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SARS-CoV-2 antigen detection by saliva; an alternative to nasopharyngeal specimen: A cross-sectional study

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Abstract

Background and Aims: Saliva samples are less invasive and more convenient for patients than naso- and/or oropharynx swabs (NOS). However, there is no US Food and Drug Administration-approved severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapid antigen test kit, which can be useful in a prolonged pandemic to reduce transmission by allowing suspected individuals to self-sampling. We evaluated the performances of High sensitive AQ$^+$ Rapid SARS-CoV-2 Antigen Test (AQ$^+$ kit) using nasopharyngeal swabs (NPs) and saliva specimens from the same patients in laboratory conditions.

Methods: The real-time reverse transcription-polymerase chain reaction (rRT-PCR) test result was used for screening the inrolled individuals and compared as the gold standard. NP and saliva samples were collected from 100 rRT-PCR positives and 100 negative individuals and tested with an AQ$^+$ kit.

Results: The AQ$^+$ kit showed good performances in both NP and saliva samples with an overall accuracy of 98.5% and 94.0%, and sensitivity of 97.0% and 88.0%, respectively. In both cases, specificity was 100%. AQ$^+$ kit performance with saliva was in the range of the World Health Organization recommended value.

Conclusion: xOur findings indicate that the saliva specimen can be used as an alternative and less invasive to NPs for quick and reliable SARS-CoV-2 antigen detection.

KEYWORDS

COVID-19 diagnosis, kit evaluation, rapid antigen test, saliva, SARS-CoV-2

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly worldwide, causing a tremendous impact on public health, economics, social activities, and lifestyle. The prolonged COVID-19 pandemic is fading due to mass vaccination, and people are adopting the "new normal" life with COVID-19.[1] However, testing and tracking SARS-CoV-2 is one of the most important approaches to controlling the spread. The virus can be spread from human-to-human via airborne transmission through the respiratory tract or conjunctival mucosa that has cells with angiotensin-converting enzyme 2 (ACE2) receptors.[2,3] Therefore, naso- and/or oropharynx swabs (NOS) are mostly used for the detection of SARS-CoV-2 using the reverse transcription-polymerase chain reaction (RT-PCR) test.[4] In contrast, there are some less explored but potential specimens type and diagnostic tests.[5]

ACE2 is highly expressed on the epithelial cells of the oral mucosa, suggesting that the oral cavity could be at high risk for SARS-CoV-2 infection and transmission.[6] Human saliva secreted by the salivary glands contains large amounts of water (94%–99%) with mucosal cells and organic and inorganic molecules. Therefore, saliva could be a potential sample for COVID-19 diagnosis with some clinical advantages. For example, saliva samples are less invasive and more convenient for patients than NOS.[4] Additionally, the NOS sampling method may cause bleeding that will reduce specimen quality and cause sneezing, increasing the risk of virus transmission to healthcare personnel.[7] Therefore, a saliva sample is recommended for COVID-19 screening, especially in children.[7,8] On the other hand, previous studies suggested that asymptomatic SARS-CoV-2 infection may originate from infected saliva[9]; therefore, it can be used for detecting early infection in asymptomatic carriers.[10]

Several meta-analyses studies compared the efficiency of PCR tests using nasopharyngeal and saliva samples and found no significant differences; however, the efficiency was correlated with the stage of infection (i.e., early) and sampling technique.[11–16] But real-time RT-PCR (rRT-PCR) requires highly trained lab personnel and well-equipped laboratory facilities that cannot be performed in field tests (remote areas from the laboratory) or point of care (POC) facilities. Also, in a resource-limited setting, specimens require transportation to a laboratory which causes delays in result delivery.[17,18] Several lateral flow devices have been introduced as quick and cost-effective methods to detect SARS-CoV-2 antigen, known as rapid antigen test (RAT), which can also be used in POC with good sensitivity and specificity.[19] Until Jun 29, 2022, US Food and Drug Administration (US-FDA) approved 49 antigen diagnostic tests for SARS-CoV-2; all were based on the nasal or naso-pharyngeal swab (NP).[20] Also, recent meta-analysis studies observed low sensitivity and specificity of RATs using saliva samples.[19,21] However, several high-sensitive RATs for saliva sample is under development.

High sensitive AQ$^+$ Rapid SARS-CoV-2 Antigen Test was developed by InTec Products, INC, China, using saliva samples. This study aimed to evaluate this highly sensitive RAT's performance (sensitivity, specificity, and accuracy) using paired NPs and saliva specimens in laboratory conditions using stored samples.

2 | MATERIALS AND METHODS

2.1 | Study design and participant

To compare the performance of RATs for detecting the SARS-CoV-2 antigen, a cross-sectional study was designed. Based on the FDA USA guideline, a minimum of 60 samples (30 negatives and 30 positives) are required to evaluate the test.[22] Between August 2021 and January 2022, 200 symptomatic hospitalized (from DNCC Dedicated Covid-19 Hospital and Kuwait Bangladesh Friendship Government Hospital, Dhaka, Bangladesh) individuals were enrolled with known COVID-19 status (100 positive, 100 negative) within 24 h of their SARS-CoV-2 rRT-PCR test with NP specimens. The inclusion criteria were as (i) all age groups, (ii) either sex; (iii) acute onset of fever or cough; OR (iv) acute onset of any three or more of the presented signs or symptoms, general weakness/fatigue, headache, myalgia, sore throat, runny nose, nasal congestion, dyspnea, anorexia/nausea/vomiting, diarrhea, altered mental status.

2.2 | Clinical samples and data collection

Two NP swabs were collected from two nostrils and placed in one cryo-tube containing 500 µL of sterile 0.9% sodium chloride solution (normal saline). An additional ~1 mL of saliva was collected in a sterile plastic tube. All samples were stored at −80°C until the laboratory test was in batch. Clinical data were also collected and recorded in SPSS.

2.3 | Sample preparation and laboratory test

All stored (−80°C) specimens were kept on dry ice inside a BSL-2 safety cabinet to prevent rapid thawing until the solid-to-liquid phase change was almost complete. Then the specimens were placed in racks with an opening at the bottom that allows air to circulate, thawing samples at room temperature.

After thawing, 100 µL NP specimens were pipetted into 100 µL of extraction buffer of InTec AQ$^+$ Rapid SARS-CoV-2 Antigen Test and mixed homogeneously. In a saliva collection tube, 500 µL of saliva was added into 1.5 mL extraction buffer for processing and mixed homogeneously. Finally, 100 µL processed specimens were applied to each cassette (in the sample well). After 15 min, the test result was recorded by comparing the test band color (which appeared on the T line) with the color intensity rating card, which

has color intensity ranging from L1 to L10. L0 was used for the antigen test negative sample.

Although the COVID-19 status was known during the sample collection, we performed another rRT-PCR (new rRT-PCR) for SARS-CoV-2 using these stored samples on the same day of RAT. Upper respiratory specimens (NP and oropharyngeal swabs or wash in ambulatory patients) are considered appropriate specimens for COVID-19 diagnosis using rRT-PCR, according to World Health Organization (WHO) guidelines.[23] Therefore, rRT-PCR results of NP swabs were used as the gold standard. A total of 200 µL of NP specimen was used for viral RNA extraction using the chemagic viral NA/gDNA kit (PerkinElmer). The extracted RNA was then tested for SARS-CoV-2 by rRT-PCR using ORF1ab (RdRp) specific primers and probes.[24]

2.4 | Data analysis

Participants were categorized based on the new rRT-PCR result as strong to moderate positive (Ct ≤30), weak positive (>30 Ct ≤36), and negative. The InTec AQ$^+$ Rapid SARS-CoV-2 Antigen Test's sensitivity, specificity, and accuracy were calculated with a 95% confidence interval (95% CI) as described previously.[25]

2.5 | Ethical statements

The study was funded by InTec PRODUCTS, INC and approved by the Institutional Review Board of icddr,b (PR-20146). Written consent was obtained from the participant before collecting data or specimens. Standard biosafety and biosecurity protocols were followed during sample collection and transportation. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

3 | RESULTS

3.1 | Characteristics of study participants

Among 200 participants, 106 were male, the mean age of the participants was 49.2 years, and the median was 52.0 (interquartile range: 35.2–61.0). Most of the samples (n = 169, 84.5%) were collected at the late stage of the illness (>7 days). For clinical data, it was observed that most of the participants were present with fever (n = 142, 71%), influenza-like illness (shortness of breath/respiratory distress/chest pain/lower level of oxygen) (n = 23, 11.5%), and cough (n = 21, 10.5). Other recorded symptoms were runny nose, vomiting, diarrhea, abdominal pain, muscle aches or pain, weakness, altered smell, headache, loss of appetite, and altered consciousness. The complete clinical data of the participants are presented in Table 1, representing unbiased sampling for both COVID-19 negative and positive participants.

3.2 | Evaluation of AQ$^+$ COVID-19 Ag Rapid Test

Among 100 rRT-PCR positive NP specimens, 97 were positive by InTec AQ$^+$ COVID-19 Ag Rapid Test, and all rRT-PCR negative (n = 100) remained negative using InTec rapid kits. Thus, comparing with rRT-PCR results, the overall sensitivity of InTec for NP was 97% (95% CI: 91.5–99.4), the specificity 100.0% (95% CI: 96.4–100.0), and the accuracy 98.5% (95% CI: 95.7–99.7) (Table 2). The kit sensitivity was increased with strong to moderate (Ct ≤30) positive NP specimen (100%; 95% CI: 95.1–100.0) and slightly decreased with weak (>30 Ct ≤36) positive (88.9%; 95% CI: 70.8–97.7).

The sensitivity, specificity, and accuracy of the kit using saliva specimens were 88.0% (95% CI: 80.0–93.6), 100% (95% CI: 96.4–100), and 94.0% (95% CI: 989.8–96.9), respectively. Like NP specimens, saliva specimens also showed high (93.2; 95% CI: 84.7–97.7) sensitivity with strong to moderate positive specimens and low (74.1%; 95% CI: 53.7–88.9) with weak positive specimens (Table 2).

4 | DISCUSSION

Previous studies showed that saliva samples could be used for PCR tests[11–16]; however, there is no US-FDA-approved saliva-based rapid test.[20] In this study, we evaluated the performance of the AQ$^+$ COVID-19 Ag Rapid Test for the identification of SARS-CoV-2 in saliva. The kit showed strong performance using both NP and saliva specimens in the laboratory.

For the performance analysis, all specimens were collected from the symptomatic hospitalized individual. Therefore, the mean age of the participant was as high as 49.2 years. The kit provides overall accuracy of 98.5% versus 94.0%, where sensitivity was 97.0% versus 88.0% for NP versus saliva. In both specimens, specificity was 100%. Therefore, the kit fulfilled the minimum performance limit for sensitivity (>80%) and specificity (>97%) set by WHO for the COVID-19 antigen test.[26]

Enrolling symptomatic cases only was a limitation of this study as asymptomatic individuals could also carry SARS-CoV-2 infection.[27] Due to mass vaccination, COVID-19 is fading and might be converted to endemic with more mild symptoms[28]; therefore, further studies should include asymptomatic populations to observe the kits' performances. Although the study considered new rRT-PCR results for stored specimens as the gold standard for comparison, the result may not be consistent for the fresh specimen. However, we anticipated that with freshly collected specimens, the kit will provide a better result by eradicating factors related to freeze-thaw. Conducting tests in the laboratory is another limitation of this kit evaluation study. The field experiment might not be the same as the controlled laboratory experiment. Finally, the time of eating might affect the consistency of COVID-19 test results using saliva[29]; while this study did not record such data during specimen collection.

In conclusion, the kit performance was in the range of WHO recommended value and can be used for rapid SARS-CoV-2

TABLE 1 Clinical data of the study participants.

Variable	Total, unless otherwise stated Col. % (*n*)	rRT-PCR positive	rRT-PCR negative
Sex			
Female	47.0 (94)	48.0 (48)	46.0 (46)
Male	53.0 (106)	52.0 (52)	54.0 (54)
Age			
12–25 years	10.0 (20)	12.1 (12)	8.1 (8)
26–35 years	14.1 (28)	16.2 (16)	12.1 (12)
36–45 years	13.6 (27)	11.1 (11)	16.2 (16)
46 years and above	62.1 (123)	60.6 (60)	63.6 (63)
Mean (*SD*)	49.2 (16.9)	47.9 (17.5)	50.5 (16.2)
Median (IQR)	52.0 (35.2–61.0)	50.0 (34.0–60.7)	54.5 (40.0–62.7)
Study arm			
0–3 days of illness episode	3.0 (6)	1.0 (1)	5.0 (5)
4–7 days of illness episode	11.0 (22)	11.0 (11)	11.0 (11)
>7 days of illness episode	85.5 (171)	87.0 (87)	84.0 (84)
Not recorded	0.5 (1)	1.0 (1)	0.0 (0)
Symptoms			
Fever	87.5 (175)	92.0 (92)	83.0 (83)
Cough	86.0 (172)	90.0 (90)	82.0 (82)
Runny nose	58.0 (116)	65.0 (65)	51.0 (51)
Shortness of breath	53.5 (107)	48.0 (48)	59.0 (59)
Vomiting/diarrhea	34.5 (69)	31.0 (31)	38.0 (38)
Muscle aches/joint aches	71.5 (143)	79.0 (79)	64.0 (64)
Sore throat	23.0 (46)	19.0 (19)	27.0 (27)
Nausea	28.5 (57)	38.0 (38)	19.0 (19)
Headache	60.5 (121)	62.0 (62)	59.0 (59)
Altered smell	67.0 (134)	76.0 (76)	58.0 (58)
Loss of appetite	70.5 (141)	71.0 (71)	70.0 (70)
Respiratory rate			
Mean (*SD*)	20.1 (2.3)	20.1 (1.8)	20.1 (2.7)
Median (IQR)	20.0 (19.0–21.0)	20.0 (19.0–21.0)	20.0 (19.0–21.0)
Co-morbidities			
Diabetes	38.0 (76)	36.0 (36)	40.0 (40)
Asthma (requiring medication)	6.5 (13)	6.0 (6)	7.0 (7)
Heart disease	13.0 (26)	13.0 (13)	13.0 (13)
Chronic kidney disease	8.0 (16)	6.0 (6)	10.0 (10)
The visited outpatient treatment facility in the past 14 days	28.0 (56)	31.0 (31)	25.0 (25)

Abbreviations: IQR, interquartile range; rRT-PCR, real-time reverse transcription-polymerase chain reaction.

TABLE 2 Sensitivity, specificity, and accuracy of InTec AQ$^+$ COVID-19 Ag Rapid Test.

Real-time RT-PCR (ORF1ab)			InTec AQ$^+$ COVID-19 Ag Rapid Test using NP					InTec AQ$^+$ COVID-19 Ag Rapid Test using saliva				
	Positive	Negative	Positive	Negative	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	Positive	Negative	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
Ct ≤30	73	N/A	73	N/A	100 (95.1–100.0)	N/A	N/A	68	N/A	93.2 (84.7–97.7)	N/A	N/A
>30 Ct ≤36	27	N/A	24	N/A	88.9 (70.8–97.7)	N/A	N/A	20	N/A	74.1 (53.7–88.9)	N/A	N/A
Overall	100	100	97	100	97.0 (91.5–99.4)	100.0 (96.4–100.0)	98.5 (95.7–99.7)	88	100	88.0 (80.0–93.6)	100.0 (96.4–100.0)	94.0 (89.8–96.9)

Abbreviations: CI, confidence interval; RT-PCR, reverse transcription-polymerase chain reaction.

detection. Our findings indicate that the saliva specimen can be used as an alternative and less invasive to NPs for quick and reliable SARS-CoV-2 antigen detection.

AUTHOR CONTRIBUTIONS

Mohammad Jubair: data curation; formal analysis; investigation; methodology; validation; visualization; writing—original draft. **Sezanur Rahman**: data curation; formal analysis; methodology; software; validation; visualization; writing—original draft. **Mst Noorjahan Begum**: data curation; investigation; methodology. **Muhammad Talha**: data curation; investigation; methodology. **Raisha Musarrat**: data curation; investigation; methodology. **A. K. M Shafiqur Rahman**: project administration; supervision. **Mohammed Shehab Uddin**: project administration; supervision. **AiMin Wen**: writing—review & editing. **YaoHui Ning**: writing—review & editing. **Kamrun Nahar**: project administration; supervision. **Mohammed Ziaur Rahman**: writing—review & editing. **Mustafizur Rahman**: conceptualization; funding acquisition; investigation; methodology; resources; supervision; validation; visualization; writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interests except two co-authors, Aimin Wen, and YaoHui Ning. They work at the funder company InTec PRODUCTS, INC, Fujian, China. They were involved in the manuscript review.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article, Additional data related to this study are available from the corresponding author upon reasonable request.

TRANSPARENCY STATEMENT

The lead author Mustafizur Rahman affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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