Metropolitan Journal Of Environmental And Health Research

ISSN: 1813-4270

Vol. 3 Issue 3, March - 2024, Pages: 627-638

Assessment of Saliva, Nasopharyngeal and Oropharyngeal Swabs in the Detection of Sars-Cov-2 among Patients Attending Selected Healthcare Facilities in Mbarara City South Western – Uganda

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Abstract:

While Nasopharyngeal swab (NPS) has consistently been the suggested sample for diagnosing Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the collection process often leads to discomfort and irritation in patients, potentially lowering the likelihood of accurate detection. Hence, there is a need for an alternative diagnostic specimen. To assess the suitability of nosaphangeal, saliva, oropharyngeal swabs and time taken for the COVID-19 Antigen Rapid Diagnostic Test (Cov-2 RDT Ag) to turn positive in relation to the polymerase chain reaction (PCR) Ct value. A cross-sectional study among 301 study participants in four selected health facilities in Mbarara City. A structured questionnaire was used to collect the demographic characteristics of participants and nasopharyngeal, oropharyngeal swabs and saliva were collected. Cov-2 RDT Ag (Standard Q- South Korea) and PCR Genexert system (USA) were used in the detection of SARS-CoV-2. The time taken for RDT to turn positive and cycle threshold (Ct) values for the positive outcomes were recorded. The overall positivity rate was 06 (2.0%) by nasopharyngeal using PCR which is the gold standard. Out of the 06, saliva and oropharyngeal swabs gave 5 (83.3%) & 6 (100%) respectively using PCR. Using CoV-2 RDT Ag, the saliva, OS and NPS were 0%, 2 (33.3%), and 4 (66.7%) respectively. Samples with low Ct values took less time (mean <5 minutes) to turn positive in the CoV-2 RDT Ag kit. Saliva can only be used as an alternative sample if you are to test SARS-CoV-2 using PCR and nasopharyngeal still remains the best. The sample of choice for CoV-2 RDT Ag is nasopharyngeal swab. Further studies evaluating the suitability of saliva in the detection of SARS-COV-2 antigen and different saliva collection methods are recommended.

Keywords: Detection, SARS-COV-2, Mbarara City, Uganda.

Introduction: The global healthcare system has been under threat from the emergence of Coronavirus Disease-2019 (COVID-19), instigated by the intense Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2). As of the conclusion of 2020, approximately 10 million cases had been confirmed, resulting in nearly 5 million deaths, reflecting a 50% case fatality rate attributable to COVID-19.[1]**).** The identification of COVID-19 primarily relies on characteristic signs and symptoms, bilateral manifestations in chest radiographs, and exposure to confirmed infected individuals via an affirmed nucleic acid test detecting SARS-CoV-2 conducted on various specimen types.[2]. The predominant method for

Received: 18.03.2024 **Accepted: 23.03.2024** **Published on: 30.03.2024**

Metropolitan Journal Of Environmental And Health Research
ISSN: 1813-4270
Vol. 3 Issue 3, March - 2024, Pages: 627-638

detecting SARS-CoV-2 has been and continues to be reverse-transcriptase-polymerase-chain-reaction (RT-PCR), which targets the ORF1ab, N, or E genes. The commonly employed specimens for this detection method are oropharyngeal and nasopharyngeal swabs. Given the COVID-19 pandemic, it was essential to evaluate the suitability of various efficient diagnostic specimens for widespread testing, ensuring precise diagnosis, and devising strategies for prevention and control. Saliva, recognized as a highly adaptable and convenient specimen choice, presents significant benefits for broad screening initiatives owing to its non-invasive nature, cost-effectiveness, robust stability, and minimal risk of cross-infection. Nasopharyngeal swabs (NPS) are entirely acknowledged as suitable samples for identifying the presence of intense acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the ongoing 2019 covid-19 pandemic. Nevertheless, the collection process for Nasopharyngeal swab (NPS) samples induces sneezing and coughing in a majority of patients, leading to the generation of droplets or aerosol particles that pose a risk to healthcare workers involved in specimen collection. The frequencies of identification for deep throat saliva (DTS) (53.7%) and NPS (47.4%) samples were comparable (P = .13) after analysis of 95 patient-matched paired DTS and NPS specimens from 62 patients **.** On March 11, 2020, Uganda reported its initial case of SARS-CoV-2 infection, involving a Ugandan traveler from the United Arab Emirates. Within a month, the number of cases had escalated to 54, and laboratory testing of oropharyngeal and/or nasopharyngeal swabs was performed at the Uganda Virus Research Institute (UVRI) utilizing real-time RT-PCR.Nasopharyngeal swabs (NPS) and oropharyngeal swabs are widely recognized as suitable specimens for identifying severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the ongoing pandemic of coronavirus disease 2019. Saliva presents numerous benefits, serving as a non-invasive sample type that individuals can collect themselves, thereby reducing the risk of infection to medical staff, minimizing the need for personal protective equipment, and alleviating dependence on equipment prone to supply shortages, such as nasal, oropharyngeal (OP), or nasopharyngeal (NP) swabs [8] .It's on this background that we intend to compare the yields in saliva, nasopharyngeal & oropharyngeal swabs among the patients attending selected health facilities in Mbarara City in south-western Uganda.

Materials and Methodology

Study Design and location

A cross-sectional study design was carried out in four (4) selected healthcare facilities within Mbarara City South-western Uganda namely; Mbarara Regional Referral Hospital, Mbarara City Health Centre IV, Devine Mercy Hospital, and City Medical Chambers Mbarara. The study population comprised patients attending health care services at Mbarara Regional Referral Hospital, Mbarara City Health Centre IV, Devine Mercy Hospital and City Medical Chambers Mbarara; all within Mbarara City. The minimum sample size was 301 participants estimated using Cochran

Received: 18.03.2024 **Accepted: 23.03.2024** **Published on: 30.03.2024**

Metropolitan Journal Of Environmental And Health Research
ISSN: 1813-4270
Vol. 3 Issue 3, March - 2024, Pages: 627-638

formula (Cochran WG 1963) taking precision assumed to be +/- 0.05 at 95% level of confidence and taking 26.8% .The total number of participants was computed by adding the average number of patients attending health care at each health facility i.e. daily average number of patients 500 + 200 + 50 + 16 = 766**.** Therefore, the population proportion was determined by the formula below;

$P = \frac{x}{n}$ [9] where; p is the population proportion, x is the sample size and n is the sample population.

A consecutive sampling technique was used for all participants attending any of the four selected healthcare facilities in Mbarara City until a total of 301study participants was achieved. All adult patients who presented with manifestations and indications suggestive of COVID-19 & willing to participate in the study; having consented were recruited for the study. Study participants who; after the health talk and informed of the objectives of the study and willing to be part but were very ill with other comorbidities and unable to provide the required specimen were excluded. The principal investigator obtained consent from the participants who had signs & symptoms suggestive of COVID-19, using the language that the patient was comfortable with. After that, the patient's demographic data was recorded.

Three specimens (Saliva, nasopharyngeal and oropharyngeal swabs) were collected from all consented study participants who presented with signs & symptoms suggestive of COVID-19. All specimens were triple-packaged and shipped to Mbarara Regional Referral Hospital Laboratory. All samples (both negative & positive) were subjected to a real-time polymerase chain reaction (rt-PCR) at Mbarara Regional Referral Hospital (MRRH). 350µL of Nasopharyngeal and oropharyngeal swabs samples collected in viral transport medium (VTM) were added to the extraction buffer, mixed and then 3 drops added to the standard Q COVID-19 antigen RDT cassette device and then incubated for 15 minutes at room temperature. Test results were read at 15 minutes (a positive test indication was shown by two bars appearing on the control & test line, a negative test had only one bar at the control line and invalid results comprised of test outcome with no bar at the control line; this mainly was due to prolonged test outcome because of failure in movement of the test fluid). For the saliva specimens, a ratio of 1:1 with the extraction buffer was used. Similar procedures were followed for the nasopharyngeal and oropharyngeal samples. This is an illness triggered by the novel coronavirus in 2019, subsequently renamed Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2), impacting the respiratory tract.

Laboratory diagnosis: diagnosis made by chemical, microscopic, microbiologic, immunologic, or pathologic study of secretions, discharges, blood or tissue.

Nasopharyngeal swab specimen: is a material obtained from the nasopharynx that is used for diagnosis of respiratory infections. The swab is inserted horizontally into the nostril passage.

Oropharyngeal swab specimen: is the specimen collected by directing a sterile swab towards the rear wall of the oropharynx and it is rotated a few times before removal.

Received: 18.03.2024 **Accepted: 23.03.2024** **Published on: 30.03.2024**

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Saliva sampling: is a simple and reliable method for collecting DNA for genetic testing. It is a great alternative to blood collection, particularly for those patients who are anxious about having blood collected.

SARS-CoV-2: Part of an extensive family of viruses known as coronaviruses, capable of infecting both humans and certain animals.

Swab: is a sample collection device used for the collection of nasopharyngeal or oral specimens for the detection of bacteria or viruses that cause respiratory infections.

Statistical analysis

The percentage positivity for the individual specimen type (e.g., saliva, NP & OP swabs) was calculated and presented in form of frequency, percentages, mean and standard deviation (SD). Having cleaned and checked for completeness, errors and consistence, Data were entered into an Excel spreadsheet and then exported to STATA software version 14 for analysis.

Results

Demographic characteristics of study participants

A total of three hundred and one (301) study participants were enrolled in the study. The majority were males 171 (56.8%). Also, majority of the study participants were aged between 27-37 years 100 (33.2%). Many participants were practicing business personalities 73 (24.2%) and those with comorbidities 54 (18%), the major comorbidity was hypertension 24 (8%) followed by diabetes 15 (5.0%) as shown in (Table 1).

Table 1: Demographic characteristics of study participants

Variables		Frequency (n=301)	Percentage (%)
Gender			
	Male	171	56.8
	Female	130	43.2
Age (years)			
	≤15	3	1.0
	16-26	63	20.9
	27-37	100	33.2
	38-48	60	19.9
	49-59	40	13.3
	≥60	35	11.7
Profession			
	None	70	23.3
	Business	73	24.2

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ISSN: 1813-4270

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	Professional	58	19.3
	Farmer	40	13.3
	Others	60	19.9
Comorbidities			
	None	247	82.0
	Hypertension	24	8.0
	Diabetes	15	5.0
	Hypertension & diabetes	6	2.0
	Asthma	3	1.0
	Liver/ kidney disease	6	2.0

Note: profession: none (housewife), business (business woman, man and shopkeepers), professional (teachers and medical workers) and others (bodaboda riders, drivers, security guards).

Yield of SARS-COV-2 in saliva and OS.

On RT-PCR, nasopharyngeal, oropharyngeal and saliva samples detected 06, 06 and 05 positive cases respectively. Oropharyngeal and saliva samples each gave 1 false negative result. This gave saliva and OS a positivity of 5 (83.3%) and 6 (100%) as shown in (Table 2).

Table 2: Positivity SARS-CoV-2 in saliva and OS against NPS

		NPS		Total
		Negative	Positive	
Saliva/ OS	Negative	295	1	296
	Positive	0	5	5
Total		295	6	301

On the CoV-2 RDT Ag, nasopharyngeal, oropharyngeal and saliva samples detected 4, 2 and 0 positive cases respectively. Nasopharyngeal samples gave 2 false negative results, oropharyngeal samples gave 4 false negative results and saliva samples gave 6 false negative results on the test cassette. So the positivity of SARS-CoV-2 using the CoV-2 RDT Ag was 4 (66.7%), 2 (33.3%) and 0% using nasopharyngeal, oropharyngeal and saliva samples respectively.

Table 3: Comparison of CoV-2 RDT Ag to PCR using NPS.

		RT-PCR		Total
		Negative	Positive	
RDT	Negative	295	2	297
	Positive	0	4	4

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Total	295	6	301

The time taken by RDT to turn positive in relation to PCR Ct values

The mean time in minutes taken by NPS samples was 4.75 minutes (SD 2.0, Minimum 3 and Maximum 7). For OS samples was 4.5 minutes (SD 0.7, Minimum 4 and Maximum 5). The mean Ct E-values were 29.4 and 30.1 for NPS and OS samples respectively. The mean Ct N-values were 27.9 and 28.5 for NPS and OS samples respectively. Samples that took less time to turn positive were having lower PCR Ct values as shown in Figure 1 & 2.

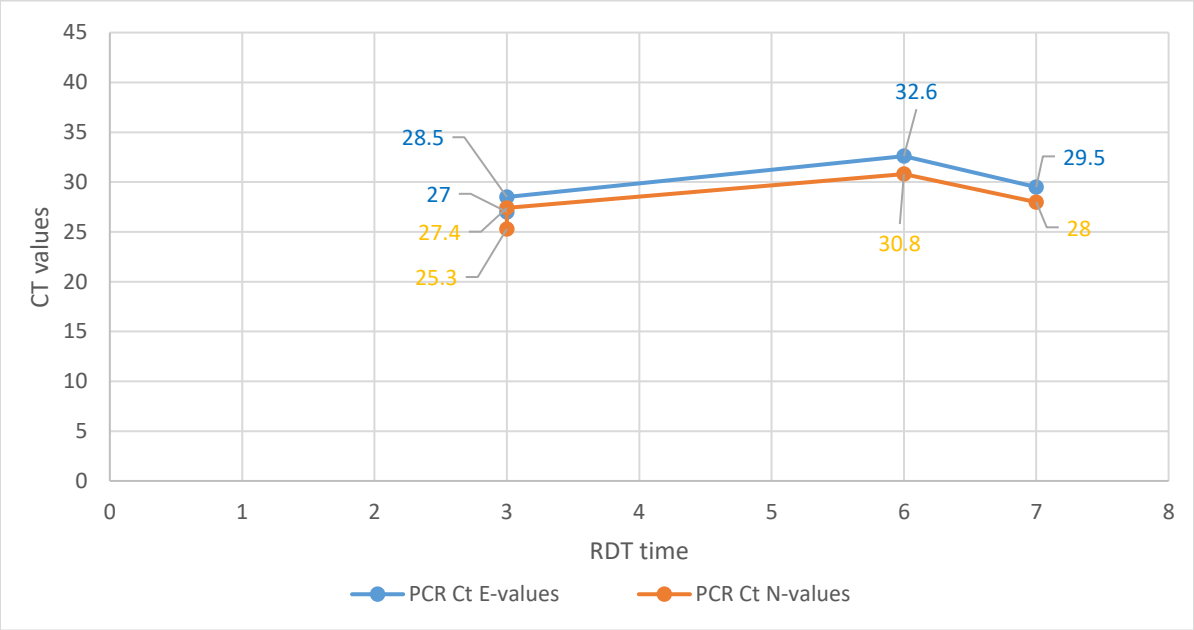

Figure 1: A plot of Ct values against the time taken for RDT to turn positive using NPS samples

Received: 18.03.2024 **Accepted: 23.03.2024** **Published on: 30.03.2024**

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ISSN: 1813-4270

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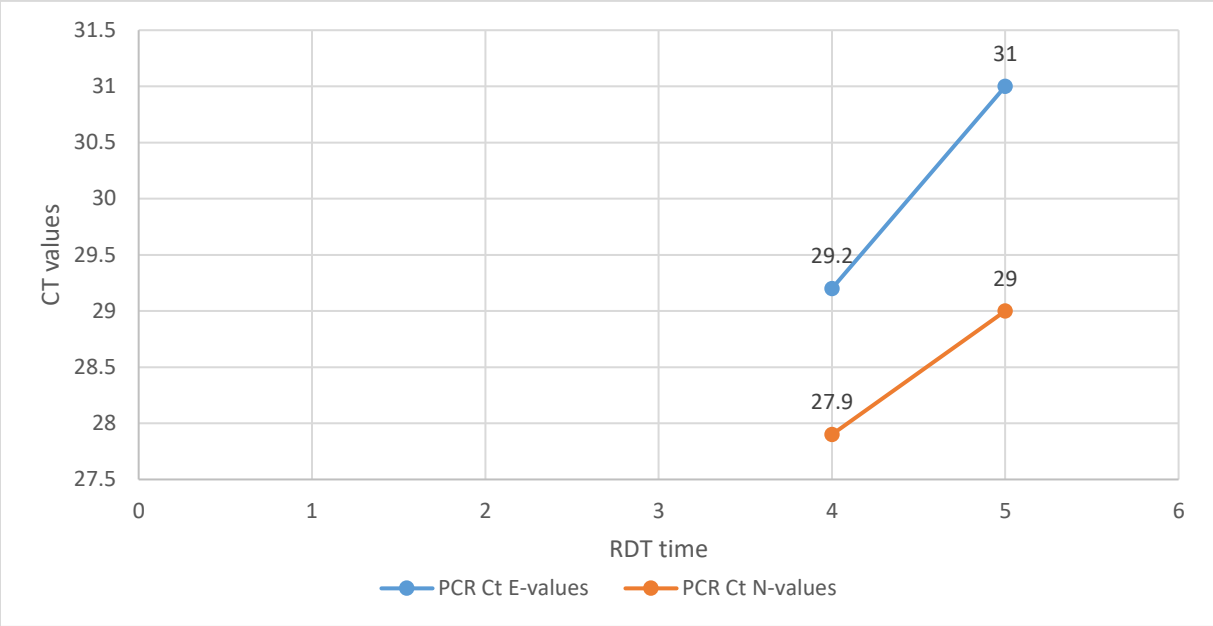

Figure 2: A plot of Ct values against the time taken for RDT to turn positive using OS samples

Discussion

The laboratory identification of SARS-CoV-2, responsible for COVID-19, usually entails identifying the virus's genetic material or proteins in respiratory specimens from patients. The two predominant laboratory tests employed for COVID-19 diagnosis are RT-PCR and CoV-2 RDT Ag [2]. Utilizing upper respiratory specimens is advised for clinically diagnosing active disease in symptomatic cases during the early stages of infection or in asymptomatic individuals. Traditionally, nasopharyngeal specimens, particularly nasopharyngeal aspirates (NPA), were considered the preferred samples for diagnosing respiratory viral infections, providing the highest likelihood of detecting viruses such as Influenza A

Positivity of saliva and OS for SARS-COV-2 detection

In regard to the findings, the positivity of oropharyngeal swabs was 6 (100%) by the RT-PCR method and 2 (33.3%) by using the Covid-19 antigen RDT method. Our finding is comparable to a study done in India, where the CoV-2 RDT Ag had a 35.3% positivity [18]and 31.6% reported in Hubei [4]. Our finding also reported 100% positivity by PCR which was as well comparable to the study by[18]. To the contrary our finding was not agreeing with a study done in Switzerland that found a positivity of 81%.

Using RT-PCR. Both specimen (saliva & OS) showed a high yield using the RT-PCR method although, oropharyngeal swabs had a higher yield using the rapid diagnostic test kit than saliva. This finding agrees with a study that found out the sensitivity of saliva at 85% using the PCR method. This study finding however also slightly correlated with a finding by the study done in Hong kong, China that reported saliva had a positivity rate of up to

Received: 18.03.2024 **Accepted: 23.03.2024** **Published on: 30.03.2024**

91.7% still by the same method [21] . The study finding also correlated with a finding from Eastern Ethiopia indicating that saliva had 83.8% sensitivity but disagreed with a finding by the same study that nasopharyngeal swab had only 68.9% sensitivity.

A systemic review and meta-analysis report that saliva had a sensitivity of 83.2% which was similar to our findings but however the sensitivity they reported for nasopharyngeal swab (84.8%) was lower than our findings. Another study also reported that the sensitivity of saliva (88%) and the oropharyngeal swabs (84%) were lower than for the nasopharyngeal swabs and referred nasopharyngeal swab as gold standard sample for Covid-19 antigen detection also was in line with our study. However, this study finding was not in line with a study that reported higher sensitivity of saliva, (94.6%) and oropharyngeal swabs (94.2%). The variation in the study finding could be attributed to the diagnostic methods, sample size variation, equipment used, sample population.

Time taken by RDT to turn positive in relation to PCR Ct values

PCR Ct value is a measure of viral load concentration in the sample. A low Ct value i.e. <29 indicates abundant target viral antigen, Ct values of 30-38 indicate a moderate abundance of the antigen and Ct values >38 indicate minimal antigens present. Our study found out that the samples that took less time on RDT to turn positive had a lower Ct value which indicated high viral load giving it a strong reaction within a short time meanwhile samples that took longer time on RDT had higher Ct values which indicated low viral load and hence a weaker reaction that needed a longer time to turn positive. This is in line with a study that also indicated lower Ct values were seen in samples with increased viral load and higher Ct values were seen in samples with low viral load which predicted their time of reaction.

Conclusions and Recommendations

Oropharyngeal swabs and Saliva showed a greater yield (100% and 83.3% respectively, higher with oropharyngeal swabs) for SARS-CoV-2 detection using the PCR method than using RDT. However nasopharyngeal swabs remain the gold standard. Hence, the detection of SARS-CoV-2 RNA through saliva samples remains feasible, contingent upon the chosen methodology. Because patients can self-collect saliva, it presents an efficient solution to address shortages in personal protective equipment (PPE) and sample collection tools like flocked swabs. Saliva emerges as a potential diagnostic specimen for SARS-CoV-2 molecular diagnosis, especially in scenarios with swab supply constraints, and proves advantageous for children and patients who may have difficulty providing nasopharyngeal swabs (NPS). It is advisable to conduct additional research to assess the suitability of saliva for detecting SARS-CoV-2 antigen, as well as to explore different methods of saliva collection.

Supplementary Materials

Word document files of Tables 1 to 3 and Figures 1 to 2 have been uploaded under supplementary materials.

Author Contributions

Received: 18.03.2024 **Accepted: 23.03.2024** **Published on: 30.03.2024**

Metropolitan Journal Of Environmental And Health Research

ISSN: 1813-4270

Vol. 3 Issue 3, March - 2024, Pages: 627-638

The following were the author contributions; Conceptualization, N.N , S.R.W and H.A ; Methodology and investigations, N.N , T.K.B, and S.R.W.; formal analysis B.B.M , N.N and S.R.W; resources N.N; S.R.W; H.A ; T.K.B and B.B.M ; data curation, N.N , H.A ; writing—original draft preparation, N.N and K.F ; writing—review and editing, R.W , B.O ; supervision, R.W and B.O ; project administration, R.W and B.O. All authors have read and agreed to the publication of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

This study was conducted in accordance with the Declaration of Helsinki, and approved by the Mbarara University of Science and Technology Faculty of Medicine Research Committee (Reference number: MUST/MLS/030, Date of approval: 28/2/2023)

Informed Consent Statement

Written informed consent was obtained from all participants involved in the study

Data Availability Statement

Data used in this study is available from the corresponding author upon request

Acknowledgements

We acknowledge the administration of Mbarara Regional Referral Hospital, Mbarara City H/CIV, Devine Mercy Hospital and City Medical Chambers-Mbarara for their cooperation and allowing us involve their patients in the study. We also extent our gratitude to Laboratory Director – technical services Mbarara Regional Referral Hospital Clinical Laboratories for allowing us to run all the RT-PCR samples from his clinical molecular laboratory section and not forgetting all the staffs in the department of Medical Laboratory Science- Mbarara University of Science and Technology.

Conflict of interest.

All authors declare no conflict of interest.

Submission declaration and verification

The authors declare that this manuscript is original, has not been published before and is not considered for publication elsewhere.

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Received: 18.03.2024 **Accepted: 23.03.2024** **Published on: 30.03.2024**

Metropolitan Journal Of Environmental And Health Research

ISSN: 1813-4270

Vol. 3 Issue 3, March - 2024, Pages: 627-638

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