested positive for the virus. A total of 17 subjects (7.1%) provided swab and saliva samples that were both positive for SARS-CoV-2. Twenty-seven (11.3%) subjects had at least either one of their provided samples (saliva or swab) positive for the virus and 194 (81.5%) subjects provided saliva and swab samples that tested negative. The resulting contingency table comparing direct saliva-to-qRT-PCR to NP swab qRT-PCR (Table 2) reveals sensitivity of 85.0% (95% CI: 62.1%, 96.79%), specificity of 96.8% (95% CI: 93.5%, 98.7%),and accuracy of 95.8% (95% CI: 92.4%, 98.0%). The estimated Cohen κ coefficient is 0.75 (95% CI: 0.60 to 0.90), indicating substantial agreement.

When NP swab qRT-PCR and direct saliva-to-qRT-PCR results were separately compared to the CRS, the use of direct saliva-to-qRT-PCR resulted to a numerically higher sensitivity, NPV, accuracy and Cohen κ compared to NP swab qRT-PCR (Table 3). However, none of these differences were statistically significant ($P > 0.05$ in all cases).

Table 4 demonstrates that, in general, *ORF1ab* gene is detected in saliva at lower CT values (*i.e.* earlier PCR cycles) compared to samples taken from the nasopharynx. *N* gene and IC in both specimens had comparable CT values.

Table 1. Characteristics of subjects who provided nasopharyngeal swab and saliva samples for the study

	Overall (n = 238)	Swab qRT-PCR result		
		Positive (n = 20)	Negative (n = 218)	P value*
Age, median (IQR)	34 (27-45)	36 (28-52)	34 (27-45)	.236
Male, n (%)	145 (60.9)	9 (45.0)	136 (62.4)	.127
Exposure to a COVID-19 case, n (%)	51 (21.4)	8 (40.0)	43 (19.7)	.034
Days since exposure, median (IQR)	8.5 (6-12)	9 (7-10)	6 (4-7)	.407
Symptomatic, n (%)	35 (14.7)	9 (45.0)	26 (11.9)	<.001
Days since onset of symptoms, median (IQR)	5 (3-7)	5 (3-7)	6 (4-7)	.136

**significant at 0.05*
IQR - Interquartile Range

Table 2. Diagnostic validity contingency table comparing direct saliva-to-qRT-PCR to nasopharyngeal swab qRT-PCR for SARS-CoV-2 detection

Direct saliva-to-qRT-PCR	Nasopharyngeal swab qRT-PCR		
	Positive	**Negative**	**Total**
Positive	17	7	24
Negative	3	211	214
Total	20	218	238
Sensitivity: 85.0% (95% CI: 62.1%, 96.8%) **Specificity:** 96.8% (95% CI: 93.5%, 98.7%) **Accuracy:** 95.8% (95% CI: 92.4%, 98.0%)			

NP, nasopharyngeal; qRT-PCR, real-time reverse transcription polymerase chain reaction

Table 3. Diagnostic validity contingency tables and parameter estimates comparing nasopharyngeal swab qRT-PCR or direct saliva-to-qRT-PCR to the CRS (positive in both or either NP swab-qRT-PCR or direct saliva-to-qRT-PCR)

Nasopharyngeal swab qRT-PCR or Direct saliva-to-qRT-PCR								
NP swab		**Positive**	**Negative**	**Total**	**Direct saliva**		**Positive**	**Negative**	**Total**
	Positive	20	0	20		Positive	24	0	24
	Negative	7	211	218		Negative	3	211	214
	Total	27	211	238		Total	27	211	238
		Measure (95% CI)					*Measure (95% CI)*		
	Sn	74.1% (53.7%, 88.9%)				Sn	88.9% (70.8%, 97.7%)		
	Sp	100.0% (98.3%, 100.0%)				Sp	100.0% (98.3%, 100.0%)		
	PPV	100.0% (100.0%, 100.0%)				PPV	100.0% (100.0%, 100.0%)		
	NPV	96.8% (94.1%, 98.3%)				NPV	98.6% (96.0%, 99.5%)		
	Accuracy	97.1% (94.0%, 98.8%)				Accuracy	98.7% (96.4%, 99.7%)		
	Cohen κ	0.84 (0.71, 0.96)				Cohen κ	0.93 (0.86, 1.00)		

CRS, composite reference standard; CI, confidence interval; NP, nasopharyngeal; NPV, negative predictive value; PPV, positive predictive value; qRT-PCR, real-time reverse transcription polymerase chain reaction; Sn, sensitivity; Sp, specificity

Table 4. CT value comparison of SARS-CoV-2-positive samples among nasopharyngeal swab- and saliva-positive subjects (n = 17)

Gene (dye)	Mean (SD) CT value		Mean Difference (95% CI)	*P* value*
	Saliva	**NP Swab**		
ORF1ab (FAM)	29.99 (4.87)	33.76 (3.93)	-3.77 (-6.62, -0.91)	0.013
N (ROX)	28.73 (5.13)	29.37 (4.05)	-0.64 (-3.41, 2.13)	0.630
RNase P (Cy5)	24.11 (2.43)	25.04 (1.58)	-0.92 (-2.36, 0.51)	0.192

**significant at .05*

Abbreviation: CI, confidence interval; CT, cycle threshold; NP, nasopharyngeal; SD, standard deviation

4. DISCUSSION

The UIUC saliva-based direct qRT-PCR technique can be used in Sansure Biotech MA-6000 Real-Time Fluorescence Quantitative PCR System. Using heat-inactivation at 95^{o}C for 30 minutes and dilution with an RNA-stabilizing buffer (TBE/Tween-20), we detected SARS-CoV-2 from the saliva of 24 out of 238 subjects.

In comparing the diagnostic validity of direct saliva-to-qRT-PCR, with the conventional NP swab qRT-PCR as the reference test, we observed an acceptable sensitivity of 85.0% (17/20) and NPV of 98.6% (211/214). Because we considered cases testing positive in the index test but negative in the reference test to be false positive, the estimated specificity, PPV, accuracy and agreement appeared to be lower than what could be expected in a qRT-PCR platform. Such finding was similarly found in another study comparing saliva and NP swab that used a qRT-PCR platform from the same manufacturer [12]. Assuming that optimal quality control procedures were observed from test sample collection to processing, the false positive rate in qRT-PCR is considered 0% because the primers designed to detect the genetic material of a pathogen is

specific to the genome sequence of the said entity [13]. It is thus more likely that these cases represent truly SARS-CoV-2-infected subjects missed by NP swab qRT-PCR rather than false positive direct saliva-to-qRT-PCR results. With this consideration in mind, we constructed a CRS wherein the criteria for a positive case is at least either a SARS-CoV-2-positive NP swab or saliva (virtually preventing every case from being considered as false positive) and, through this, separately assessed the diagnostic performance of both NP swab qRT-PCR and direct saliva-to-qRT-PCR [14]. We were able to demonstrate that direct saliva-to-qRT-PCR performed numerically better than the conventional test in terms of sensitivity, NPV, accuracy and agreement with respect to this CRS, suggesting that this index test is non-inferior at the very least to NP swab qRT-PCR in SARS-CoV-2 detection.

It has been observed by the CDC that only about 10% of the COVID-19 cases in the United States have been detected [15]. This may partly be because as a clinical test, qRT-PCR is designed for use with symptomatic people to provide definitive clinical diagnosis given a single opportunity to test.

4.1 Timing the Test

In this study, we showed that *ORF1ab* is detected in the saliva at a significantly lower CT value than in samples taken from the nasopharynx. This is consistent with the study of Wyllie et al. that shows higher viral concentrations in the saliva compared to NP swabs [7].

As a clinical test, the validity of detecting viral genetic materials in either a saliva or swab sample increases as the duration between onset of symptoms and timing of test fall within the narrow range of highest pre-test probability. In our cohort, 9 out of 20 NP swabs (45.0%), and 12 out of 24 saliva (50.0%) that tested positive reported symptoms at the time of sample collection (Table 1), suggesting that a substantial proportion of individuals with detectable SARS-CoV-2 in either saliva or nasopharynx are either asymptomatic or pre-symptomatic at the time of sample collection.

It has been shown that the window during which polymerase chain reaction detects infection before infectivity is short while the post-infection PCR-detectable window is long [16]. This well-described long tail of RNA positivity after the transmissible stage means that it is possible that many people whose NP swab turned positive long-after the onset of symptoms may no longer be infectious by the time the sample collection is done, and in some cases after the delayed test results come out.

4.2 Patient Safety and Convenience

Nasopharyngeal swab is not without complications as it is a blind procedure. Anatomic variations and operators' knowledge of anatomy and skill may affect patient experience. Prevalence of complication like bleeding has been reported to be 1 in 20 in one trial [17]. Rare serious complication of cerebrospinal fluid leak after a nasopharyngeal swab in a patient with undiagnosed skull base defect has been reported [18]. This rare complication was widely reported in Philippine media that the Department of Health (DOH) found it necessary to allay the fear associated with NP swab [19]. It is in this situation that a publicly acceptable alternative is needed to regain the momentum of adequate testing.

Optimal sampling to prevent false-negative results depends highly on the technique of the healthcare personnel involved, as well as the patients' reaction to the experience. To our knowledge, no study has established the extent of variability related to these aspects of sample collection. Multiple investigations have also pointed out that not all NP/OP (oropharyngeal) swabs, from patients confirmed to have COVID-19 through other samples, turn out positive, implying a significant risk of generating false-negative results. In our study, we documented 7 subjects with SARS-CoV-2-positive saliva, despite the absence of the virus in their NP swab.

4.3 Minimizing Quarantine

The psychological impact of the COVID-19 pandemic in the Philippines has been documented. Specifically, being quarantined by health authorities was found to be associated with moderate to severe adverse psychological impact as well as depression, anxiety and stress [20]. It is therefore mandatory for policy-makers to ensure that positive qRT-PCR results accurately identify the disease at its transmissible stage and does not result in unnecessary isolation.

A recent report revealed that more than 50% of test results that were interpreted as positive detected genetic material at cycle threshold of

mid to upper 30 [16,21]. A study has also established that patients with CT values greater than 34 are no longer infectious as there was no active SARS-CoV-2 isolated/cultured from these individuals [22]. These findings have caused the US CDC to examine the use of cycle threshold measures for policy decisions. This is important since the US FDA claimed it does not specify the cycle threshold ranges used to determine positive results, and that "commercial manufacturers and laboratories set their own" [21].

When we compared CT value of SARS-CoV-2-positive samples among our NP swab- and saliva-positive subjects, we observed that ORF1ab was detected at mean CT of 29.99 in saliva while it was detected at mean CT of 33.76 in NP specimen for a mean difference of -3.77 (-6.62, -0.91). Given the yet unclear meaning of CT values in terms of disease transmissibility, it may be worthwhile to consider a repeat testing of patients who have high CT value during a test to lessen mis-identification of people as infective long after the period has passed. This is crucial for the patients' mental health status as well as the economic cost of being unnecessarily quarantined. Saliva testing, being a convenient, easy to perform and relatively safer method, presents itself as a viable alternative to NP swab.

4.4 Minimizing Risk, Cost, and Waste

The validated technique offers several benefits including: 1) less PPE used in individualized NP swabbing; 2) plain sterilized vials will be used; and 3) cold chain transport method will no longer be required. The streamlined method of directly heating the sample at 95^{o}C upon receipt in the lab fulfills 3 important things: 1) it protects the technicians from possible exposure to the virus by not opening the sample vial prior to heat inactivation step; 2) heat deactivates all the proteolytic enzymes present in saliva that may potentially degrade the viral RNA; and 3) heat degrades the viral capsid thus releasing the viral RNA in solution. The addition of the non-ionic detergent Tween-20 enhances viral RNA release and replaces the expensive and oftentimes limiting (both in time and supply chain) process of RNA extraction [9].

4.5 Improved Testing Coverage

Mass or universal testing to detect SARS-CoV-2 has been raised as a means to "flatten the curve" of the COVID-19 pandemic. However, several issues prevent its application as a public health policy [23]. It requires that the diagnostic platform to have excellent accuracy parameters; require less specialized personnel; reduced technical complexity from sample collection to processing; have quick turnaround time; and be affordable even with frequent and/or use [23,24]. The aforementioned logistical advantages of saliva over NP swab as a sample for COVID-19 testing, together with the growing evidence of satisfactory diagnostic validity in which this study adds to, increases the acceptability of saliva-based qRT-PCR as a modality for this purpose [24]. Painless saliva collection is preferred over invasive NP swabbing by asymptomatic, presymptomatic or mildly symptomatic patients. All of these were indeed demonstrated directly in a recent study involving asymptomatic subjects recruited through contact tracing efforts and airport quarantine protocols in Japan. The difference in test accuracy between the two populations provides insights on the effects of over-all test positivity on the positive predictive value of test results. However, as in many other studies comparing NP swab against saliva, the authors admit that NP swab is not a "gold standard" in the absence of data on clinical correlations and longitudinal follow-up [25]. While this setup obviously remains far from the ideal scenario of rapid and accurate point-of-care testing, it will definitely enhance the prospects of countries and localities, especially those with meager resources, in achieving recommended metrics for testing [23].

5. CONCLUSION

The ease of saliva collection, processing and testing for SARS-CoV-2 using the Sansure Novel Coronavirus Nucleic Acid Diagnostic Kit provides a viable alternative to the conventional nasopharyngeal swab RT-PCR test in the diagnosis of COVID-19.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the Philippine Red Cross and the University of the Philippines as part of their humanitarian response to the COVID-19 pandemic.

CONSENT AND ETHICAL APPROVAL

The study has been duly approved by the University of the Philippines Manila Review Ethics Board under protocol number 2020-599-01 and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All subjects signed an informed consent form.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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