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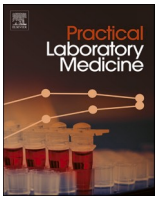

Registered Report Stage II

# Performance evaluation of influenza a rapid antigen test and PCR among nasopharyngeal and oropharyngeal samples

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ABSTRACT

*Objectives:* Rapid antigen test (RAT) and polymerase chain reaction (PCR) using nasopharyngeal (NP) or oropharyngeal (OP) swab specimens are the two main testing techniques used for laboratory diagnosis of influenza in clinical practice. However, performance variations have been observed not only between techniques, but also between different specimens. This study evaluated the differences in performance between specimens and testing techniques to identify the best combination in clinical practice.

*Methods:* Both NP and OP samples from suspected influenza patients collected in the 2023/4–2023/5 Flu-season in Xiamen, China, were tested for RAT and quantitative PCR. The testing performance of the different specimens and testing techniques were recorded and evaluated.

*Results:* Compared to PCR, RAT showed 58.9 % and 10.3 % sensitivity for NP and OP swabs, respectively. The Limit of Detection (LoD) was 28.71 the Median Tissue Culture Infectious Dose ($TCID_{50}$)/mL. Compared with PCR using NP swabs, PCR with OP swabs showed 89.5 % sensitivity and 95.4 % specificity.

*Conclusions:* There were no significant differences in performance between the specimens when PCR was used to test for influenza. However, a decrease in sensitivity was observed when the RAT was used, regardless of the specimen type. Therefore, to avoid false-negative results, PCR may be a better choice when OP swabs are used as specimens. In contrast, NP swabs should be the recommended specimens for RAT.

## 1. Introduction

Influenza is a contagious respiratory illness caused by influenza A and B viruses in humans [1]. Influenza virus infections manifest with common symptoms such as fever, headache, body aches, extreme tiredness, nausea, stuffy nose or rhinitis, and a dry cough [2,3]. Influenza has a significant impact on public health and global economy. In the US, clinical laboratories have confirmed 349,474 influenza cases from October 2022 to April 2023, according to the US CDC [4]. In China, the CDC reported 8,414,824 influenza-infected patients and 75 deaths from January to November in 2023.

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The clinical diagnosis of influenza is often inaccurate because of overlapping symptoms from infections with other cocirculating respiratory pathogens, including SARS-CoV-2. Influenza testing can aid in clinical decisions [5]. The most commonly used methods for detecting influenza in clinical facilities are RAT and PCR [6], both of which have advantages and disadvantages. Compared to PCR, RAT is much easier to perform and has a shorter turnaround time. Patients were able to perform the test at home and receive results within 10 min. However, the sensitivity of RAT is lower than PCRs' [7]. According to published studies, the sensitivity of RAT is between 51 and 67.5 % [8–10].

On the other hand, sample quality is another important factor affecting assay performance. The major specimens used in the Influenza RAT was NP and OP swabs. Alveolar lavage fluid and sputum can be used under certain circumstances. However, differences can be observed in the testing performance between different specimens. Published studies that focused on adults showed greater differences in the sensitivity of specimen type (range, 47–96 %) [11]. Nasopharyngeal wash demonstrated a significantly higher sensitivity (96 %) than NP (76 %) and OP (56 %) swabs [12]. However, in clinical practice, patients often strongly request OP swabs because of discomfort during NP swab sampling. This will result in a substantial proportion of false-negative results and finally affect infection treatment and local prevalence control.

Although much research has been conducted to evaluate the performance differences between PCR and RAT or between NP and OP samples, comprehensive studies of sample types combined with detection methods are still rare. In this study, a comprehensive performance evaluation considering both methods and specimen differences was performed using patient samples collected during the 2023 influenza season in Xiamen, China. This allows for a comprehensive discussion of the impact of different influencing factors on influenza detection performance.

## 2. Material and methods

*Ethical statement*

This study was approved by the Institutional Review Board of Zhongshan Hospital, Fudan University, Xiamen Branch. The approval number is B2023-051. All participants provided informed consent for publication of anonymized case details.

### 2.1. Inclusion criteria for patients

Patients with suspected symptoms, such as fever, vomiting, and cough, who were admitted to the fever clinic and ER of Zhongshan Hospital Fudan University Xiamen Branch between April 3rd and May 31st, 2023, were enrolled in this study. A total of 646 patients were enrolled in this study. Both NP and OP swabs were collected from each patient.

For reasons such as patient uncooperation, nasal stenosis, nosebleeds, and inappropriate sample collection, 150 patients were excluded from the study. Therefore, only 496 patients were included in this study.

### 2.2. Sample collection and transportation

NP and OP swabs were collected from enrolled patients simultaneously by a specialized nurse using a standard dry swab collection set. The collected samples were transported to the laboratory using pneumatic logistics systems immediately after sample collection.

### 2.3. Sample preparation and testing performed

Influenza RAT was performed using Wondfo Influenza A and B antigen testing on both NP and OP swabs as soon as the sample arrived at the laboratory. The assay was performed according to the manufacturer's instructions. Briefly, the swab was eluted with 500 μL elution buffer. Then 3–4 drops of eluent were added to the testing strip through the sample inlet. The results were recorded after 15 min of incubation. The residual eluent was sealed and preserved at −80 °C for further testing.

Nucleic acids for all preserved eluent samples were prepared using the Bio-Germ BG-Abot-96 automated nucleic acid sample preparation system. Then, quantitative PCR was performed using a self-developed Influenza A PCR system. The sequences of the primers and probes are listed in Table 1. The amplification program is shown in Table 2.

**Table 1**
Sequence information for PCR.

| For Influenza Virus | |
|---|---|
| IFV A-F | GATCTYGAGGCTCTCATGGA |
| IFV A-R | AACACAAATCCYAAAATCCCCTTAG |
| IFV A-P | AAAGACAAGACCAATCCTGTCACCT |
| For Inner Control | |
| IC-F | GGTGGGACTTCAGCATGGC |
| IC-R | CCTTAAAGTCAACGATATGATTGATAGC |
| IC-P | ACTGAATAGCCAAGGTGAGCGGCTG |

**Table 2**
Amplification program.

| Step | Temperature(°C) | Time |
|---|---|---|
| 1 | 50 | 5 min |
| 2 | 95 | 2 min |
| 3 | 95 | 15 s |
| 4 | 55 | 45 s |
| 5 | Go to Step 3 | 45 cycles |

### *2.4. Data collection and analysis*

The RAT and PCR results were recorded for each sample. RAT results were presented as qualitative results, while PCR results were presented as quantitative results with median tissue culture infectious dose ($TCID_{50}$/mL) as a unit. The result for the inner control (IC) of the PCR system was also recorded using the Ct value. All data were analyzed and plotted using GraphPad Prism 9. The data was presented as Mean ± SD.

## 3. Results

### *3.1. Population characteristics of research participants*

A total of 496 patients were enrolled in this study. The study population comprised 269 females and 227 males, accounting for 54.2 % and 45.8 %, respectively. The mean age of the participants was 33.16 ± 15.56 years old. Fever was the most common symptom, accounting for 84.5 % of the patients, and the remaining 15.5 % were cough, sore throat, vomiting, diarrhea, fatigue, and so on.

### *3.2. Testing performance analyzing among specimen types and methods*

As shown in Fig. 1, the Ct value for IC, which represents the quality of sample collection, was 29.79 ± 4.61 and 26.76 ± 7.11 for NP and OP swab, respectively. And as shown in Fig. 2, the quantitative PCR result for NP and OP swab was 251.9 ± 776.0 and 3.618 ± 9.896 $TCID_{50}$/mL, respectively. When the NP swab was used as the gold standard, the consistency of PCR results in OP swabs was 89.5 %.

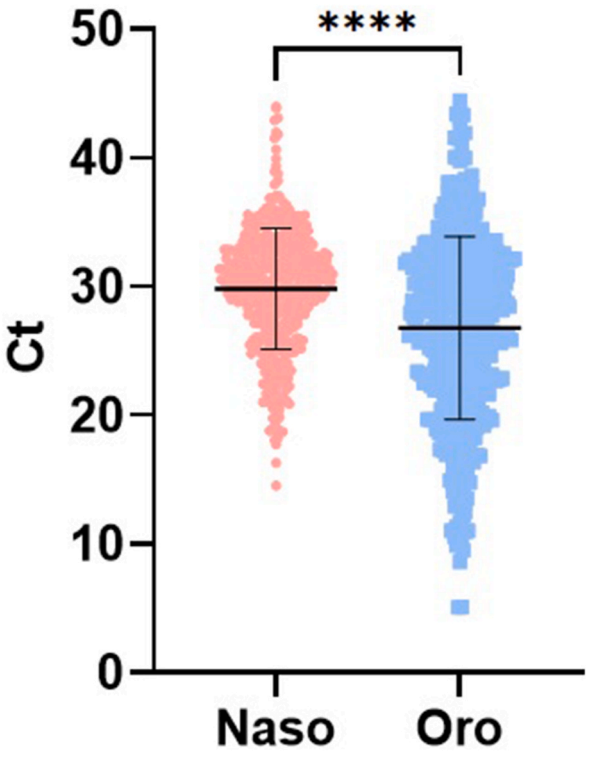


**Fig. 1.** Ct of IC in nasopharyngeal and oropharyngeal swabs

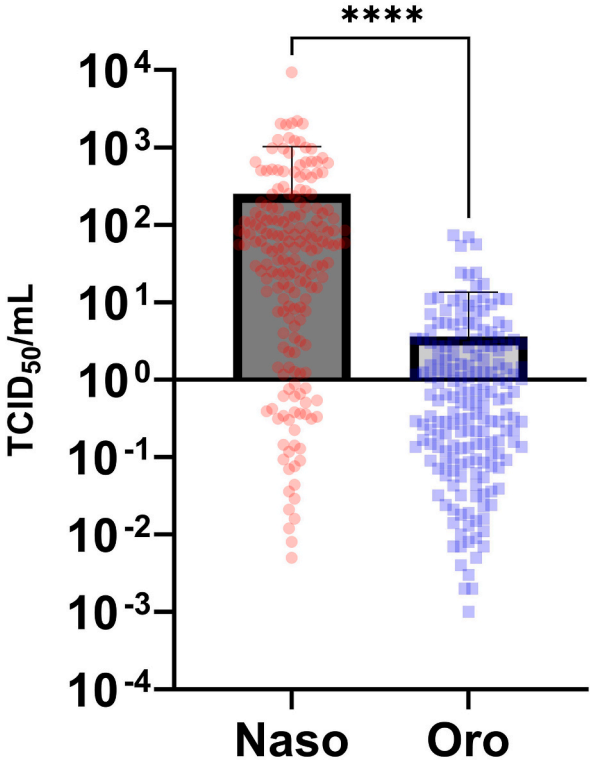


**Fig. 2.** Viral Load in nasopharyngeal and oropharyngeal swabs.

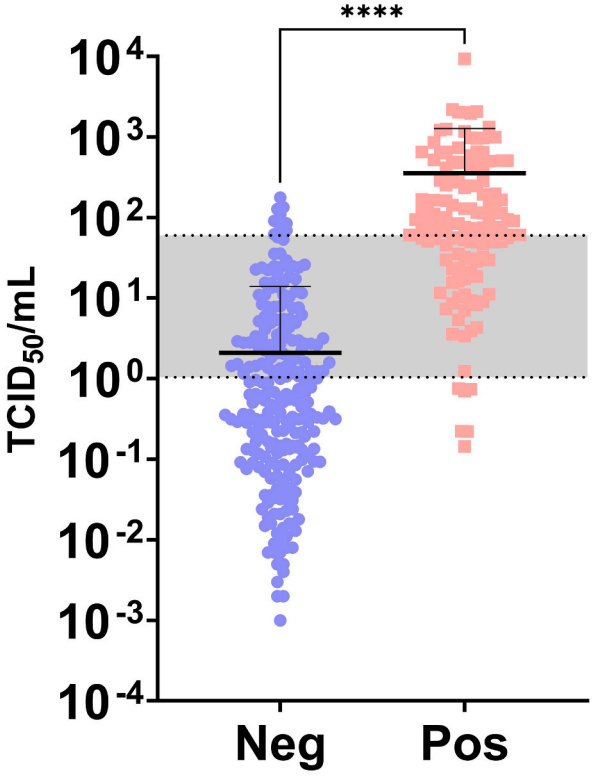


**Fig. 3.** Viral load among antigen negative and positive samples.

Using PCR as a reference method, the sensitivity and specificity of antigen testing of NP swabs were 58.9 % and 100 %, respectively. The sensitivity and specificity of antigen testing of OP swabs were 10.3 % and 100 %, respectively.

### 3.3. Limit of detection of rapid antigen testing

As shown in Fig. 3, the quantitative PCR results showed significant differences between the antigen-positive and antigen-negative samples. The viral loads for RAT-negative and positive groups are 2.095 ± 11.90 and 356.7 ± 916.1 TCID50/mL, respectively. The LoD of the rapid antigen test used in this study was 28.71 $TCID_{50}$/mL. The gray zone of the test can range from 1.04 to 60.63 $TCID_{50}$/mL.

## 4. Discussion

### 4.1. Samples can affect testing performance of respiratory viruses

In this study, different specimens could have affected the test performance of the methods for detecting respiratory viruses has been verified. The sensitivity of both PCR and antigen tests was significantly higher in nasopharyngeal swabs. According to these data, the sensitivity of the antigen test was much more affected by sample variation than by PCR. The PCR method showed an 89.5 % agreement between NP and OP swabs, whereas the sensitivity of the rapid antigen test was approximately six times lower for OP swabs.

On the other hand, although the agreement of qualitative PCR results between NP and OP swabs was 89.5 %, the quantitative results showed a huge difference. As shown in Fig. 2, viral concentrations in the OP group were significantly lower. These results indicated that samples from OP swabs contained fewer viral particles.

Another finding was based on the Ct value of the IC in the PCR. The Ct value of the OP swabs was lower than that of the NP swabs. As shown in Fig. 1, the difference in Ct values between these two groups was −3.035 ± 0.3957, indicating that the number of cells from OP swabs was approximately eight times higher.

It is generally accepted that one of the main reasons for the superior detection performance of NP swabs compared to OP swabs is due to the samples contain more cells with viral particles. However, the data from this study does not support this hypothesis. According to the data, the number of cells obtained from NP swabs was only one-eighth of that from OP swabs, but the concentration of the virus was still much higher than that from OP swabs. This result suggests that the sample collection efficiency is not the main reason for the differences in test performance. This difference may have been due to the specificity of the sampling sites. Compared to NP, OP samples are more influenced by daily activities, such as diet, smoking, and oral hygiene. Therefore, the samples may contain more interference or inhibitors for PCR and immunological testing, but fewer virus-infected cells.

In summary, the testing performance of PCR in NP and OP samples varied but was generally within acceptable limits. This may be due to the sample preparation step, which allowed for the purification and concentration of the sample. However, the results were not acceptable if the testing technique did not remove any interference or concentrate the sample. Therefore, use of OP swabs is not recommended if the assay does not include a sample pre-treatment step.

There is no significant correlation between test results and the risk of transmission.

Assessing viral activity is crucial in determining a patient's risk of transmission. However, current technical limitations prevent PCR and RAT from discriminating the survival status of the virus. Therefore, diagnosing the infection status of patients through these simple methods is not yet possible. Retrospective testing is no longer sufficient for clinical diagnosis, treatment, and public health prevention and control of virulent infectious diseases, such as influenza virus infection. Therefore, it is important to study in vitro diagnostic techniques that can determine the survival status of pathogens for the prevention and control of respiratory infectious disease epidemics.

### 4.2. Limitations and perspectives

This study has some limitations that are limited by existing conditions. First, only one type of quick antigen detection reagent and one self-developed PCR reagent were used in this study. Therefore, the results of this study do not adequately represent the performance of all commercially available reagents. More evaluations using different reagents are needed to improve the credibility of the study findings. Second, fewer than 10 samples out of 496 patients were positive for Influenza B virus strain. Due to the financial constraints and the low positive rate of Flu B, PCR for Flu B was not conducted. More evaluation testing for Flu B strain needs to be done in the future to minimize the potential of research bias.

## 5. Conclusion

In this study, NP and OP swab specimens from 496 patients with suspected influenza were analyzed using both RAT and PCR to evaluate performance characteristics. Compared to NP swabs, PCR using OP swabs showed 89.5 % positive concordance, which is generally within acceptable limits. In contrast, the sensitivity of the RAT was only 58.9 % and 10.3 % for the NP and OP swabs, respectively. The LoD of the Wondfo RAT was 28.71 $TCID_{50}$/mL. To increase the diagnostic accuracy for influenza, OP swabs should be avoided when RAT testing is performed by the clinician. If patients strongly declined NP sampling, PCR testing was considered. According to the CDC's recommendation, this RAT will lead to a high to moderate false-negative rate in areas with a low to moderate prevalence of influenza. Therefore, interpretation of negative results should consider the clinical characteristics of the patient and the

prevalence of influenza in the patient population being tested. If an important clinical decision is affected by the test result and influenza is still suspected, then the RIDT result should be confirmed using a molecular assay, such as RT-PCR [13].

## Financial support

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## Ethical approval statement

This study was approved by Institutional Review Board of Zhongshan Hospital Fudan University Xiamen Branch. The approval number is B2023-051.

## Patient consent statement

All participants provided informed consent for publication of anonymized case details.

## CRediT authorship contribution statement

**Xiaosong Su:** Writing – original draft, Visualization, Methodology, Investigation. **Jiaye Zhou:** Writing – review & editing, Supervision, Resources, Formal analysis. **Ling Liu:** Resources, Data curation. **Hongzhi Gao:** Data curation. **Yan Lin:** Data curation. **Zhile Wang:** Resources, Data curation. **Xin Zhang:** Software. **Baishen Pan:** Supervision, Project administration, Conceptualization. **Beili Wang:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Chunyan Zhang:** Funding acquisition. **Wei Guo:** Writing – review & editing, Validation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00416.

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