

## Spit or Swab? The Diagnostic Accuracy of Saliva-based Testing as a SARS-CoV-2 Surveillance Tool

Raymundo Lo, Farrah Kristine Santiago, Grig Misiona, Melani Sionzon, Danielle Anne Gonong, Jill Jaime, Jan Roman Ayco, Aquiles Joseph Lira, Erik Francis Rarugal, Jon Carlo Crisostomo, Manuel Bautista, Kenneth Chrysostom Ibarra, Eunice Jane Ciriaco, Arielle Trish Zamora, Kimberly Abundo, Emmylou Tarnate, Jonathan Nario, Jan Joseph Hernandez, Kathleen Mae Montes, Anabella Recio

*Philippine Children's Medical Center, Quezon City, Philippines*

### ABSTRACT

**Background.** Nasopharyngeal swab/oropharyngeal swab (NPS/OPS) qRT-PCR is the gold standard for detecting SARS-CoV-2. However, it has its own limitations including cost and invasiveness. As an alternative, individual qRT-PCR testing of saliva samples was validated and shown to be comparable in sensitivity and specificity with NP-OP qRT-PCR. To further maximize its utility, the researchers wish to explore antigen and pooled testing methods.

**Objective.** The study aimed to evaluate the diagnostic accuracy of detecting SARS-CoV-2 infection using saliva-based pooled qRT-PCR and rapid antigen test compared with individual saliva qRT-PCR.

**Methodology.** In this retrospective cross-sectional study, saliva specimen from individuals aged 18 years old and above from the outpatient specimen collection station at the Philippine Children's Medical Center were tested individually using qRT-PCR (Mag-bind RNA Extraction Kit/MACURA, Allsheng Extraction Machine, Sansure PCR kit, and MA-600 Sansure Biotech). Non-probability convenience sampling was utilized. Based on the individual results, pools of five (5) individual specimens, which includes one (1) positive sample were tested with qRT-PCR for sensitivity. DNK-2150-1S Dynamiker SARS-CoV-2 Ag Rapid Test (Dynamiker Biotechnology Co., Ltd., Tianjin, China) was also used to test individual saliva specimens.

**Results.** Out of 196 individual saliva specimens, 73 were detected to have SARS-COV-2 by qRT-PCR, while the remaining 123 were negative. Compared with the individual saliva qRT-PCR, rapid antigen tests done showed sensitivity of 46.58% (95% CI 35.13%, 58.02%), specificity of 86.18% (95% CI 80.08%, 92.28%), positive and negative predictive value of 66.67% (95% CI 53.71%, 79.60%) and 73.10% (95% CI 65.89%, 80.32%) respectively. Based on the results of individual saliva-based qRT-PCR, 62 pools were tested and showed sensitivity of 98.39% (95% CI 91.34%, 99.96%).

**Conclusion and Recommendation.** Pooled saliva-based testing for SARS-CoV-2 is comparable with individual saliva-based rapid antigen testing. The use of rapid antigen testing is less sensitive and less specific compared with qRT-PCR consistent with prior reports. Additional studies are recommended to determine optimal conditions for testing.

*Key words: qRT-PCR, rapid antigen test, pooling, SARS-CoV-2, COVID-19*

ISSN 2507-8364 (Online)

Printed in the Philippines.

Copyright© 2022 by the PJP.

Received: 19 August 2022.

Accepted: 30 September 2022.

Published online first: 18 November 2022.

<https://doi.org/10.21141/PJP.2022.14>

*Corresponding author: Raymundo W. Lo, MD*

*E-mail: raychrislo@gmail.com*

*ORCID: <https://orcid.org/0000-0002-6194-430X>*

### INTRODUCTION

In October 2020, the Philippines entered the top 20 countries with the highest number of COVID-19 cases in the world.<sup>1</sup> Due to the economic harm that this pandemic caused the country, Filipinos are obliged to enter the “new normal” despite the threat of COVID-19 infection. As more Filipinos return to work, demand for a less invasive, more efficient, timely and affordable means of testing for the SARS-CoV-2 virus is at its pinnacle. At present, qRT-PCR of nasopharyngeal (NP) and/or oropharyngeal (OP) specimens is the accepted standard for diagnosis of SARS-CoV-2. This method of testing albeit specific and sensitive incurs high costs limiting the method's availability to the public. The intensive need for trained swabbers and the use of costly materials such as NP and OP swabs, viral transport media, and personal protective equipment increased the cost of these tests. Hence, only a



few sectors of the population can afford standard qRT-PCR SARS-CoV-2 testing.

On August 17, 2020, the Philippine Society of Pathologists Inc. (PSP) published a statement that pooled testing strategies of NP/OP samples can be used to enable mass testing at a lower cost.<sup>2</sup> These strategies allowed asymptomatic individuals in a low positivity rate population to be tested, to be diagnosed faster, and to be reintegrated back to work at reduced costs. Despite pooling strategies, several areas must be improved to adapt to the pandemic. One would be on the issue of safety, as NP and OP swabbing are invasive procedures which may be harmful to patients when done by poorly-trained personnel. Though most patients experience mild discomfort, it is, in general, not a pleasant experience which causes fear and hesitancy in getting the test. Another area that may be improved is the use of disposable materials such as swabs, viral transport media and personal protective equipment (PPE) which not only incur higher costs but also burden the environment with pathologic wastes.

A possible answer to these issues may lie in the utilization of saliva. Saliva qRT-PCR has been reported to have diagnostic sensitivity that ranges from 69.2% to 100.0% in detecting COVID-19.<sup>3</sup> These numbers are better than the sensitivities found in qRT-PCR of NP and OP specimens which range from 63.0% to 73.0% and 32.0% to 61.0% respectively.<sup>3</sup> However there are few studies done on saliva and its potential in the field of COVID-19 diagnosis.

On April 6, 2021, the Department of Health (DOH) issued Department Memorandum No. 2021-0161 which established the guidelines for saliva-based RT-PCR and allowed COVID-19 laboratories certified by the Research Institute for Tropical Medicine (RITM) to perform saliva-based RT-PCR.<sup>4</sup> In compliance with the DOH memorandum, a validation study comparing NP/OP and saliva samples for qRT-PCR was done prior to the commencement of this study. The validation study yielded a sensitivity of 96.77% (CI: 96.58%-96.97%) and specificity of 100% (CI: 100%). The result of the study was submitted to the RITM and the PCMC's COVID laboratory was authorized to perform saliva-based qRT-PCR testing on September 15, 2021(Appendix 3 and 4).

The study aims to answer two questions: (1) How does saliva-based SARS-CoV-2 rapid antigen test and pooled saliva qRT-PCR compare to individual saliva qRT-PCR? (2) What is the diagnostic utility of saliva-based SARS-CoV-2 rapid antigen test and pooled saliva qRT-PCR?

### Significance of the study

Utilization of saliva instead of NP and OP specimens will support patient safety and decrease patient anxiety towards an invasive procedure. More so, validation of pooled saliva qRT-PCR and SARS-CoV-2 rapid antigen testing will decrease the cost of testing by eliminating the need for swabs, viral transport media, and minimize use of PPE as well as hasten the rate of testing by simplifying the method of collection. In addition, pooled saliva-based qRT-PCR when proven to have acceptable sensitivity will allow more individuals to be tested due to the ease of specimen collection and lower cost of testing.

As we ease into a “new normal” transitioning from a pandemic to an endemic phase, there may still be outbreaks that can lead to the rise of new variants if not controlled. However, we predict that many COVID-19 testing laboratories will either shut down or transition to other targets for testing due to the low demand for SARS-CoV-2 testing. Likewise, the teams of trained swabbers consisting mainly of nurses and medical technologists will be demobilized and assigned elsewhere for more productive work. Thus, there may be a scenario where there is an urgent need to test a specific community experiencing an outbreak but the laboratory resources may not be available anymore.

Once saliva pooled testing is found to be a safe, effective and accurate method, it will serve to bridge the gap between the transition phases. Swabbers need not be called back which will disrupt their newly assigned duties and the few remaining laboratories can cope with the volume of testing required. Those who are not symptomatic nor close contacts will just be asked to self-collect saliva samples which will be pooled-tested.

This study focuses on the pooled testing sensitivity since biomarker tests have less than perfect sensitivity, and this is further reduced by the dilution of the specimens to create pools. This is also the most important parameter when pooled testing is applied, since a positive pool will undergo individual testing. Because of this, specificity, positive predictive value and negative predictive value will not be tested.

This study also uses the individual saliva-based testing as the index test as it has been shown that saliva is comparable with NP/OP specimens in sensitivity, using a less invasive method of specimen collection.

### Review of related literature

The gold standard for COVID-19 is still RT-PCR of nasopharyngeal swab. However, this mode of testing has some disadvantages. It is time consuming, entails a certain degree of patient discomfort, requires a health care professional to do the invasive, aerosol-generating procedure, as well as swab collection kits. The above factors may hinder this method of specimen collection from being the regularly done, affordable and convenient test that is needed for COVID-19 diagnosis and monitoring.<sup>5</sup>

Mina et al., explained that due to the long duration of RNA-Positive tail, most infected people are identified as positive after the infectious period has passed and are subjected to unwarranted quarantine measures. Rapid lateral-flow antigens tests and other antigen tests have analytic limits of detection 100 to 1000 times higher than that of RT-PCR. This coincides well with the exponential growth phase of the SARS-CoV2 virus and its most infectious period. Antigen testing serves many purposes: identifying infectious individuals and reducing isolation periods for previously infected persons who had positive Rt-PCR tests thereby allowing more economic activity and downgrading of quarantine regimens without the threat of an outbreak. The key is to perform more frequent testing with the lower sensitivity assays that are cheap, fast and easy to perform in community wide surveillance regimens for SARS-CoV-2.<sup>6</sup>

Saliva is an attractive sample for detection of SARS-CoV-2 due to the documented process of oral shedding of the virus and the rapid and convenient nature of saliva collection which minimizes the need for direct contact between the patient and health care provider with notable savings of PPE. Many reports have shown that saliva contains higher viral loads than in NP swabs which is highest during the first week of infection and consequently the most infectious period of the disease. These reports also suggest that the viral load found in saliva is a good reflection of the transmission potential of a COVID-19 patient. NP qRT-PCR on the other hand remains positive long after the infectious period of the disease.<sup>7</sup>

The median viral load in posterior oropharyngeal saliva and other respiratory specimens at presentation is 5.2 copies per milliliter.<sup>8</sup> The viral load in saliva peaks during the first week of symptoms and subsequently declines in the next few days.

### Use of saliva as qRT-PCR sample

The study of Wyllie et al., demonstrated that saliva samples had comparable sensitivity to nasopharyngeal samples.<sup>9</sup> Their study population consisted of 70 SARS-CoV-2 confirmed cases who were diagnosed 1 to 5 days prior via NP qRT-PCR. They collected secondary NP samples and saliva samples for molecular testing which revealed a positivity rate of 71% and 81% respectively.<sup>9</sup>

The potential of saliva for SARS-CoV-2 testing was cemented by Watkins et. al 2020, when their study on pooled saliva-based qRT-PCR showed promising diagnostic utility. The potential of pooling saliva samples by 5, 10 and 20 prior to qRT-PCR was evaluated and showed sensitivities of 92.52%, 88.89% and 85.19%, respectively.<sup>10</sup> When the prevalence rate exceeds 3%, pools of 5 showed greatest resource savings.

SARS-CoV-2 rapid antigen test is a faster and simpler diagnostic method which enables result reporting in less than 30 minutes.<sup>3</sup> Though it is acknowledged that antigen tests exhibit lesser sensitivity than RT-PCR, the cases that were not detected by the antigen test but were detected by RT-PCR had higher CT values and in about half of the discordant samples that were cultured, none resulted in cytopathic effect indicating absence of viable virus.<sup>3</sup> Subsequent RT-PCR testing of culture supernatants were also negative. Thus, even if antigen tests are not as sensitive as RT-PCR, they still pick up most of the cases that are actually infectious.

The study of Nagura-Ikeda et al., demonstrated the presence of SARS-CoV-2 viral RNA via qRT-PCR in saliva samples of more than half of the asymptomatic individuals included in their study which detected ORF-gene and E-gene in 53.3% and 60.0% of cases respectively.<sup>11</sup> CT values for E-gene were also noted to be significantly lower in positive cases ( $25.4 \pm 1.8$ ) than in negative cases ( $30.8 \pm 2.7$ ). The rapid antigen test (Espline SARS-CoV-2; Fuji Rebio Inc., Tokyo, Japan), however, detected only 11% of the submitted saliva specimens.

Barat et al., evaluated the sensitivity of pooled saliva testing compared to individual nasopharyngeal testing via three

RT-PCR platforms. They noted an average signal loss of 2 to 3 cycle thresholds on pooled saliva specimens with an average sensitivity of 92.6% on all three platforms.<sup>12</sup> The study also noted that the sensitivity of pooled saliva testing increased when evaluating patients with moderate to high viral loads (cycle threshold  $\pm 34$ ).<sup>12</sup>

Rainey et al., adapted the pooled saliva protocol of Ranoa et al., and while adapting the protocol they noted that some individual samples contained an unidentified PCR inhibitor which blocked SARS-CoV-2 gene amplification as well as the amplification of the control RNA.<sup>13</sup> The same individual samples can also block amplification when they are added to pools leading to RT-PCR test failure.<sup>13</sup>

### Use of saliva as rapid antigen test sample

DNK-2105-1SDynamikerSARS-CoV-2(DynamikerBiotechnology (Tianjin) Co., Ltd.) is a lateral flow assay that utilizes SARS-CoV-2 Nucleocapsid protein (N protein) as the target analyte.<sup>14,15</sup> Based on manufacturer's data, it facilitates rapid and dependable testing with a test to result turnaround time of 10 minutes and a 92.98% and 99.07% sensitivity and specificity, respectively.<sup>15</sup> This high specificity is further supported by a multi-center study of Diao et. al wherein they found that all immunochromatographic assays that tested positive and negative for the N protein after three days of fever were in concordance with RT-PCR results.<sup>16</sup>

De Marinis et al., evaluated the sensitivity of four saliva rapid antigen testing platforms (Flowflex, PCL, Pabio and Joinstar) compared to nasopharyngeal and saliva RT-PCR. They noted that for RT-PCR sample cycle threshold values lower than 25, the sensitivity of saliva rapid antigen tests ranged from 43% to 64% while for cycle thresholds higher than 29, the sensitivity ranged from 9% to 19%.<sup>17</sup> The sensitivity of saliva rapid antigen tests also increased when the patient's symptom duration was 5 days or less (average 39.7%) and 10 days or less (average 47.0%).<sup>17</sup> However, for patients with symptom duration longer than 10 days the positive detection rate is 11%.<sup>17</sup> The lower rate of positive case detection of saliva rapid antigen testing was attributed to several factors such as subjects being recruited in the latter part of their COVID illness, some positive PCR tests may not reflect the presence of viable virus and freeze and thaw effect on saliva samples used in the rapid antigen testing.<sup>17</sup>

## OBJECTIVES

This study aimed to evaluate the diagnostic accuracy of saliva as the specimen of choice for detecting SARS-CoV-2 infection and specifically answered the following research objectives: (1) Evaluate the sensitivity of pooled saliva-based qRT-PCR compared with individual saliva qRT-PCR; (2) Evaluate the sensitivity, specificity, positive predictive value and negative predictive value of saliva-based rapid antigen test compared with individual saliva qRT-PCR.

## METHODOLOGY

### Design

This is a retrospective cross-sectional diagnostic accuracy study that determines the diagnostic utility of saliva-based testing using pooled qRT-PCR and Rapid Antigen Test compared with individual saliva qRT-PCR (Figure 1).

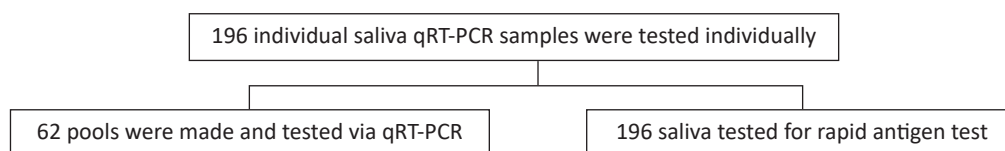


Figure 1. Study workflow diagram.

### Subjects and sample size

Included in this study were 196 saliva specimens taken from individuals aged 18 years old and above from the outpatient specimen collection station at Philippine Children's Medical Center (PCMC). Based on the results of the individual qRT-PCR saliva specimens, non-probability (convenience) sampling was done to determine the pools. Exclusion criteria includes indeterminate saliva qRT-PCR result and contaminated specimen.

The computed sample size for both pooled testing and rapid antigen testing is 73 based on a paper by Hajian-Tilaki which estimates the sample size in diagnostic studies and using the following assumptions: 95% sensitivity, marginal error of 10%, and 25% prevalence based on the Philippine SARS-CoV-2 positivity rate.<sup>18,19</sup>

### Collection and storage of saliva samples

Collection of saliva specimens from an individual is based on the PCMC's standard procedure for saliva-based SARS-CoV-2 qRT-PCR testing. The study participants included in this study were instructed not to eat, drink, brush their teeth, nor gargle for 30 minutes prior to collection. Morning saliva was preferred but not required. Sterile, screw-capped, wide mouth containers were given to the participants, and they were requested to self-collect 5.0 ml of saliva.

The collected saliva specimens were stored and transported to the laboratory at room temperature within 24 hours. Once received in the laboratory, individual saliva-based SARS-CoV-2 qRT-PCR and Rapid Antigen Testing were done immediately. The remaining saliva specimens were stored at 2-8° Celsius while waiting for the individual saliva-based SARS-CoV-2 qRT-PCR results. Based on the results of the individual saliva-based SARS-CoV-2 qRT-PCR, pools were done and tested using qRT-PCR. The remaining samples were kept for 14 days from the time of collection, then discarded using the hospital's biosafety waste disposal policy.

### Saliva processing for individual qRT-PCR

The individual saliva-based qRT-PCR was performed using 200  $\mu$ l aliquot of saliva. The samples underwent nucleic acid extraction using Mag-Bind RNA Extraction Kit/MACCURA and ALLSHENG Extraction Machine. SANSURE PCR KIT and MA-6000/Sansure Biotech were used for qRT-PCR amplification. The samples were subjected to magnetic bead extraction and placed in SANSURE master mix and template prior to qRT-PCR amplification. The rest of the procedures were performed according to the manufacturer's instructions.

### Interpretation of results for individual saliva-based qRT-PCR

All test controls were validated prior to interpretation of patient results. Tests without valid controls were interpreted

as invalid. The Cycle threshold (Ct) cutoff value was set at 38 as per SANSURE kit specification. All results with Ct values  $\leq 40$  and typical S-shape amplification curves as assessed were interpreted as SARS-CoV-2 positive. Results with Ct values  $\geq 40$  were interpreted as SARS-CoV-2 negative.

### Rapid antigen testing of saliva specimens

After an adequate aliquot was obtained for individual saliva-based qRT-PCR test, the saliva specimen was tested within an hour of collection using DNK-2105-1S Dynamiker SARS-CoV-2 Ag Rapid Test (Saliva) using the following instructions:

1. 1.0 ml of saliva was mixed with the rapid antigen kit buffer and placed into the extraction tube.
2. The extraction tube was recapped and mixed completely.
3. 3 drops of the solution were added to the test card.

### Interpretation of results for rapid antigen testing of saliva specimens

The result for the saliva antigen test was interpreted 10 minutes after dropping the saliva and buffer mix to the test card. The presence of two lines: one control and one test, even if faint, were considered as positive results. The presence of a control line only was read as negative. If the control line is not present, the result is invalid. Rapid antigen test kits were identified with the subject's accession number and photographed for data recording purposes and were kept in a secured data file.

### Saliva specimen processing for pooled qRT-PCR

A master list for all individuals selected and the individual saliva-based qRT-PCR results was prepared. From the list, 62 individuals with detected SARS-CoV-2 using saliva-based qRT-PCR and all the saliva samples of individuals which were negative were selected for pooled qRT-PCR.

To create a 1ml pool, one (1) 200  $\mu$ l aliquot of SARS-CoV-2 qRT-PCR-detected saliva specimen was mixed with four (4) aliquots of 200 $\mu$ l SARS-CoV-2 qRT-PCR-negative saliva samples. The 1ml pool was mixed using a vortex mixer for 30 seconds. From the mixed pooled sample, 200  $\mu$ l was obtained to undergo qRT-PCR using the same procedure and interpretation as the individual saliva-based SARS-CoV-2 qRT-PCR test.

### Data processing and analysis

Data was collated and analyzed using Microsoft Excel. The mean age, percentage of male and female participants and presence of symptoms were tabulated to illustrate the characteristics of all the participants.

### Pooled saliva-based RT-PCR

The sensitivity of the pooled saliva-based RT-PCR was computed through the following formula:

$$\text{Sensitivity} = \frac{\text{number of individual samples that were detected/positive using the pooling method}}{\text{total number of individual samples that tested positive using individual RT-PCR}}$$

Comparison of the Ct values of the ORF gene and N gene between the individual positive saliva specimen and the pooled specimen were done using paired T test, however, interpretation is limited due to use of non-probability sampling (Figure 2).

**Saliva-based rapid antigen test**

Diagnostic test evaluation of the saliva-based rapid antigen test was done using a 2 x 2 table. The formula for the calculation of sensitivity, specificity, PPV and NPV were as follows:

$$\begin{aligned} \text{Sensitivity} &= \text{True Positive (TP)} / (\text{TP} + \text{False Negative (FN)}) \\ \text{Specificity} &= \text{True Negative (TN)} / (\text{TN} + \text{False Positive (FP)}) \\ \text{Positive Predictive Value (PPV)} &= \text{TP} / (\text{TP} + \text{FP}) \\ \text{Negative Predictive Value (NPV)} &= \text{TN} / (\text{TN} + \text{FN}) \end{aligned}$$

Box plot was used to illustrate the Ct values of the specimen compared with the rapid antigen test result. Mann-Whitney U test was used to analyze if there is a statistically significant difference between the Ct values of those who tested positive and those who tested negative for the rapid antigen test. Interpretation is limited, however, due to use of non-probability sampling.

**ETHICAL CONSIDERATIONS**

The research was developed in compliance with the Data Privacy Act (2012) and National Ethical Guidelines for Health and Health-Related Research. Informed consents were requested from the participants to allow the use of stored saliva samples for rapid antigen and pooled testing prior to the commencement of the study.

To ensure the protection of the study participants, each data was treated with utmost confidentiality. No personal identifiable information was included and each data set was coded with a control number. Only the investigators were allowed to retrieve and have access to the data.

This study was funded by the Philippine Center for Entrepreneurship Foundation. The individual and pooled saliva-based SARS-CoV-2 qRT-PCR tests and rapid antigen tests were free of charge to the subjects. Official results

for the individual saliva-based SARS-CoV-2 qRT-PCR tests were given to the subjects. The results of the pooled saliva qRT-PCR and saliva rapid antigen testing were not disclosed to the subjects.

Approval was obtained from the Institutional Review Board prior to the commencement of the study (PCMC-IREC 2021-004).

**RESULTS**

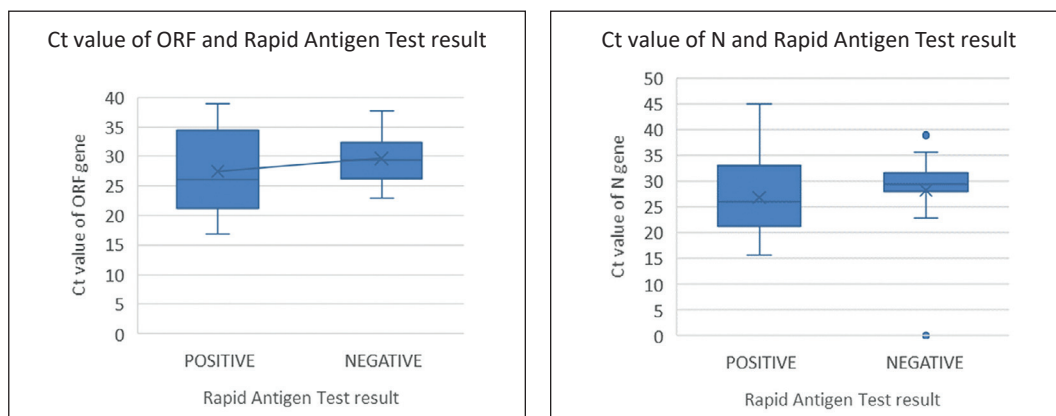
One hundred ninety-six (196) freshly collected saliva were obtained for the study. The characteristics of the study participants are summarized in Table 1. Of the saliva specimens tested, seventy-three (73) were detected to have SARS-CoV-2, while the remaining one hundred twenty-three (123) were not detected to have SARS-CoV-2 by qRT-PCR.

Table 1 describes the characteristic of participants. Among the participants who were detected to have SARS-CoV-2 by saliva qRT-PCR, the mean age is 38 years old. 37% is composed of female participants and 63% are male. 70% reported to have symptoms such as fever, cough, colds and sore throat. Among participants who were not detected to have SARS-CoV-2, 33% are females, 67% are males while only 3% presented with symptoms.

The diagnostic accuracy of the saliva rapid antigen test compared with saliva qRT-PCR as the standard is illustrated in Table 2. Overall, the saliva rapid antigen test has a sensitivity of 46.58% (95% CI 35.13%, 58.02%), specificity of 86.18% (95% CI 80.08%, 92.28%), positive predictive

**Table 1.** Characteristics of participants based on SARS-CoV-2 detection by saliva qRT-PCR

	SARS-CoV-2 Detected (n=73)	SARS-CoV-2 Not Detected (n=123)
Mean Age (years)	38	35
% Female participants	37% (27)	33% (41)
% Male participants	63% (46)	67% (83)
% Symptomatic participants	70% (53)	3% (4)



**Figure 2.** Distribution of the Ct values of the ORF gene and N gene compared with Rapid Antigen Test result.

**Table 2.** Diagnostic accuracy of saliva rapid antigen test compared with saliva qRT-PCR

Rapid Antigen test	Individual Saliva-based qRT-PCR		Total
	Positive	Negative	
Positive	34	17	51
Negative	39	106	145
Total	73	123	196
Sensitivity: 46.58% (95% CI 35.13%, 58.02%)			
Specificity: 86.18% (95% CI 80.08%, 92.28%)			
Positive Predictive Value: 66.68% (95% CI 53.71%, 79.60%)			
Negative Predictive Value: 73.10% (95% CI 65.89%, 80.32%)			

value of 66.67% (95% CI 53.71%, 79.60%), and negative predictive value of 73.10% (95% CI 65.89%, 80.32%).

The mean Ct values of the ORF gene were lower in those who tested positive (27.45) than those who tested negative (29.64) in the rapid antigen test. This is also true for the Ct values of the N gene, in which rapid antigen positive saliva has a lower mean Ct value (26.75) compared with rapid antigen negative saliva specimens (28.23). However, analysis using Mann-Whitney U test showed no statistically significant difference between the rapid antigen test results and the Ct values of the ORF gene ( $p=0.09296$ ) or the N gene ( $p=0.09692$ ). Interpretation of this statistical test may be limited due to use of non-probability sampling.

Among the 73 saliva specimens detected to have SARS-CoV-2, sixty-two (62) were included in the 1:5 dilution for the pooling part of the study since the other eleven (11) saliva specimens were fully consumed. Of the 62 pooled samples, sixty-one (61) were detected to have SARS-CoV-2 by saliva qRT-PCR. Based on this, the overall sensitivity of the 1:5 dilution of pools of saliva specimen is 98.39% (95% CI 91.34%, 99.96%). The ORF Ct value of the individual saliva specimen in the pool not detected is 35.37. From the day of collection up to the pooled saliva qRT-PCR, the number of days of interval ranges from 0 to 2 days, with the specimen stored at 2-8°C. Table 3 details the Ct values of the original individual specimen compared with the pooled saliva samples and the number of days of interval between the specimen collection and pooling.

Using the paired t-test of two sample for means, there was no significant difference ( $p>0.05$ ) noted between the Ct values of the ORF and N gene of the individual saliva specimen compared with the 1:5 dilution of pooled saliva specimen (Table 4). Interpretation of the result is limited by the use of non-probability sampling

## DISCUSSION

At present the use of nasopharyngeal and oropharyngeal (NP/OP) swab specimens for qRT-PCR is the standard for testing. Unfortunately, the use of NP/OP may hinder mass testing since collection will cause patient discomfort and pain and delays due to the involved collection procedure. Furthermore, the use of biosafety barriers and need for additional personnel is costly. These limitations in NP/OP sample collection prompted researchers to explore alternative samples for SARS-CoV-2 testing such as saliva. In the study of Oguri et al., antigen titers and Ct values of saliva samples remain unchanged after 72-hour storage in ambient temperature while Nasopharyngeal and oropharyngeal (NP/OP) swabs specimen displayed

decreased antigen titers when stored at the same temperature and duration. However, they noted that the Ct values in NP/OP swabs remain unchanged.<sup>20</sup>

In a meta-analysis of 51 studies done by Buban et al., it was shown that pooled researches of saliva samples have sensitivity of 84% and specificity of 96%.<sup>21</sup> These data were consistent with the meta-analysis done by Butler-Laporte et al., which showed that saliva NAAT has pooled sensitivity of 83.3% and specificity of 99.2%.<sup>22</sup>

On March 31, 2021, the DOH issued a memorandum on the interim guidelines for the conduct of saliva-based RT-PCR for the detection of SARS-CoV-2. In this memorandum, the use of saliva specimens is allowed for nucleic acid amplification (NAAT) based test, however, strict regulatory requirements, in-house verification and implementation arrangements were developed to ensure that laboratories that will offer saliva-based RT-PCR will perform the procedure correctly and accurately.<sup>4</sup> In relation to this, the Health Technology Assessment Council (HTAC) of the DOH also recommends the use of saliva specimen for RT-PCR as an alternative, provided that the healthcare workers assigned shall provide instructions and directly observe patients on the proper collection of saliva specimens.<sup>23</sup> The recommendation also suggests that the cost of using saliva specimens for public institutions should be significantly less than the government price cap for RT-PCR using NPS/OPS. The same DOH memorandum however does not allow the use of saliva specimens for antigen or antibody tests.<sup>4</sup>

### Saliva rapid antigen tests

As of January 17, 2022, nine (9) saliva-based antigen test kits including the kit used in this study, Dynamiker SARS-CoV-2 Ag Rapid Test is on Stage 2 (Pre-evaluation Stage) of the evaluation being done by the RITM.<sup>24</sup> The findings of this study showed a higher sensitivity for the Dynamiker SARS-CoV-2 Antigen Test compared with the kit used by Nagura et al., in their 2020 study which yielded only 11% sensitivity.<sup>11</sup> The authors attributed the low sensitivity to probable incompatibility of the saliva specimen to the test kits and the freeze-thaw and centrifugation process in their methodology.<sup>11</sup> The sensitivity of the rapid antigen test in this study is closer to the findings by Seitz et al., using COVID-19 Antigen Test Cassette (hypersensitive colloidal gold, Xiamen Zhongsheng Langjie Biotechnology Co., Ltd).<sup>25</sup> Seitz et al., documented an overall sensitivity of 44.4% and concluded that saliva antigen test is not a reliable substitute to RT-PCR.<sup>25</sup>

The results of this study also showed a specificity of 86.16% and positive predictive value of 66.68% for the saliva antigen test. The United States Food and Drug Administration (USFDA) published a report on the Potential for False Positive Results with Antigen Tests for Rapid Detection of SARS-CoV-2, and recommended following the manufacturer's instructions on the appropriate time to read the test result and minimizing the risk for cross-contamination.<sup>26</sup> A study by Patriquin et al., showed that direct sample testing (without the kit buffer) resulted in false positive signals in rapid antigen test kits, and the likely explanation is nonspecific interactions between the SARS-CoV-2 specific conjugated and capture antibodies as pH-induced conformational changes under

**Table 3.** Ct values of the individual saliva specimen compared with the 1:5 saliva pools and the number of days of interval between the specimen collection and pooling

Pool Accession Number	SARS-CoV-2 gene	Ct Values of individual SARS-CoV-2 detected specimen	Ct Values of Pools (1:5 dilution)	Result	Number of days from collection to pooling	Pool Accession Number	SARS-CoV-2 gene	Ct Values of individual SARS-CoV-2 detected specimen	Ct Values of Pools (1:5 dilution)	Result	Number of days from collection to pooling
1102CS-P5A	ORF	30.63	29.43	DETECTED	0	0111RS-P5AF	ORF	23.36	26.44	DETECTED	1
	N	29.10	29.06				N	21.29	23.73		
1102CS-P5B	ORF	31.90	28.17	DETECTED	0	0111RS-P5AG	ORF	38.88	37.17	DETECTED	1
	N	30.59	27.49				N	34.08	36.16		
1105RS-P5C	ORF	22.72	26.72	DETECTED	0	0111RS-P5AH	ORF	22.81	27.88	DETECTED	1
	N	22.45	29.44				N	21.44	27.17		
1105RS-P5D	ORF	23.97	24.54	DETECTED	0	0111RS-P5AI	ORF	17.51	18.69	DETECTED	1
	N	23.08	27.32				N	15.74	17.31		
1105RS-P5E	ORF	29.42	31.26	DETECTED	0	0111RS-P5AJ	ORF	27.22	26.24	DETECTED	1
	N	29.19	35.22				N	26.84	24.55		
0108RS-P5F	ORF	22.90	23.02	DETECTED	0	0111RS-P5AK	ORF	27.59	33.02	DETECTED	1
	N	24.86	25.24				N	26.15	31.91		
0108RS-P5G	ORF	26.26	27.00	DETECTED	0	0111RS-P5AL	ORF	29.40	33.43	DETECTED	1
	N	29.34	29.77				N	28.05	32.62		
0109RS-P5H	ORF	24.02	26.81	DETECTED	1	0111RS-P5AM	ORF	33.01	34.73	DETECTED	1
	N	27.59	25.33				N	31.34	34.06		
0109RS-P5I	ORF	27.38	29.11	DETECTED	1	0111RS-P5AN	ORF	31.13	32.95	DETECTED	1
	N	30.77	28.13				N	28.21	31.25		
0109RS-P5J	ORF	32.27	35.89	DETECTED	0	0111RS-P5AO	ORF	34.62	24.34	DETECTED	1
	N	35.31	34.11				N	31.53	23.95		
0109RS-P5K	ORF	26.90	33.59	DETECTED	0	0111RS-P5AP	ORF	—	39.17	DETECTED	1
	N	30.04	31.74				N	33.80	36.48		
0109RS-P5L	ORF	25.23	29.04	DETECTED	0	0111RS-P5AQ	ORF	34.10	37.83	DETECTED	1
	N	28.75	28.28				N	31.77	36.37		
0109RS-P5M	ORF	24.31	28.01	DETECTED	0	0111RS-P5AR	ORF	24.30	29.09	DETECTED	1
	N	28.53	27.25				N	22.88	27.02		
0109RS-P5N	ORF	16.84	18.54	DETECTED	0	0111RS-P5AS	ORF	37.14	31.63	DETECTED	1
	N	20.16	16.97				N	34.17	31.18		
0109RS-P5O	ORF	27.13	—	DETECTED	0	0111RS-P5AT	ORF	20.82	22.33	DETECTED	1
	N	30.36	33.82				N	18.13	20.72		
0109RS-P5P	ORF	29.33	29.62	DETECTED	0	0111RS-P5AU	ORF	31.98	30.10	DETECTED	1
	N	31.45	29.25				N	28.27	28.62		
0109RS-P5Q	ORF	24.31	25.63	DETECTED	0	0111RS-P5AV	ORF	27.23	26.60	DETECTED	1
	N	26.77	24.50				N	24.38	25.16		
0109RS-P5R	ORF	31.01	30.79	DETECTED	0	0111RS-P5AW	ORF	34.40	39.48	DETECTED	1
	N	33.73	29.93				N	33.98	35.73		
0109RS-P5S	ORF	28.83	33.43	DETECTED	0	0111RS-P5AX	ORF	21.08	26.04	DETECTED	1
	N	33.55	31.77				N	20.32	21.02		
0109RS-P5T	ORF	21.52	23.29	DETECTED	0	0111RS-P5AY	ORF	36.76	22.01	DETECTED	1
	N	23.27	21.37				N	35.58	20.84		
0109RS-P5U	ORF	35.68	38.05	DETECTED	0	0111RS-P5AZ	ORF	34.06	40.47	DETECTED	2
	N	38.87	37.06				N	33.27	36.55		
0109RS-P5V	ORF	24.46	31.27	DETECTED	0	0112RS-P5BA	ORF	30.92	29.31	DETECTED	1
	N	28.67	29.74				N	31.67	27.24		
0109RS-P5W	ORF	25.71	40.97	DETECTED	0	0112RS-P5BB	ORF	30.41	30.55	DETECTED	1
	N	28.23	36.72				N	33.24	28.94		
0109RS-P5X	ORF	35.37	—	NOT DETECTED	0	0112RS-P5BC	ORF	34.03	26.08	DETECTED	1
	N	—	—				N	31.42	24.34		
0111RS-P5Y	ORF	21.40	24.57	DETECTED	1	0112RS-P5BD	ORF	19.81	24.62	DETECTED	1
	N	19.06	22.54				N	21.18	19.74		
0111RS-P5Z	ORF	20.80	30.52	DETECTED	1	0112RS-P5BE	ORF	21.01	25.01	DETECTED	1
	N	19.28	24.37				N	24.46	23.41		
0111RS-P5AA	ORF	37.36	—	DETECTED	1	0112RS-P5BF	ORF	33.25	—	DETECTED	1
	N	34.01	39.32				N	39.21	32.87		
0111RS-P5AB	ORF	36.92	34.16	DETECTED	1	0112RS-P5BG	ORF	26.03	26.81	DETECTED	1
	N	33.55	32.07				N	24.40	22.30		
0111RS-P5AC	ORF	35.99	33.44	DETECTED	1	0112RS-P5BH	ORF	35.05	25.45	DETECTED	1
	N	33.16	32.69				N	29.40	21.76		
0111RS-P5AD	ORF	23.35	26.70	DETECTED	1	0112RS-P5BI	ORF	27.82	32.53	DETECTED	1
	N	22.79	26.06				N	30.88	27.83		
0111RS-P5AE	ORF	24.54	28.51	DETECTED	1	0112RS-P5BJ	ORF	26.29	25.18	DETECTED	1
	N	24.19	27.48				N	27.66	23.60		

**Table 4.** Paired t-test of two sample for means of Ct values of ORF and N gene between individual and pooled saliva specimen (interpretation is limited due to use of non-probability sampling)

SARS-CoV-2 gene	Individual Sample		Pooled Sample		T statistic	df	P (Tst) two-tail
	Mean	Variance	Mean	Variance			
ORF gene	27.7496	43.1424	27.5364	78.3749	0.1523	61	0.8794
N gene	27.8297	40.4573	27.8785	40.8710	-0.0930	61	0.9262

conditions promoting artifact formation.<sup>22</sup> The authors who performed the rapid antigen test strictly followed the manufacturer's instruction on the addition of the buffer, appropriate time interval from application to test kit to reading of the results, and minimized cross-contamination by using clean single-use pipettes.

### Pooled saliva qRT-PCR

In order to provide high-volume screening using saliva, this study looked into the utilization of pooling strategies in saliva samples. Pooling using NP/OP samples was previously demonstrated to be cost-effective and efficient at PCMC with pools of five (5) having the highest sensitivity.<sup>12,27</sup> In this study, pooling of saliva was done using five (5) saliva samples comprising of one (1) aliquot of SARS-CoV-2 detected saliva mixed with four (4) aliquots of SARS-CoV-2 negative saliva. This yielded a sensitivity of 98.39%, and there were no statistical differences observed in the ORF and N-gene Ct-values of the individual and pooled saliva test results.<sup>27</sup>

A study by Barat et al., which included 449 individuals showed that the positive and negative agreement of saliva samples compared to NP swabs were 81.1% and 99.8% respectively. Pooling of the saliva samples using pools of five (5) showed sensitivities of 94%, 90%, and 94% using easyMAG/ABI 7500, Hologic Panther Fusion, and Roche Cobas 6800, respectively. An average decline in pooled Ct-value of 2 to 3 was noted in comparison with individual saliva testing.<sup>12</sup>

In a study done by Sahajpal et al., which used twenty (20) positive and negative pools consisting of five (5) samples per pool, testing done demonstrated 95% positive agreement and 100% negative agreement. The N and ORF1ab gene Ct values of pooled samples were found to be significant compared to individual saliva testing Ct values.<sup>28</sup>

A study by Esteves et al., which used 279 saliva samples for qRT-PCR yielded sensitivity, specificity, positive predictive value, and negative predictive value of 96.6%, 96.8%, 96.6% and 96.8% respectively. Furthermore, pooling strategies were done using 10-sample pools and 20-sample pools producing a sensitivity of 96.9% and 87.5% respectively.<sup>29</sup>

As shown by prior studies, the use of saliva for qRT-PCR showed high sensitivity and specificity when contrasted with NP/OP qRT-PCR. Moreover, pooled saliva testing showed no significant decline in testing sensitivity and congruence of Ct-values between individual and pooled saliva samples was observed in this study.

Despite the promising results of saliva pooling strategies, it is important to take note that in this study one positive sample was not detected using pooled testing. Prior studies also reflected that few samples were not detected using

pooling strategies and some noticed a significant difference in the Ct-value of pooled and individual saliva samples. The small error in detecting SARS-CoV-2-detected saliva samples and incongruence of Ct-values may be due to the limitation of using saliva as a specimen and/or using pooling as a diagnostic strategy.

Saliva as a specimen may differ in viscosity, hence under pipetting may be an issue due to the presence of bubbles and variation in viscosity which may lead to a false-negative result.<sup>7</sup> Moreover, an inherent limitation of pooled testing is the minute risk of not catching weak positive individuals due to sample dilution and the advance technical factors needed to produce consistent pools.<sup>12</sup>

Overall, the results show that pooled saliva testing may be a useful and economical strategy to initiate mass testing in asymptomatic and non-close contact populations such as in work settings and social gatherings. This strategy will eliminate the need for NP/OP swabs, viral transport media, and minimize usage of personal protective equipment thereby lowering the overall cost for the testing laboratory and tested individuals.

## CONCLUSION AND RECOMMENDATIONS

The importance of accurate testing for SARS-CoV-2 during the ongoing pandemic cannot be understated. Reliable diagnostic options should be widely available and commonly known to both clinicians and patients alike. In any setting, but especially the local one, the decision to use a particular testing method needs to take into account the test's sensitivity, potential risks to healthcare providers or patients, estimated costs, simplicity and convenience, and the general population. Scientific literature has demonstrated the effectiveness of using saliva to detect viral load and that it compares favorably with nasopharyngeal swab specimens. The ease with which it can be collected and subsequently tested helps to provide a solution as to how more testing and contact tracing can be done. As testing laboratories become certified to provide this option, it becomes imperative to find ways to improve the process to be more efficient and cost-effective. The data from this study clearly demonstrates that pooled saliva-based testing for SARS-CoV-2 is a reliable and accurate tool to help augment testing. However, it must be emphasized that this study is brand and method-specific and needs to be replicated by other laboratories using their own kits and methods.

For rapid antigen testing using saliva specimens the data is not as clear, though the results from this study are in line with previously published reports. Further studies are needed to determine if there are optimal conditions including particular test kits, reagents, specimen volume, and time to test that can support this applicable method for screening and diagnosis.



Pooled saliva qRT-PCR testing may be an invaluable tool in a pandemic scenario where lower test costs and accurate, faster results are necessary to embark on more widespread testing in outbreak scenarios. Pooled testing is not designed to test symptomatic individuals or their close contacts; rather it is meant to rapidly screen large numbers of people in a locality where community transmission is suspected to prevent further spread of the virus. This method has been and still is being successfully deployed in several countries like China, South Korea and some European countries. But it takes time, effort, personnel and resources to effectively swab and screen large populations that are not at high risk of infection. Using saliva as a sample for pooled testing changes the dynamics with decreased demand for all these factors thus effecting a faster response when results are released earlier.

With the increasingly relaxed restrictions necessary for promoting increased economic activity comes the risk of further outbreaks as people start to congregate in large numbers and indoors. We believe saliva pooled testing will become an important tool to suppress these outbreaks should they occur. We are by no means totally safe when a large segment of the population is still unvaccinated, necessitating control measures such as saliva pooled testing.

Even in a post-pandemic scenario, saliva pooled testing can be useful in routine surveillance for possible outbreaks. In a statement of the PSP on Strategic Testing to Manage Local Outbreaks, it states that limited testing and contact tracing will not address the rapid spread of the virus and thus, more widespread testing in a particular locality under lockdown should be done to effect control of the virus.<sup>30</sup> It proposed pooled testing as a means to mass test at a faster rate, achieve economy of scale with test savings within a certain range of positivity and still achieve regular turnaround time as individual testing. The use of saliva samples for pooled testing will further decrease costs as well as time and personnel resources if used in these situations.

Likewise, it can be a model for mass testing in future pandemics of respiratory pathogens with a significant presence in saliva. There will be no need to mobilize large numbers of swabbers who will have to be trained to perform the procedure safely and efficiently thus ensuring a faster response and may be crucial in determining the course of the pandemic in a particular country. In an archipelagic nation such as the Philippines, immediate border closures followed by mass testing in suspect populations should be sufficient in delaying if not stopping the entry and/or community transmission of a virus and allowing time for the health system to gear up and be prepared for the pandemic.

In conclusion, we have demonstrated the viability of saliva samples for pooled SARS-CoV-2 qRT-PCR testing as a means of fast, efficient and accurate determination of infection status for use in asymptomatic, low positivity rate populations. It will be very useful in both the current pandemic situation as well as in transitioning to an endemic situation. Further, it will also serve as a model for future pandemic response with respiratory pathogens with significant presence in saliva.

## STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

## AUTHOR DISCLOSURE

The authors declared no conflict of interest.

## FUNDING SOURCE

The study was sponsored by the Philippine Center for Entrepreneurship Foundation.

## REFERENCES

1. World Health Organization. Laboratory testing for coronavirus disease. Interim Guidance; 2020. <https://apps.who.int/iris/bitstream/handle/10665/331329/WHO-COVID-19-laboratory-2020.4-eng.pdf>.
2. Lo R, Barrientos A, Espiritu B, Santiago FK, et al. An Evaluation of pooling strategies for RT-qPCR testing for SARS-CoV-2 infection: a pragmatic multi-site parallel operational study. *Philipp J Pathol.* 2020;5(2):12–33. <https://doi.org/10.21141/PJP.2020.12>.
3. Cerutti F, Burdino E, Milia MG, et al. Urgent need of rapid tests for SARS CoV-2 antigen detection: evaluation of the SD-Biosensor antigen test for SARS-CoV-2. *J Clin Virol.* 2020;132:104654. PMID: 3305349. PMCID: PMC7522649. <https://doi.org/10.1016/j.jcv.2020.104654>.
4. Duque FT. Interim guidelines for the conduct of saliva-based RT-PCR testing for the detection of SARS-CoV-2; 2021. Department of Health. <https://doh.gov.ph/sites/default/files/health-update/dm2021-0161.pdf>. Accessed February 14, 2021.
5. Fogarty A, Joseph A, Shaw D. Pooled saliva samples for COVID-19 surveillance programme. *Lancet Respir Med.* 2020;8(11):1078–80. PMID: 32976755. PMCID: PMC7508511. [https://doi.org/10.1016/S2213-2600\(20\)30444-6](https://doi.org/10.1016/S2213-2600(20)30444-6).
6. Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 test sensitivity - a strategy for containment. *N Engl J Med.* 2020;383(22):e120. PMID: 32997903. <https://doi.org/10.1056/NEJMp2025631>.
7. Ranoa DRE, Holland RL, Alnaji FG, et al. Saliva-based molecular testing for SARS-CoV-2 that Bypasses RNA extraction. *bioRxiv.* <https://doi.org/10.1101/2020.06.18.159434>.
8. To KK-W, Tsang OT-Y, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis.* 2020;20(5):565–74. PMID: 32213337. PMCID: PMC7158907. [https://doi.org/10.1016/S1473-3099\(20\)30196-1](https://doi.org/10.1016/S1473-3099(20)30196-1).
9. Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med.* 2020;383(13):1283–6. PMID: 32857487. PMCID: PMC7484747. <https://doi.org/10.1056/NEJMc2016359>.
10. Watkins AE, Fenichel EP, Weinberger DM, et al. Increased SARAS-CoV-2 testing capacity with pooled saliva samples. *Emerg Infect Dis.* 2021;27(4):1184-7.

- PMID: 33755009. PMCID: PMC8007323. <https://doi.org/10.3201/eid2704.204200>.
11. Nagura-Ikeda M, Imai K, Tabata S, et al. Clinical evaluation of self-collected saliva by quantitative reverse transcription-PCR (RT-qPCR), direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19. *J Clin Microbiol.* 2020;58(9):e01438-20. PMID: 32636214. PMCID: PMC7448663. <https://doi.org/10.1128/JCM.01438-20>.
  12. Barat B, Das S, De Giorgi V, et al. Pooled saliva specimens for SARS-CoV-2 testing. *J Clin Microbiol.* 2021;59(3): e02486-20. PMID: 33262219. PMCID: PMC8106731. <https://doi.org/10.1128/JCM.02486-20>.
  13. Rainey A, Pierce A, Deng X, et al. Validation and deployment of a direct saliva real-time RT-PCR test on pooled samples for COVID-19 surveillance testing. *PLoS One.* 2021;16(12):e0261956. PMID: 34969053. PMCID: PMC8718011. <https://doi.org/10.1371/journal.pone.0261956>.
  14. Dynamiker Biotechnology (Tianjin) Co. L. DNK-2105-1S Dynamiker SARS-CoV-2. [http://en.dynamiker.com/index/index/pro\\_info/aid/640.html](http://en.dynamiker.com/index/index/pro_info/aid/640.html). Accessed October 20, 2021.
  15. Dynamiker SARS-CoV-2 AG rapid test (saliva). FIND Diagnosis for all. <https://www.finddx.org/test-directory/?subpage=variants-panel>. Accessed October 20, 2021.
  16. Diao B, Wen K, Chen J, et al. Diagnosis of acute respiratory syndrome coronavirus 2 infection by detection of nucleocapsid protein. medRxiv. <https://doi.org/10.1101/2020.03.07.20032524>. Accessed January 1, 2020.
  17. De Marinis Y, Pesola A-K, Söderlund Strand A, Norman A, Pernow G, Aldén M, et al. Detection of SARS-CoV-2 by rapid antigen tests on saliva in hospitalized patients with COVID-19. *Infect Ecol Epidemiol.* 2021;11(1):1993535. PMID: 34745449. PMCID: PMC8567870. <https://doi.org/10.1080/20008686.2021.1993535>.
  18. Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. *J Biomed Inform.* 2014;48:193–204. PMID: 24582925. <https://doi.org/10.1016/j.jbi.2014.02.013>.
  19. Department of Health Philippines. Department of Health - nCOV tracker; 2020. <https://ncovtracker.doh.gov.ph/>. Accessed September 18, 2020.
  20. Oguri S, Fujisawa S, Kamada K, et al. Effect of varying storage conditions on diagnostic test outcomes of SARS-CoV-2. *J Infect.* 2021;83(1):119–45. PMID: 33823203. PMCID: PMC8018904. <https://doi.org/10.1016/j.jinf.2021.03.026>.
  21. Buban JMA, Villanueva PN, Gregorio GE. Should RT-PCR of saliva samples be used for diagnosis of COVID19? Philippine COVID-19 Living Clinical Practice Guidelines Inst Clin Epidemiol Natl Institutes Heal UP Manila; 2021. [https://www.psmid.org/wp-content/uploads/2021/05/SALIVA-RT-PCR-CPG-FINAL\\_031521\\_MMA.pdf](https://www.psmid.org/wp-content/uploads/2021/05/SALIVA-RT-PCR-CPG-FINAL_031521_MMA.pdf).
  22. Butler-Laporte G, Lawandi A, Schiller I, et al. Comparison of saliva and nasopharyngeal swab nucleic acid amplification testing for Detection of SARS-CoV-2: a systematic review and meta-analysis. *JAMA Intern Med.* 2021;181(3):353-60. PMID: 33449069. PMCID: PMC7811189. <https://doi.org/10.1001/jamainternmed.2020.8876>.
  23. The Health Technology Assessment Council (HTAC). HTAC recommendation on the use of saliva as an alternative specimen for RT-PCR testing; 2021. [https://hta.doh.gov.ph/wp-content/uploads/2021/05/Evidence\\_Summary\\_RT-PCR\\_Testing\\_for\\_COVID-19-Recommendation.pdf](https://hta.doh.gov.ph/wp-content/uploads/2021/05/Evidence_Summary_RT-PCR_Testing_for_COVID-19-Recommendation.pdf).
  24. Product evaluation status antigen test kits. Research Institute for Tropical Medicine. <https://ritm.gov.ph/covid-19-kit-evaluation/ongoing-evaluations/product-evaluation-status-antigen-test-kits/>. Accessed January 29, 2022.
  25. Seitz T, Schindler S, Winkelmeyer P, et al. Evaluation of rapid antigen tests based on saliva for the detection of SARS-CoV-2. *J Med Virol.* 2021;93(7):4161–2. PMID: 33788280. PMCID: PMC8251355. <https://doi.org/10.1002/jmv.26983>.
  26. Potential for false positive results with antigen tests for rapid detection of SARS-CoV-2 - letter to clinical laboratory staff and health care providers. USFDA. <https://www.fda.gov/medical-devices/letters-health-care-providers/potential-false-positive-results-antigen-tests-rapid-detection-sars-cov-2-letter-clinical-laboratory>. Accessed January 29, 2022.
  27. Gonong DA, Misiona G, Sionzon M, Santiago FK, Lira AJ, Lo R. Analysis of SARS-CoV-2 RT-PCR testing and pooling strategies for screening of asymptomatic individuals - the Philippine Children's Medical Center experience. *Philipp J Pathol.* 2021;6(1):18–25. <https://doi.org/10.21141/PJP.2021.03>.
  28. Sahajpal NS, Mondal AK, Ananth S, et al. SalivaSTAT: direct-PCR and pooling of saliva samples collected in healthcare and community setting for SARS-CoV-2 mass surveillance. *Diagnostics (Basel).* 2021;11(5):904. PMID: 34069462. PMCID: PMC8159081. <https://doi.org/10.3390/diagnostics11050904>.
  29. Esteves E, Mendes AK, Barros M, et al. Population wide testing pooling strategy for SARS-CoV-2 detection using saliva. *PLoS One.* 2022;17(1):e0263033. PMID: 35089942. PMCID: PMC8797214. <https://doi.org/10.1371/journal.pone.0263033>.
  30. Padua Jr. R. Diagnostic testing strategies to manage COVID-19 pandemic: proposed by the Philippine Society of Pathologists, Inc. *Philipp J Pathol.* 2020; 5(1):5–8. <https://doi.org/10.21141/PJP.2020.08>.

**Disclaimer:** This journal is **OPEN ACCESS**, providing immediate access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. As a requirement for submission to the PJP, all authors have accomplished an **AUTHOR FORM**, which declares that the ICMJE criteria for authorship have been met by each author listed, that the article represents original material, has not been published, accepted for publication in other journals, or concurrently submitted to other journals, and that all funding and conflicts of interest have been declared. Consent forms have been secured for the publication of information about patients or cases; otherwise, authors have declared that all means have been exhausted for securing consent.