

Research Article

Diagnostic evaluation of nasopharyngeal swab and saliva kits against SARS-CoV-2: Adequate rapid screening is deemed necessary to overcome COVID-19 Pandemic

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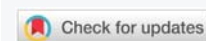
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Keywords: SARS-CoV-2; COVID-19; PCR; RDT kits; Nasopharyngeal swab; Saliva-based kits



Abstract

SARS-CoV-2 is the virus associated with the disease called COVID-19 and become a global pandemic. The only way to prevent its severe scenarios is through timely and rapid testing. In comparison to more time taking gold-standard RT-PCR testing, rapid diagnostic kits are used. For better prevention and diagnosis of SARS-CoV-2, the analysis of rapid diagnostic kits' accuracy and specificity is necessary. This study is meant to assess and examine the viability, responsiveness, and explicitness of quick antigen distinguishing nasopharyngeal swabs (NPS), and saliva-based units. The study was conducted on 200 suspected COVID-19 patients from Islamabad, 100 of which were RT-PCR positive while 100 were RT-PCR negative. For the analysis of Rapid diagnostic COVID-19 kits (RDT), nasopharyngeal swabs (NPS) and saliva samples were taken from the RT-PCR positive and negative patients. Among 100 RT-PCR positive patients, 62% were males (19 - 91 years), 34% were females (20 - 78 years) and 4% were children (6 - 17 years). False-negative results were significantly more observed in saliva-based RDTs of the sample (49%) as compared to nasopharyngeal swab RDT (38%). There were 2% invalid results in saliva-based RDT and 3% invalid results in Nasopharyngeal swab RDT. While among 100 RT-PCR negative patients 69% were males (19 - 80 yrs), 27% were females (18 - 77 yrs) and 4% were children (12 - 16 yrs.). False positive results were significantly more in saliva-based RDT (22%) as compared to Nasopharyngeal swab RDT (13%). The sensitivity and specificity of saliva-based RDT were 67% and 87% respectively while that of Nasopharyngeal swab (NPS) was 72% and 82% respectively, both of which were less than the gold standard RT-PCR sensitivity demanding the introduction of more sensitive RDT kits in Pakistan for accurate detection of COVID-19.

Introduction

The seventh human coronavirus is known as a severe acute respiratory syndrome (SARS-CoV-2) first outbreak in Wuhan, Hubei province, China [1,2]. The deadly virus spread rapidly all over the world and infected 4,806,299 people and caused 318,599 deaths as of 20 May 2020 [3]. The deadly SARS-CoV-2 virus is a beta coronavirus and its subgenus is Sarbecovirus [4]. There were 43,820,929 positive cases of SARS-CoV-2 globally and were rising rapidly and the number of deaths in that period was 1,165,189, India and United States

were the most badly influenced by COVID-19 [5]. On July 22, 2020, there were more than 1,47,65,256 confirmed cases of COVID-19 infection, and there were more than 6,12,054 fatalities in 200 countries (mortality rate of about 3.7%) [6].

Genomes investigation and examination with recently known COVID genomes show that SARS-CoV-2 presents exceptional highlights that discern it from other COVIDs: ideal partiality for angiotensin changing over catalyst 2 (ACE2) receptor and a polybasic cleavage site at the S1/S2 spike intersection that decides infectivity and host range [7,8]. The

novel SARS-CoV-2, on the other hand, has an RNA genome size of 29.9 kb [9]. SARS-CoV-2 exhibits 88 % nucleotide sequence identity to the two SARS-like coronaviruses generated from bats (bat-SL-CoVZC45 and bat-SL-CoVZXC2), as well as 79% and 50% similarity to the SARS-CoV and MERS-CoV, respectively [10]. A rising number of publications suggest that during the process of geographical diffusion, the SARS-CoV2 genome has undergone evolutionary alterations and heterogeneity. Global SARS-CoV-2 isolates' pan-genomic study has identified numerous genomic areas with higher genetic variation and a distinctive mutation pattern [11].

Since a large number of infected cases were asymptomatic [12]. For the early diagnosis of cases, controlling the pandemic situation large-scale testing was proposed to be crucial. The availability of the complete genome of the SARS-CoV-2 virus within outbreak declaration two weeks, allowed the production and introduction of a broad range of RT-PCR kits by numerous developers and manufacturers. The process of Emergency Use Authorization (EUA) was used for the clinical application of these kits by regulatory agencies to deal with the demand of large-scale testing, instead of using the basic authorization process of granting full clearance for diagnostic applications [13,14].

To avoid viral pathogenicity early detection and isolation of affected cases were necessary [15]. The globally recommended and approved gold standard for SARS-CoV-2 detection and analysis refers to the nasopharyngeal swab (NPS) which is followed by real-time reverse-transcription polymerase chain reaction (RT-PCR) of the RNA extracted from the suspect [16,17]. But in the pandemic situation analyzing large-scale cases using RT-PCR within a small time limit was challenging and burdensome demanding for the other fast, correct and cost-efficient diagnosis of SARS-CoV-2 for resource-limited countries like Pakistan to fulfill national and international requirements [18]. Although there may be some doubt about the validity and efficacy of these assays in the real world, Rapid diagnostic kits (RDTs) for COVID-19 are cost-efficient, easy, and safe to use [19]. The NPS method is intrusive, potentially bleeding, and there is an increased likelihood that SARS-CoV-2 may be transmitted to healthcare employees [20]. While the collecting of saliva samples is safe to handle outside of hospitals and is non-intrusive [21]. Additionally, taking saliva samples on one's own can lower the risk of healthcare workers being transmitted with SARS-CoV-2 than NPS [22]. Notably, there was no discernible difference in the viral level of SARS-CoV-2 in NPS or saliva samples [23].

Materials and methods

The current study was conducted by the International Center of Medical Sciences Research (ICMSR), Islamabad (44000), Pakistan, after getting IRB approval, from 1st Jan 2022 to 30th April 2022, as per standard operating procedures described previously [24]. Finding the best reliable diagnostic

assay for SARS-CoV-2 RDTs based on saliva (INVBIO, INV-1047) or NPS (INVBIO, INVBIO-COVID) is difficult without sacrificing the validity of test results. We aimed to evaluate the specificity and sensitivity of NPS and Saliva based kits used in Pakistan for COVID-19 diagnosis, which could be helpful to formulate effective testing procedures for SARS-CoV-2 in Pakistan.

Results

A total of 200 COVID-19 suspected patients were selected for evaluation of SARS-CoV-2 to antigen rapid test kits. Among selected individuals 130 (65%) were males, 62 (31%) were females and 8 (4%) were children. The average age of individuals was 41.5 years. Out of 200 patients, 99 showed positive results on saliva-based RT PCR i.e mean CT value less than 40, but on saliva-based antigen rapid test kits 62 (44 males, 15 females, and 3 children) individuals showed reactivity i.e positive results. In comparison with RT PCR for saliva, antigen rapid test kits for saliva showed sensitivity and specificity of 67% and 87% respectively, as shown in Table 1 and Figure 1.

Furthermore, the same 200 patients' nasopharyngeal specimen was used for RT-PCR and antigen rapid test. Now out of 200 patients, 100 showed positive results on RT-PCR i.e mean Ct value less than 40 and 81 (50 males, 27 females, and 4 children) showed reactivity i.e positive results on nasal-based antigen rapid test kits. In comparison with RT-PCR and antigen rapid test kit for saliva, showed sensitivity and specificity of 72% and 82% respectively, as shown in Table 1.

These sensitivities of our lollipop style SARS-CoV-2 Antigen rapid test kits are not appreciable and so these rapid diagnostic kits cannot be used as a reliable and accurate screening kit.

Discussion

The COVID-19 monitoring and diagnosis of SARS-CoV-2 are significant public health issues in third-world nations with severe socioeconomic gaps and inferior healthcare systems. According to WHO recommendations, the SARS-CoV-2 RDTs

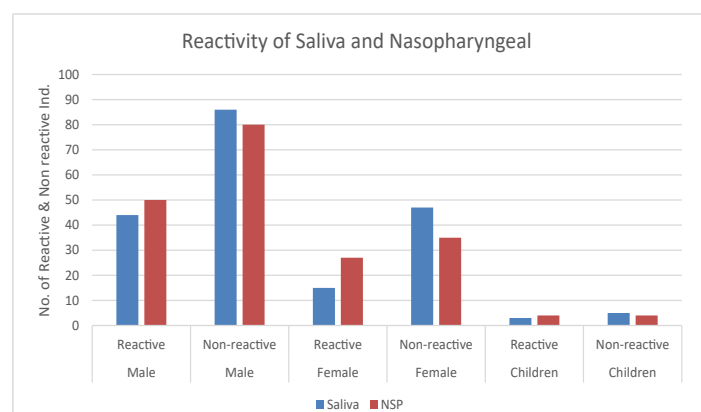


Figure 1: Reactivity of Saliva and Nasopharyngeal Testing.



Table 1: Characteristics of patients and clinical features.

Samples	Age (av)	RT-PCR Saliva	RT-PCR NSP	Saliva-ARTK	NSP-ARTK
Male (n = 130)	42.84 (19-91)	Reactive (n = 62) Non-reactive (n = 68)	Reactive (n = 62) Non-reactive (n = 68)	Reactive (n = 44) Non-reactive (n = 86)	Reactive (n = 50) Non-reactive (n = 80)
Female (n = 62)	42.48 (18-78)	Reactive (n = 34) Non-reactive (n = 28)	Reactive (n = 34) Non-reactive (n = 28)	Reactive (n = 15) Non-reactive (n = 47)	Reactive (n = 27) Non-reactive (n = 35)
Children (n = 8)	13.25 (6-17)	Reactive (n = 3) Non-reactive (n = 5)	Reactive (n = 4) Non-reactive (n = 4)	Reactive (n = 3) Non-reactive (n = 5)	Reactive (n = 4) Non-reactive (n = 4)
Total (n = 200)	41.54			Sensitivity = 67% Specificity = 87%	Sensitivity = 72% Specificity = 82%

testing should have a minimum sensitivity of $\geq 80\%$ and specificity of $\geq 97\%$ [18].

Since the same sample materials were used to compare RT-PCR to RDTs rather than utilizing distinct specimens, there was no chance for distribution error, which is a benefit of this study. Because SAR-CoV-2 replication is greater in the pharynx in the early days following infection and then diminishes [16,25]. It might be the reason, that the sensitivity of nasopharyngeal swab-based antigen tests is high during the early stages of infection. Considering that the findings of both rapid tests were unsatisfactory, a combination-test strategy is also recommended for reliable COVID-19 diagnosis.

The number of viruses is growing, thus innovative molecular techniques should be investigated to consider signal transduction pathways and potential host proteins that affect viral replication (Saeed U, Piracha ZZ) [26-30]. The results extracted in the current study are crucial for national strategic organizations that make policy decisions, but it also satisfies a global need for precise COVID-19 diagnostic testing. Using the findings of this study, improved testing procedures may be developed that would solve technical and economical problems. Executing the COVID-19 RDT testing approach involves several hurdles, such as developing policies, finding qualified staff, creating quality assurance standards, and addressing technical challenges. Hence, it is important to regularly assess RDT-based COVID-19 kits among Pakistani populations. Before marketing, the COVID-19 RDT kits, proper usage, and quality must be assured. Furthermore, it is strongly advised that the government ensure the adoption of standard operating procedures for the validation of national testing methods regularly throughout time.

Conclusion

Rapid and precise detection of COVID-19 is needed to avoid the worst health scenarios. In developing countries like Pakistan having a delicate health care system, poor quality RDT kits can cause severe health issues. The study investigated that the sensitivity and specificity of saliva-based kits are 67% and 87% respectively, and that of NPS-RDT is 72% and 82% respectively, which need further research and improvements.

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