International Journal of Research in Medical Sciences
Saeed A et al. Int J Res Med Sci. 2023 Nov;11(11):3969-3975
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20233363

Original Research Article

Comparing the accuracy of nasopharyngeal COVID-19 PCR alone versus combined nasopharyngeal and oropharyngeal COVID-19 PCR tests

Alan Saeed[1]*, Anas Kalfah[2], Zhila Mohammed[3]

[1]Department of Family Medicine, Primary Health Care Corporation, Doha City, Qatar
[2]Department of Medical Education, Hamad Medical Corporation, Doha City, Qatar
[3]Department of Basic Science, College of Medicine, Qatar University, Doha City, Qatar

Received: 17 September 2023
Revised: 12 October 2023
Accepted: 16 October 2023

***Correspondence:**
Dr. Alan Saeed,
E-mail: alsaeed@phcc.gov.qa

ABSTRACT

Background: Efficient containment of the SARS-CoV-2 virus, responsible for the COVID-19 pandemic, is heavily reliant on precise diagnostic methodologies. Amidst prevalent use of nasopharyngeal (NP) and oropharyngeal (OP) swabs for detection, the superiority in sampling method effectiveness remains debated.
Methods: A retrospective study within Qatar's Primary Health Care Corporation (PHCC) contrasted the accuracy of combined NP/OP PCR tests versus NP-only tests, utilizing inconclusive test rates, primarily attributed to sampling adequacy, as a crucial accuracy measure. A total of 179,694 NP/OP and NP-only samples were analyzed across two phases: pre and post-16/01/2022, the latter marking a transition predominantly to NP-only swabs and increased reliance on rapid antigen testing. With a notable disparity in sample size between methods, a 1% simple random sample was extracted for analysis. Patients aged 18 years and below were excluded in this study.
Results: The dual NP and OP swab approach registered a 2% inconclusive rate pre-cut off, while a 5% inconclusive rate was observed with the NP-only technique post-cut off, presenting a statistically significant 3% differential ($p<0.001$). Subgroup analyses divulged a mere 1% inconclusive rate disparity between age groups and a 1% lower rate amidst symptomatic individuals, with chronic allergic rhinitis patients exhibiting a 2% elevation ($p=0.086$).
Conclusions: Combined NP/OP swabbing produced fewer inconclusive PCR results relative to NP-only swabbing, offering a 3% improvement in conclusive diagnostics. Notably, symptomatic presentation and chronic allergic rhinitis most significantly influenced accuracy, indicating potential avenues for further diagnostic refinement research, thus bolstering our current understanding and mitigation approaches towards COVID-19.

Keywords: COVID-19, Inconclusive results, Nasopharyngeal swabs, Oropharyngeal swabs, PCR, SARS-CoV-2

INTRODUCTION

During December 2019, the World Health Organization (WHO) was knowledgeable about an epidemic of pneumonia in Wuhan city, Hubei Province, China, and the origin and the aetiology were not identified yet.[1] On March 11, 2020, WHO declared that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak is a public health emergency of high international concern.[2] In February the 11th, 2020, the WHO legitimately named the current epidemic of coronavirus illness as Coronavirus Disease-2019 (COVID-19) and the International Committee on Taxonomy of Viruses (ICTV) named the virus that causes

it as SARS-CoV-2.[3] It turns out that bats are the natural reservoir of SARS-CoV-2.[4,5]

SARS-CoV-2 is spread via objects, fomites, and droplets during close vulnerable contact between the infected and the healthy ones.[6] Symptomatic and asymptomatic affected patients are the core source of contagion.[7] The virus can also spread over unintended contact transmission.[8] The droplets containing the virus can contaminate hands, people then contact the mucous membranes of the mouth, nose, and eyes, causing infection.[9] The most common clinical manifestations of COVID-19 are fever and dry coughing.[10] Most of the infected people presented bilateral pneumonia. Old males with comorbidities are more expected to be affected by SARS-CoV-2. The blood counts of patients showed leukopenia and lymphopenia. The content of cytokines and biomarker (IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFa) in the plasma of intensive care unit (ICU) patients is higher than non-ICU patients.[11,12] Real-time reverse transcription-PCR (RT-PCR) assays remain the molecular test of choice for the etiologic diagnosis of SARS-CoV-2 infection while antibody-based techniques are being introduced as supplemental tools.[13]

For diagnostic testing for current SARS-CoV-2 infections, CDC recommends collecting and testing an upper respiratory specimen for PCR, but it must be noted that collecting those specimens may carry a theoretical risk of transmitting SARS-CoV-2, particularly if the airborne transmission is demonstrated as the investigation of the COVID-19 outbreak continues.[14-16]

According to CDC guidelines, nasopharyngeal swabs (NPS) and oropharyngeal swabs (OPS) are suitable respiratory specimens for detection of SARS-CoV-2 RNA.[17] Later, the US CDC is presently admonishing the collection of only nasopharyngeal swabs (NP), not NP and oropharyngeal swabs (OP).[18]

The nasopharyngeal swab is also commonly used for the detection of several viral and bacterial infections, and it has been the standard testing technique for the diagnosis of COVID-19.[19-21]

In nasopharyngeal swabs, a sterile swab stick is used for the sampling procedure, the stick is inserted impenetrably into the nasopharynx, far from the hard-soft palate transition, and in the end, direct contact is achieved with the mucosal wall of the posterior nasopharyngeal.[22] The cerebral spinal fluid (CSF) leakage consequential to iatrogenic skull base injury is a possibly rare fatal adverse event of NPs swabs.[23] The infectious state could be comorbid with cellulitis mastoidites, osteitis, and even aggravate to sepsis.[19,24]

OP specimens can be collected with a wider range of swab products.[21] OP swabs are less specific than NP swabs and the FDA recommends that OP swabs must be collected by a healthcare professional.[24,25] Oropharyngeal swabs, despite being more tolerated by patients, are the slightest desirable since data suggests this specimen sort exhibits a higher false negative percentage.[26] Oropharyngeal sampling is easier to achieve. The swab is directed toward the rear barrier of the oropharynx, and it is rotated a few times before removal.[27]

When comparing the accuracy of oropharyngeal and nasopharyngeal swabs for diagnosis of COVID-19, some studies found that about 60% of COVID-19 infected people were positive using OP, whereas nearly 70% were positive on NP, and it was stated that the difference was most notable at days 8 plus after the onset of the disease.[18] Other studies demonstrate that the detection of SARS- CoV-2 by NP and OP/N sampling for RT-PCR was equivalent, and the sensitivity of both sampling moods was very high in hospitalized patients.[28] On a very large-scale study (13988 swabs), it was found that OP/N swabbing was acceptable, repeatable, and more tolerated in children for screening purposes, despite slightly reduced test performance.[25] According to another study, it has been found that NP swabs had higher sensitivity, viral load, and detection rate for SARS-CoV-2 than OP swabs.[24] However, none of these studies were conducted in the GCC or MENA region and thus their generalizability to these populations is limited. Given that Qatar has a racially and ethnically diverse population, studying this in health centres in Qatar is potentially valuable with regards to generalizability of results for the wider region.

Other factors have also been demonstrated to influence the accuracy of detection for SARS-CoV-2 such as swabbing technique and timing of test relative to phases of illness.[29,30]

Laboratory tests are validated by the power of detection of a positive case (sensitivity) and a negative case (specificity). The high accuracy of the results under ideal conditions samples from patients' high viral load (peak of the symptoms) or from no exposure completely. However, under the real-world condition in which patients are presenting at different timing of their illnesses and sample collection, storage and analysis loopholes may result in variable Sensitivity which is lower than the ideal conditions.[29,30]

There are two main types of tests for COVID-19. The first detects viral RNA using molecular methods such as polymerase chain reaction (PCR). These tests are highly specific because they are based on the unique genetic sequence of SARS-CoV-2.[31]

The CDC recommends use of nasopharyngeal (NP) swabs for molecular testing because in most patients, the nasopharynx, or the space above the soft palate at the back of the nose, as it yields highest viral concentration.

However, NP swab samples are technically challenging to obtain, and a suboptimal collection may reduce test

sensitivity and increase the likelihood of obtaining a false-negative result in a patient with the virus. On the other hand, oropharynx swabs alone have shown reduce sensitivity, other sampling methods such as saliva or blood likely result in even lower sensitivity. For patients with frank pneumonia, bronchoalveolar lavage sampling from the lower respiratory tract may have sensitivity equal to or better than an NP swab, although collection of these types of samples increases risks of occupational biological hazards to the health worker.[31]

The timing of the sampling is a determinant factor of the COVID 19 sensitivity, testing samples should be collected near the time of the symptoms onset for a higher sensitivity rate, as infected asymptomatic patients have a higher false negative rate due to low viral loads. Again, the longer the timing from the onset of the illness to sampling time the higher the false negative rates.[31]

Testing influenza viruses NP typically had a higher sensitivity than OP, but "a combination of two less-invasive swabbing methods, such as nasal and oropharyngeal swabs, had about the same sensitivity as did nasopharyngeal specimens.[32]

In the primary health care corporation health centers in Qatar, COVID testing was conducted by two swabs NP and OP samples, and from 16/01/2022 the new policy implemented according the corporation policy code LAB-P197V01.0: Issue date 02/10/2022, this has changed to one Nasopharyngeal swab only.

This study seeks to rigorously compare the diagnostic efficacy of nasopharyngeal (NP) swabs against a combined nasopharyngeal and oropharyngeal (NP + OP) swabbing approach for detecting SARS-CoV-2 in the diverse population of Qatar. Anchoring on variables such as sensitivity, specificity, and swabbing technique, the investigation aspires to illuminate which of the two methodologies-NP alone or NP + OP-manifests superior reliability and accuracy in real-world testing scenarios within the regional context, thereby contributing to the refinement of diagnostic practices and policy development amidst the ongoing pandemic.

METHODS

This study employed a retrospective cohort design utilizing electronic health records (EHR) to compare the accuracy of nasopharyngeal (NP) COVID-19 polymerase chain reaction (PCR) testing alone versus combined NP and oropharyngeal (OP) PCR testing. The study aimed to investigate the rate of inconclusive test results between these two testing approaches.

Participants

The study population included individuals who underwent COVID-19 screening between 16/11/2021 and 16/4/2022 at PHCC. The inclusion criteria encompassed individuals aged above 18 who had undergone both NP and OP swab testing during the initial period of the study compared to those who underwent NP swab testing alone during the later period. Patients aged 18 years and below were excluded in this study.

Data collection

Data for the study were obtained from the EHR system, which provided comprehensive medical records of the participants. The electronic records included age demographic information, medical history, laboratory results, and COVID-19 testing outcomes. All data were de-identified to ensure participant confidentiality and compliance with relevant privacy regulations.

Testing procedures

During the study period, which spanned from [16/10/2021] to [16/04/2022], two different testing procedures were implemented for COVID-19 screening. In the initial phase of the study, both nasopharyngeal (NP) and oropharyngeal (OP) swabs were collected from each participant by trained healthcare professionals wearing appropriate personal protective equipment.

The collection of NP and OP swabs involved inserting a swab into the nasal cavity (for NP) and the back of the throat (for OP), respectively. The swabs were rotated gently to collect adequate samples, ensuring proper contact with the mucosal surfaces. Following collection, the swabs were carefully placed into separate vials containing appropriate transport media. These vials were then securely sealed and transported to the laboratory for subsequent PCR testing.

The second phase of the study commenced on [16/01/2022], when only NP swabs were collected from each participant for COVID-19 detection. Trained healthcare professionals performed the collection using the same technique as in the previous phase, ensuring consistency in sampling methodology. The NP swabs were placed in vials with transport media and transported to the laboratory for PCR testing.

Inconclusive test results were defined as cases where the PCR test did not yield a definitive positive or negative result for SARS-CoV-2 infection. These results could be due to factors such as low viral load, inadequate sample collection, or technical issues during the testing process. The rates of inconclusive results were calculated separately for the period of combined NP and OP swab testing and the period of NP swab testing alone.

Descriptive statistics, including frequencies and percentages, were used to summarize the demographic characteristics of the study population. The rates of inconclusive results were compared between the two testing periods using appropriate statistical tests, which

included the chi-square and Fisher's exact test, depending on the distribution of the data.

Subgroup analyses were performed to examine potential variations in the rate of inconclusive results based on demographic factors, including age, smoking status, and the presence of rhinitis. Additional analyses were conducted to assess the impact of the revised testing protocol on the overall accuracy of COVID-19 screening, considering the rates of inconclusive results and their implications for diagnostic accuracy. By comparing the rates of inconclusive results before and after the implementation of NP swab testing alone, we aimed to evaluate the effectiveness of each testing procedure in providing accurate and reliable COVID-19 diagnostic outcomes.

This study was conducted in accordance with the ethical guidelines and regulations governing retrospective research studies and the use of electronic health records. The study protocol was reviewed and approved by the institutional review board or ethics committee of PHCC. Informed consent was not required, as the study involved the analysis of de-identified data from routine healthcare records. Participant confidentiality and privacy were strictly maintained throughout the study, and all data were securely stored and accessed only by authorized personnel.

Statistical analysis

The Statistical analysis was performed by STATA 11.2 (College Station TX USA). Chi square test for goodness of fit were used to measure the association between the age groups, symptoms, smoking status, C_ALRHINITIS and the cut-off date with conclusive and inconclusive results respectively and it's expressed as frequency and percentage. P<0.05 considered as statistically significance.

RESULTS

Our study provides crucial insights into the efficacy of two diagnostic methods and their impact on inconclusive PCR results for SARS-CoV-2. A comparison was made between combined nasopharyngeal (NP) and oropharyngeal (OP) swab testing, and NP swab testing alone. The rate of inconclusive results was found to be significantly different between the two testing methods.

Of the 1,550 patients who underwent the combined NP and OP swab testing, 31 (2%) returned inconclusive results. In stark contrast, the single NP swab testing, conducted on 1,050 patients, yielded a higher inconclusive rate of 5% (51 patients). Statistical significance was established at p<0.001, underscoring a considerable difference between the two testing strategies.

Our investigation also delved into the effects of various demographic and health-related factors on the rate of inconclusive results, in both the testing groups. The subgroup analysis unearthed important associations.

We found a significantly higher rate of inconclusive results (6%) among patients with allergic rhinitis (C_ALRHINITIS positive) compared to those without this condition (C_ALRHINITIS negative) which stood at 3% (p = 0.024) (Table 1).

Table 1: Total population tested for COVID 19 PCR tests 3 months prior, using two sample testing method, the cut off date 16/01/2022 and three months after, using nasal swabs only.

	Conclusive	Inconclusive	Total	P-value
Cutoff Date 16/01/2022				
Before (NP/OP)	166,321 (98%)	2,915 (2%)	169,236	<0.001
After (NP Only)	13,504 (95%)	639 (5%)	14,413	

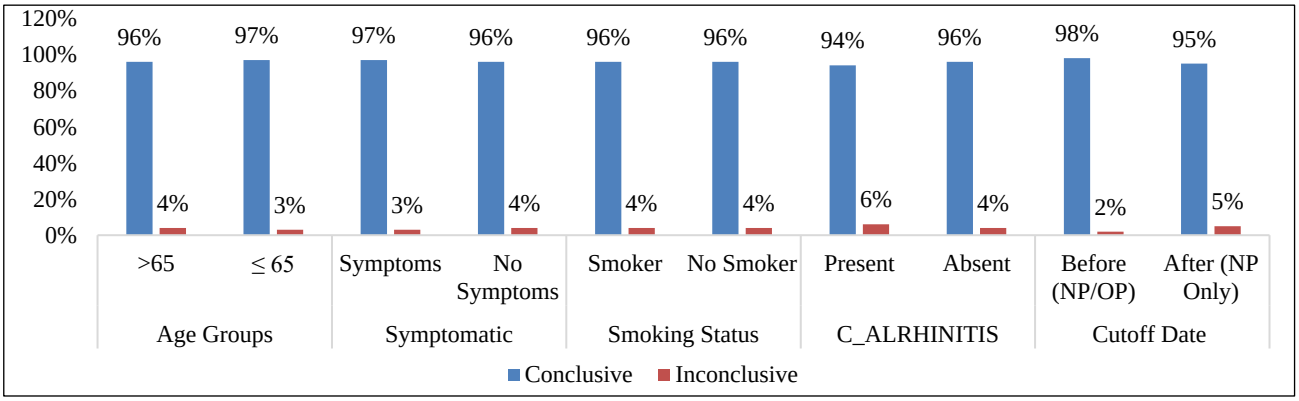

Figure 1: A graph demonstrates the results of the study showing the effects of different variables on inconclusive rates of COVID 19 test.

pISSN 2320-6071 | eISSN 2320-6012

Smoking status did not present a significant disparity in the rate of inconclusive test results. Both smoker and non-smoker subgroups had an inconclusive rate of 4%, with a p-value of 0.715 indicating no significant difference. Furthermore, symptomatic status did not impact the inconclusive rates considerably. Symptomatic individuals showed an inconclusive rate of 3% while those without symptoms reported an inconclusive rate of 4% ($p = 0.703$).

Lastly, age did not seem to significantly influence the inconclusive result rates. Patients older than 65 years had an inconclusive rate of 4%, compared to those under 65 years who showed a 3% rate, with the difference being statistically insignificant ($p = 0.896$) (Figure 1).

DISCUSSION

The impetus for this study stemmed from the urgent need for accurate diagnostic methods in the fight against COVID-19. As the world's healthcare systems strive for efficiency and accuracy, identifying the most reliable swabbing technique for SARS-CoV-2 PCR testing is paramount. Our findings contribute to this endeavor by highlighting the difference in inconclusive rates between NP-only and combined NP/OP swabbing methods. The sample, the test procedure, including the reagents, equipment, and people, as well as the test subject's health are some of the causes of equivocal results. Retesting with the original specimen is thought to be less beneficial in terms of prevention and effectiveness compared to the quantity of testing resources input, though, if contamination is properly managed in a typical laboratory. In addition, depending on the subject's epidemiologic history and the positive rate at the time, results that are not definitive may be interpreted in a variety of ways.[35]

An indeterminate result in a patient without a documented COVID-19 history may indicate an incubation period or an early stage of infection, thus if the retest result is the same, it is categorized as an inconclusive result, and a new sample is advised to be taken and examined.[35]

Our results demonstrate that combined NP and OP swabbing yields fewer inconclusive results, with a statistically significant 3% difference compared to NP-only swabs. This suggests that the combined swabbing method provides a more comprehensive and possibly accurate sample, thereby reducing the risk of inconclusive or false-negative results. The reduction in inconclusive outcomes is particularly significant given the sample size and retrospective nature of our study, which makes the findings robust and clinically relevant.

The subgroup analyses further nuanced our findings. Notably, age and smoking status showed no significant impact on inconclusive rates, thereby suggesting that these factors are less likely to influence the accuracy of PCR tests. Interestingly, symptomatic individuals demonstrated a 1% lower inconclusive rate compared to asymptomatic subjects, which could be due to a higher viral load in symptomatic cases, thereby making detection more straightforward. The most striking result was in patients with chronic allergic rhinitis, who had a 3% higher rate of inconclusive results, although this was not statistically significant. This subgroup could warrant further investigation to understand any underlying mechanisms that might affect PCR test accuracy. In conclusion, our findings suggest that combined NP and OP swabs yield more accurate results compared to NP-only swabs. However, it is essential to conduct further research to corroborate these results and explore other variables affecting the diagnostic accuracy of COVID-19 PCR tests. Future research should focus on prospective studies that also consider variables like healthcare worker proficiency, patient compliance, and swabbing technique to better understand the complexities surrounding PCR test accuracy.

In summary, our study highlights significant variations in the inconclusive rates depending on the testing approach and the presence or absence of allergic rhinitis. However, variables such as smoking status, symptomatic status, and age did not demonstrate a substantial effect on the rates of inconclusive results.

A sizable part of the contradictory findings should be interpreted as positive samples at the start or conclusion of the infection. However, there are also a lot of false-positive results, therefore each patient's should be examined separately after the clinical symptoms and epidemiological information.[34]

Our findings support the notion that the combination of NP and OP swab testing may lead to a lower rate of inconclusive test results compared to NP swab testing alone. This observation aligns with previous research that has highlighted the complementary nature of NP and OP swabs in detecting SARS-CoV-2 infection. The inclusion of OP swabs in the testing protocol appears to enhance the diagnostic accuracy of COVID-19 screening by potentially capturing viral particles that may be missed by NP swabs alone, thereby reducing false-negative results.

On all platforms, NP/OP and NP swab samples are valid specimen types for SARS-CoV-2 RNA testing by PCR. Although the NP only swab may be less sensitive than the NP/OP swab, it is less intrusive for patients. The quality of the sample, the presence of interfering compounds, and unsuitable or delayed transport circumstances are only a few of the numerous factors that affect the SARS-CoV-2 test's sensitivity.[33]

On another comparative study published 2020 during the COVID-19 outbreak, showed that nasopharyngeal swabs may be more appropriate than oropharyngeal swabs.[3]

It is crucial, however, to consider the limitations inherent in our study. The retrospective design and the predominance of combined NP/OP samples in the pre-cut-off period could potentially introduce selection bias. Also, external factors, such as the skill of the healthcare worker performing the swab, were not accounted for and could affect the inconclusive rates. Future investigations should encompass larger and more diverse populations to further validate and strengthen these findings.

Inconclusive PCR covid sampling is due to many factors mainly insufficient sampling (which was the core theme of our study), during the initial phase of the illness and towards recovery the low viral load level would also contribute to inconclusive results, however it is not possible to separate these factors causing the inconclusive, our study shows the effect of different sampling methods alone.

CONCLUSION

In conclusion, our study demonstrates a significant reduction in the rate of inconclusive test results when combining NP and OP swab testing for COVID-19 screening, as compared to NP swab testing alone. These findings imply that the inclusion of OP swabs in the testing protocol may contribute to improved accuracy in diagnosing COVID-19. The implications of this research are considerable, as minimizing inconclusive test results can enhance the efficiency and effectiveness of COVID-19 management. Future directions in this field could involve assessing the impact of combined swab testing on other performance metrics, such as sensitivity and specificity, as well as investigating the cost-effectiveness and feasibility of implementing this approach in diverse healthcare settings. Overall, our study provides valuable insights into optimizing testing strategies for COVID-19 and emphasizes the significance of incorporating multiple sampling methods to enhance diagnostic accuracy and reduce the occurrence of inconclusive results.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Saeed A, Kalfah A, Mohammed Z. Comparing the accuracy of nasopharyngeal COVID-19 PCR alone versus combined nasopharyngeal and oropharyngeal COVID-19 PCR tests. Int J Res Med Sci 2023;11:3969-75.