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JCIMCR OPEN ACCESS Journal of Clinical Images and Medical Case Reports

ISSN 2766-7820

Research Article

Open Access, Volume 5

Saliva as a tool for SARS-CoV-2 genomic and immunological surveillance in the Republic of Congo

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Received: May 08, 2024

Accepted: May 28, 2024

Published: Jun 04, 2024

Archived: www.jcimcr.org

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DOI: www.doi.org/10.52768/2766-7820/3094

Abstract

Background: The design of this study was intended to evaluate the use of saliva as a reliable non-invasive tool for the genomic and immunological surveillance of SARS-CoV-2 infection in the Republic of Congo.

Methods: During this cross-sectional study, the active infection was determined by detecting SARS-CoV-2 RNA using RT-PCR in 220 paired saliva and oropharyngeal samples (OPS), and by sequencing SARS-CoV-2 genome using the Oxford nanopore technology. The detection of anti-SARS-CoV-2 IgG antibody was done in 148 pair saliva and plasma samples using an in-house developed ELISA, and the reproductivity of the assay based on saliva were assessed in two independent laboratories.

Results: Overall, saliva (22/220) and OPS (23/220) showed similar rates of viral detection (p=1.00). The sensitivity and specificity of detecting SARS-COV-2 active infection in saliva were 95.7% (95% CI: 79.0-99.8%) and 100% (95% CI: 98.1-100%) respectively, with the mean cycle threshold values similar to those of oropharyngeal samples (p>0.05). The genome sequencing revealed a mean coverage of 95.5±2.8 %, finding omicron as the main variant. The anti-SARS-COV-2 antibody detection in saliva showed a sensitivity of 92.0% (95% CI: 85.0-96.0%) and specificity of 93.3% (95% CI: 78.0-99.2%) compared to plasma. There was a high agreement in antibody detection results between Congolese Foundation for Medical Research and the Institute of Tropical Medicine laboratories (Cohen's kappa 0,94; p=0.0001).

Conclusion: These findings demonstrate that saliva can be used as a surrogate to Oropharyngeal or plasma for surveillance of SARS-COV-2 infection in the Republic of Congo.

***Keywords*:** SARS-COV-2 surveillance; Saliva; Reliability; Republic of Congo.

Citation: Mouzinga FH, Lissom A, Heinzel C, Kreidenweiss A, Ntabi JDM, et al. Saliva as a tool for SARS-CoV-2 genomic and immunological surveillance in the Republic of Congo. J Clin Images Med Case Rep. 2024; 5(6): 3094.

Background

The world was recently threatened by COVID-19, a new pandemic caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) [1,2]. Globally, COVID-19 is the leading cause of death by an infectious disease since the first cases were reported in late December 2019 in Wuhan, China [3]. The worldwide negative impact of the pandemic has called for the rapid development of public health measures to control the spread of the virus including, diagnostic tools for a rapid and mass screening of SARS-COV-2 variant and antibodies [4]. In some ways, however, the virus is under better control as most countries are lifting COVID-19 restrictions. As of January, 2022, the number of confirmed COVID-19 cases in Africa stood at around 7 million and 155.505 of deaths representing respectively around 2% and 3% of the worldwide situation [5]. These low rates have been mainly attributed to a good campaign policy to combat the spread of the virus, the youth of its population, whose median age is 19 according to United Nation data, and to undetected COVID-19 cases [6,7]. WHO calculated that the true number of infections was seven times higher than the reported figure in Africa [6].

The Republic of Congo was among the affected sub-Saharan countries by SARS-CoV-2 pandemic with Brazzaville being the epicentre of the pandemic in the country. Our institution, the Fondation Congolaise pour la Recherche Médicale (FCRM) and the National Laboratory in Brazzaville were the only centres equipped to carry out the SARS-COV-2 PCR test. In addition, FCRM is also involved in the surveillance of SARS-COV-2 variants and serological testing [8,12]. Since the beginning of the pandemic, we have noted a very low number of individuals participating in voluntary testing or in the study designed to report on the real-time situation of the pandemic in the country. Most of the people tested are either travelers or medically-referred patients. Preliminary data from our KAP (Knowledge, attitudes, and practices toward COVID-19) study show high correlation trends between reluctance and the invasive nature of the sample recommended for molecular (Oropharyngeal or nasopharyngeal) and immunological (blood sample) testing (results not yet published). This prompted us to explore another type of sample that might be more comfortable for participants and better suited to monitoring current or future epidemic surveillance interventions for SARS-COV-2 infections. Numerous previous studies from across the world had already evaluated the efficacy of saliva-based SARS-CoV-2 molecular and immunological testing as a non-invasive sample in comparison to invasive recommended sample 4 [13,20]. However, there are controversy results surrounding its sensitivity [21,22]. Unlike sampling with blood and swabs, collection and processing methods for saliva are largely unstandardized, thus studies evaluating the efficacy of saliva-based SARS-CoV-2 testing have been conflicting and incommensurate, and often unclear or inconsistent about testing procedures and analyses [23]. Therefore, more studies are needed, as part of the integration of laboratory testing services with saliva as a non-invasive tool for genomic and immunological surveillance of upper respiratory pathogens including SARS-COV-2. The use of saliva in surveillance interventions, at least as a surrogate for the recommended samples, could help to better overcome the problem of reluctance observed alongside the pandemic while also serving as a basic tool for the surveillance of other diseases such as Monkeypox [23]. In addition, since WHO is developing recommendations for integrating SARS-CoV-2 surveillance into already existing respiratory disease surveillance systems, saliva could also better help in this framework [24]. The present study aimed at evaluating the use of saliva as a reliable tool for the surveillance of SARS-CoV- 2 infection in the Republic of Congo.

Materials and methods

Study design and participants: A cross-sectional study using the STARDreporting guidelines [25] was conducted, from 15th April 2022 to 13th January 2023, at the health centre of the Fondation Congolaise pour la Recherche Médicale (FCRM), Brazzaville, Republic of Congo, jointly in collaboration with the Institute of Tropical Medicine (ITM), Universität Tübingen, Germany. Any volunteer able to provide his own saliva living in Brazzaville was included in the study. Prior to sample collection, the participants were advised to refrain from consuming any food, beverages, or tobacco products for a minimum of 30 minutes. Trained personnel were responsible for overseeing and giving instructions to the subjects. A well-structured questionnaire was used to collect socio-demographic and clinical data including as the age, sex, weight, fever or fever history in the last 48 hours, headache, vomiting, nausea etc. Fever was defined based on axillary temperature ≥37.5°C [26,27]. Three types of samples were collected from each participant including oropharyngeal swab (using a cultiplast swab) and saliva excretion (self-collection by spitting in the 50 ml sterile conical tube) according to the recommended international protocol of COVID-19 specimen collection (CDC, 2021), and venous blood in EDTA tubes. Oropharyngeal swab and saliva excretion samples were processed and tested for COVID-19 using RT-PCR technique on one hand, plasma extracted from whole blood samples and saliva sample were used for anti-SARS-CoV-2 antibody measurement.

The assessment of the potency and reliability of saliva in the detection of active infection was done based on result of SARS-CoV-2 RNA analyses in comparison with result of oropharyngeal samples (gold standard) and by processing sequencing in saliva sample. The assessment of the potency and reliability of saliva in the exposure to the infection was done based on the results of anti-SARS-CoV-2 IgG antibody measurement in comparison with result obtained with gold standard (plasma samples).

The minimum sample size (N) was determined based on the following formula [28]:

$$N=\frac{Z^2 Sp(1\text{-}Sp)}{d^2 x(1\text{-}Prev)}$$

For α=0.05, Z is inserted by 1.96; Sp is the specificity of the evaluated test and was estimated at Sp=0.99. d was the precision of estimate (i.e. the maximum marginal error) and was assumed at 7% with 95% confidence level. Prev was the prevalence of Covid-19 in Congo and was estimated at 7.4% during the outbreak [10].

Then the based on all these parameters, the sample size of participant of this study was N=105 participants

Assuming we could have a rate of 10% of non-responses, the adjusted minimal sample size was: N1= 450/ (1-0.1) = 116 individuals.

SARS-CoV-2 RNA extraction and detection: RNA extraction from saliva and OPS samples was done using the QIAamp RNA mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Due to their high viscosity, the saliva samples were vortexed 3-5 minutes before processing.

For the detection of SARS-CoV-2 RNA, 10 µl of RNA template were tested in a master mixt using a RealStar® SARS-CoV-2 RT -PCR KIT 1.0 (Altona Diagnostics, Germany), which targets the B-β CVv specific RNA E gene and the SARS-CoV-2 specific S gene respectively. Samples were classified as positive for SARS-CoV-2 when the probes for the E and S genes were detected with a cycle threshold (Ct) value below 32 as recommended by the manufacturers. All tests were performed in the Light Cycler 480 real-time PCR system (Roche).

Saliva SARS-CoV-2 NGS sequencing: Oxford Nanopore sequencing Technology (ONT) was used to sequence saliva samples as we have previously described using the appropriate primers (S1 Table) [8-10]. Briefly, after amplicon quality control using the Qubit DNA BR, Thermo Scientific, libraries were prepared as described in Freed et al. (RAPID barcoding, 1200 bp amplicon) [29] and sequenced on GridION of the ONT platform. Sample-specific FastQ files, representing all reads generated for that sample, were aligned using the ARTIC field bioinformatics network [Github. https://github.com/artic-network/feldbioinformatics] pipeline for ONT and online tool (https://edge-covid19.edgebioinformatics.org/) [30]. Lineages of these genomes were annotated by Pangolin online tool and consensus genome sequences deposited in the GISAID database available under serial number (EPI_ISL_17287483, EPI_ISL_17287484, EPI_ISL_17287487, EPI_ISL_17287488, EPI_ISL_17287485, EPI_ISL_17287486, EPI_ISL_17287489).

Anti-SARS-CoV-2 IgG antibody detection in plasma and saliva sample: The measurement of IgG specific to SARS-CoV-2 RBD was done using in-house ELISAs techniques [17,31]. Briefly, high-binding plates (Corning) were coated overnight with 50 µl per well of 2 µg/ml of the SARS-CoV-2 RBD antigen suspended 1x PBS (Gibco). Wells were then washed once with 1x PBS without detergent, 200 µl per well, and blocked with 100 µl of the Blocking Solution (Candor Bioscience GmbH) for 2 hours at Room Temperature (RT) on a microplate shaker (700 rpm). Plasma samples and controls were serially diluted from 1:100 to 1:62500 using the blocking solution. The plates were washed three times with 200 µl per well with wash solution (1x PBS + 0.1% Tween 20). Diluted samples (100 ml) were added in each well and the plates were incubated for 1 hour (at RT with checking of 700 rpm). The HRP-coupled anti-human IgG (detection antibody) was diluted in 1:10.000 (first dilution 1:100 in 1x PBS tablet clean and second dilution 1:100 in 1x Roti Block (Carl Roth)). After plate washing, 50 µl per well of diluted detection antibody were added (Jackson Immuno Research Laboratories) and the plates were then incubated for 30 min (RT, 700 rpm). For visualization, 100 µl TMB substrate solution were added after four times washing with washing solution as described above, and the reaction was stopped with 50 µl/well of 1 M HCl. The colorimetric signal was measured at 450 nm and 620 nm using microplate reader (CLARIOstar, BMG LABTECH). The IgG concentration was expressed as µg/ ml and the cut-off value was previously set to 4.0 µg/ml [17].

The SARS-CoV-2 IgG detection in saliva was performed as described above, but with some modifications. Saliva samples were diluted from 1:3 up to 243 using the same blocking solution. Biotinylated anti-human IgG was diluted at 1:20.000 (first dilution 1:100 in 1x PBS tablet clean and second dilution 1:200 in 1x Roti Block) and used for IgG detection. The 1:1000 diluted Avidin-HRP (Biolegend) was applied for 30 min. The cut-off for salivary SARS-CoV-2 IgG positivity was previously set to 6.3 ng/ml [17].

Multi-centre comparison of Anti-SARS-CoV-2 IgG detection results with saliva: To be ensured of the reproducibility of anti-SARS-CoV-2 IgG detection results with saliva, the assay of Anti-SARS-CoV-2 IgG was done using the same in-house ELISA protocol in two independent laboratories located at the Institute of Tropical Medicine (ITM) of Tübingen University (Germany) and the FCRM laboratory in Brazzaville (Republic of Congo).

Ethics approval and consent to participate: This study received ethical approval from the independent Institutional Ethics Committee of Fondation Congolaise pour la Recherche Medical (No.038/CIE/FCRM/2022), the administrative authorizations from Marien Ngouabi University (No.081/UMNG. FST. DFD.FDSBIO). Prior to enrolment, a written consent form was signed by the participants or the parents (or guardian) of a minor child (<18 years of age). An assent form was signed by children aged between 15 to 17 years.

Statistical analysis: Participant characteristics were summarized using standard descriptive statistics. RStudio (Version 1.2.5001), running R (version 4.2.1.), Graph Pad Prism (Version 8.0.2) and IBM SPSS software version 22 were used for statistical analyses. Categorical variables were displayed as numbers and/or percentages and continuous data as median (interquartile range, IQR). The difference in antibody concentrations or RNA Ct values between the groups was analyzed with Mann–Whitney test. Kappa test was used to determine the degree of result concordance of SARS-CoV-2 test of saliva samples compared to the gold standards samples on one hand, and according to the baseline laboratory measurement (ITM of Tübingen University versus FCRM laboratory). Saliva sensitivity and specificity were determined. Sensitivity (Se) of the test is the ability of the test to correctly identify those who have the disease (true positive rate). Specificity (Sp) of the test is the ability of the test to correctly identify those who do not have the disease (true negative rate). Positive predictive value (PPV) is the probability that a disease is present when the test is positive. Negative predictive value (NPV) is the probability that the disease is not present when the test is negative. The following formula were used to determine each parameter.

Sensitivity = true positive / (true positive + false negative) × 100%

Specificity = true negative / (true negative + false positive) × 100%

PPV = true positive / (true positive + false positive) × 100%

NPV = true negative / (true negative + false negative) × 100%

Results

Characteristics of study population: A total of 220 participants were enrolled in this study, with a sex ratio of 0.8 (97/123) female/male (Table 1). The age range was between 7 to 83 years old, with the predominance of participant aged between 21 and 30 years that represented 30% (66) of the total population. The participants having the symptoms of a common cold represented 4% of the study population, while the less recording clinical sign was fever (2.7%).

Table 1: Characteristics of the study population.

	Number	Frequency (%)
Overall population	220	-
Sex ratio (Female/male)	0.8 (97/123)	
Median age (range) (year)	21 (7-83)	
Age ranges (year)		
≤10	6	2.7
[11-21]	79	35.9
[21-31]	66	30.0
[31-41]	17	7.7
[41-51]	11	5.0
[51-61]	16	7.3
≥61	25	11.4
Symptomatic	14	6.4
Clinical parameters		
Fever	6	2.7
Headache	7	3.2
Cough	8	3.6
common cold	9	4.0

Prevalence of SARS-CoV-2 in saliva and oropharyngeal swab (OPS) samples: Out of 220 individuals tested for SARS-CoV-2 active infection, 23/220 (10.5%) were positive with oropharyngeal swab while 22/220 (10%) were found positive in saliva samples (Table 2). The proportions of positive cases with saliva and OPS samples were similar in all age groups, except in individuals of ≥61 years old. Here, one sample was negative using saliva sampling which was positive in the OPS sampling (saliva 24% vs OPS 28%), but not statistically significant (p=0.7471). Regardless of the participant's clinical sign, the proportions of SARS-CoV-2 positive cases with saliva samples were comparable to those of positive cases with OPS samples.

Performance of saliva samples use in SARS-CoV-2 detection and its genome sequencing: The result of SARS-CoV-2 RT-PCR with OPS confirmed 100 % (22/22) positive and 99.5% (197/198) negative results found with saliva samples (Table 3). Among the saliva samples thought to be SARS-CoV-2 negative, 1 (0.5%) was confirmed to be positive with OPS samples. No SARS-CoV-2 positive saliva sample was diagnosed negative with OPS sample. According to the result from the gold standard (OPS samples), detection of SARS-CoV-2 from saliva samples showed a sensitivity of 95.7% (95% CI: 79.0-99.8%) and a specificity 100% (95% CI: 98.1-100%). There was 100% (95% CI: 85.1-100%) of chance to get a good positive result and 99.5% (95% CI: 97.2-100%) of chance to have true negative result with saliva samples. The result of the Cohen's kappa coefficient (k) showed a high agreement (kappa=0.95) between the SARS-CoV-2 detection in saliva samples and the result obtained from OPS samples. The mean cycle threshold (Ct) values of the SARS-CoV-2 PCR diagnostic with saliva samples (E gene: 23.6±3.9 and S gene: 23.8±4.3) and OPS (E gene: 22.5±3.3 and S gene: 22.3±3.3) were similar (p=0.2049 for E gene; p=0.0690 for S gene). To assess whether saliva is a reliable tool for genomic surveillance, we performed SARS-CoV-2 genome sequencing from 8 positive saliva samples. The sequencing result revealed an average Coverage of 95.5±2.8% with omicron as the main variant (S2 Table).

Table 2: Distribution of the proportion of SARS-CoV-2 positive saliva and OPS samples according to the participant's age and clinical signs.

	Saliva samples n (%)	OPS samples n (%)
Overall prevalence (n=220)	22(10.0)	23(10.5)
Age groups (years)		
≤10 (n= 6)	4(66.7)	4(66.7)
[11-21] (n= 79)	3(3.8)	3(3.8)
[21-31] (n= 66)	4(6.1)	4(6.1)
[31-41] (n= 17)	1(5.9)	1(5.9)
[41-51] (n= 11)	2(18.2)	2(18.2)
[51-61] (n= 16)	2(12.5)	2(12.5)
≥61 (n= 25)	6(24.0)	7(28.0)
Symptomatic		
Yes (n=14)	4(28.6)	4(28.6)
No (n=206)	18(8.7)	19(9.2)
Clinical signs		
Fever (n= 6)	2(33.3)	2(33.3)
Headache (n= 7)	2(28.6)	2(28.6)
Cough (n= 8)	3(37.5)	3(37.5)
common cold (n= 9)	3(33.3)	3(33.3)

OPS: Oropharyngeal Swab.

Performance of saliva use for anti- SARS-CoV-2 IgG antibody detection: Out of 148 paired plasma and saliva sample used to detect the biomarker of past infection (anti-SARS-CoV-2 IgG), 74.32% (110/148) were positive with saliva compared to a seroprevalence of 79.72% (118/148) found in plasma samples. Plasma samples were used as a gold standard to assess the performance of the use of saliva samples in the detection of anti-SARS-CoV-2 IgG. The result of anti-SARS-CoV-2 IgG detection in plasma confirmed 98.2% (108/110) positive and 73.7% (28/38) negative results found with saliva samples (Table 4). Among the saliva samples found anti-SARS-CoV-2 antibody positive, 2 (1.8%) were negative according to the plasma ELISA results. The saliva samples detected negative with anti-SARS-CoV-2 IgG represented 26.3% of plasma samples diagnosed positive to anti-SARS-CoV-2 IgG. Based on the gold standard results (plasma), anti-SARS-CoV-2 IgG detection from saliva samples showed the sensitivity of 92% (95% CI: 85.0-96.0%) and a specificity 93.3% (95% CI: 78.0-99.2 %). The result of the Cohen's kappa coefficient (k) showed a high agreement (kappa=0.78) between the anti-SARS-CoV-2 IgG detection in saliva samples and the result obtained from plasma samples (Table 4).

Multi-centre comparison of Anti-SARS-CoV-2 IgG detection in saliva: To ensure the reproducibility of the in-house saliva ELISA, 50 well characterized saliva samples were processed in two independent laboratories (ITM, Tübingen, Germany and FCRM, Brazzaville, Republic of Congo). Overall results (Figure 1) indicated a high agreement between result from ITM and FCRM laboratories 49/50 (Cohen's kappa 0.94; p=0.0001). One sample was positive at ITM lab and negative at FCRM lab. There anti-SARSCoV-2 IgG concentrations were similar (p=0.6641) between the two laboratories, with a significant positive correlation between the two parameters (r=0.9309; p<0.000).

Table 3: Performance parameters of SARS-CoV-2 detection with saliva samples compared to the oropharyngeal swab sample.

		OPS sample		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Kappa
		Positive	Negative					
Saliva sample n (%)	Positive	22(100)	0(0)	95.7	100	100	99.5	0.95
	Negative	1 (0.5)	197(99.5)	(79.0-99.8)	(98.1-100)	(85.1-100)	(97.2-99.9)	

OPS: Oropharyngeal Swab; PPV: Positive Predictive Value; NPV: Negative Predictive Value; CI: Confidence Interval.

Table 4: Performance parameters of the detection of anti-SARS-CoV-2 antibody with saliva samples compared to the oropharyngeal swab sample.

		Plasma		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Kappa
		Positive	Negative					
Saliva n (%)	Positive	108(98.2)	2(1.8)	92.0 (85.0-96.0)	93.0 (78.0-99.2)	98.2 (93.6-99.8)	73.7 (57.0-86.6)	0.78
	Negative	10(26.3)	28(73.7)					

OPS: Oropharyngeal Swab; PPV: Positive Predictive Value; NPV: Negative Predictive Value; CI: Confidence Interval.

Discussion

Substantial evidence has been reported on the non-inferiority of saliva compared to the Naso-Oropharyngeal Swab (NOS) as a specimen for conventional RNA extraction-dependent RT-qPCR detection of SARS-CoV-2 [32,33]. However, there is still limited data on the reported in sub-Saharan Africa including the republic of Congo, mainly for the detection of anti- SARS-CoV-2 antibody. Two major reasons justify the preference on saliva use for disease diagnostic, including non-invasive property of the sample and its collection does not need a well-trained health personnel. The present study was conducted to address the above-mentioned issues in the Republic of Congo.

Overall, the assessment of active infection showed similar rates of viral detection between saliva and OPS, as well as according to the clinical signs and the age group of participants. Furthermore, there was a high sensitivity (95.7%) and specificity (100%) of SARS-CoV-2 detection in saliva, indicating that this specimen is as sensitive and reliable as oropharyngeal for the surveillance of SARS-CoV-2 active infection. Our findings were similar to what was reported in a study performed in Russia 34, but were slightly higher compared to a study conducted in Philippines that reported a sensitivity of 91.3% and specificity of 98.9% for SARS-CoV-2 detection in saliva [35]. The high level of agreement between saliva and the gold standard OPS samples found here was consistent with the result of other studies [4,36,37].

When comparing the viral concentration (Ct values) between saliva and OPS samples based on the RT-qPCR results, we noticed no difference. These results are in line with previous reports in the literature showing the reliability of using saliva in SARS-CoV-2 detection by RT-qPCR [4,15,38].

Knowing that the genome completeness is rarely 100%, the 95.5% mean genome completeness found from sequencing results with saliva clearly indicates that, this specimen is useful for SARS-CoV-2 genomic studies, confirming previous reports [30]. The majority of RNA samples sequenced in this study showed that Omicron was the main strain circulating in the study population, which is in accordance with our sequencing results made on samples obtained across the country during the study period and published on GISAID platform. Here is some serial accession number of those sequences in GISAID: [EPI_ISL_13191428, EPI_ISL_13191427, EPI_ISL_13163557, EPI_ISL_11621477, EPI_ISL_11621476, EPI_ISL_11621475, EPI_ISL_11621468)]. A study conducted in South Africa reported that saliva was the most reliable tool for the detection of the Omicron variant due to higher viral shedding in saliva compared to mid-turbinate swabs [39].

Only few studies reported the use of saliva for SARS-CoV-2 antibody detection [18,19,31,40,42], while there was substantial evidence of assessing antibody profile against this virus in plasma sample even in republic of Congo [8,10]. The assessment of the performance of saliva in anti-SARS-CoV-2 antibody detection showed a similar rate of detection compared to plasma, with high sensitivity (92%) and specificity (93%). The same trend was observed with the finding of MacMullan and collaborators who reported the sensitivity and specificity of 84.2% and 100% respectively [40]. Compared to our study where we used a validated in-house ELISA, these authors used the commercial ELISA kits for the detection antibody. The fact that these two-study reached to similar result using different protocol of ELISA sustains the reliability of saliva as a surrogate diagnostic tool of plasma. Furthermore, a high agreement between saliva and plasma samples in antibody detection was observed in this study. We also found a high agreement when comparing the saliva ELISA results between the Institutes in Germany and the Republic of Congo.

This study has some limitations: Our sample set was not large enough to add different combination of analysis. So, future studies could improve the robustness by including a larger sample size at all time points. The sample size of participant tested positive was relatively small. Only four symptomatic cases were identified in this study so could not helped to compare the sensitivity of the two types of samples among symptomatic COVID-19 cases. We did not have information about the drug consumption by the participant, since it is well known that some drug might reduce the concentration of the virus, affecting the quality of the assays with saliva.

Conclusion

Overall, the findings of this study indicate that saliva can be used as a surrogate to oropharyngeal for SARS-CoV-2 RNA detection and genomic studies, as well as to plasma for SARS-CoV-2 antibody detection in the Republic of Congo. The non-

invasive property of saliva samples makes it more acceptable to people, which reduces reluctance to testing.

Declarations

Availability of data and materials: All data are fully available without restriction. Data are available from the FCRM Institutional Data Access. All request for Data should be addressed to the Executive Director of FCRM reachable by the following address Prof. Francine Ntoumi, villa D6, Cité OMS Djoué, Brazzaville, République du Congo (Tel: +242069977980, email: fntoumi@fcrm-congo.com). The genomic data of the samples, EPI_ISL_17287483, EPI_ISL_17287484, EPI_ISL_17287487, EPI_ISL_17287488, EPI_ISL_17287485, EPI_ISL_17287486, EPI_ISL_17287489 are available in GISAID.

Funding: This study was supported by CANTAM (EDCTP-CSA2020NoE-3100) and the Central Africa Humboldt research Hub (HR-Coca) of the Alexander von Humboldt foundation, ORCHESTRA (GRANT NUMBER 101016167), Bill and Melinda Gates Foundation (INV-036514) and the institute of Tropical Medicine, University of Tübingen. AL and JCD are supported by the Fondation Merieux.

Acknowledgments: We thank all the participants for their support during the sample collection. We thank Nick Desy MOUHINGOU, Armel Landry BATCHI-BOUYOU and all laboratory team for their help in sample processing in FCRM laboratory. We also thank, Georges MISSONTSA and Steve DIAFOUKA KIETELA for the administrative assistance.

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Supporting information:

S1 Table: List of primer used for Saliva SARS-CoV-2 NGS sequencing.

Primer pool 1

Primer names	Nucleotide sequence	Start	Pool	Size	Tm
SARSCoV_1200_1_Sens	ACCAACCAACTTTCGATCTCTTGT	30	1	24	60.69
SARSCoV_1200_1_Anti-sens	GGTTGCATTCATTTGGTGACGC	1205	1	22	61.49
SARSCoV_1200_3_ Sens	GGCTTGAAGAGAAGTTTAAGGAAGGT	2153	1	26	61.19
SARSCoV_1200_3_ Anti-sens	GATTGTCCTCACTGCCGTCTTG	3257	1	22	61.5
SARSCoV_1200_5_ Sens	ACCTACTAAAAAGGCTGGTGGC	4167	1	22	60.55
SARSCoV_1200_5_ Anti-sens	AGCATCTTGTAGAGCAGGTGGA	5359	1	22	61.16
SARSCoV_1200_7_ Sens	ACCTGGTGTATACGTTGTCTTTGG	6283	1	24	60.8
SARSCoV_1200_7_ Anti-sens	GCTGAAATCGGGGCCATTTGTA	7401	1	22	61.53
SARSCoV_1200_9_ Sens	AGAAGTTACTGGCGATAGTTGTAATAACT	8253	1	29	60.59
SARSCoV_1200_9_ Anti-sens	TGCTGATATGTCCAAAGCACCA	9400	1	22	60.29
SARSCoV_1200_11_ Sens	AGACACCTAAGTATAAGTTTGTTCGCA	10343	1	27	60.74
SARSCoV_1200_11_ Anti-sens	GCCCACATGGAAATGGCTTGAT	11469	1	22	61.8
SARSCoV_1200_13_ Sens	ACCTCTTACAACAGCAGCCAAAC	12450	1	23	61.55
SARSCoV_1200_13_ Anti-sens	CGTCCTTTTCTTGGAAGCGACA	13621	1	22	61.38
SARSCoV_1200_15_ Sens	TTTTAAGGAATTACTTGTGTATGCTGCT	14540	1	28	60.06
SARSCoV_1200_15_ Anti-sens	ACACACAACAGCATCGTCAGAG	15735	1	22	61.12
SARSCoV_1200_17_ Sens	TCAAGCTTTTTGCAGCAGAAACG	16624	1	23	61.28
SARSCoV_1200_17_ Anti-sens	CCAAGCAGGGTTACGTGTAAGG	17754	1	22	61.19
SARSCoV_1200_19_ Sens	GGCACATGGCTTTGAGTTGACA	18596	1	22	61.91
SARSCoV_1200_19_ Anti-sens	CCTGTTGTCCATCAAAGTGTCCC	19678	1	23	61.62
SARSCoV_1200_21_ Sens	TCTGTAGTTTCTAAGGTTGTCAAAGTGA	20553	1	28	60.58
SARSCoV_1200_21_ Anti-sens	GCAGGGGGTAATTGAGTTCTGG	21642	1	22	60.95
SARSCoV_1200_23_ Sens	ACTTTAGAGTCCAACCAACAGAATCT	22511	1	26	60.18
SARSCoV_1200_23_ Anti-sens	TGACTAGCTACACTACGTGCCC	23631	1	22	61.52
SARSCoV_1200_25_ Sens	TGCTGCTACTAAAATGTCAGAGTGT	24633	1	25	60.51
SARSCoV_1200_25_ Anti-sens	CATTTCCAGCAAAGCCAAAGCC	25790	1	22	61.45
SARSCoV_1200_27_ Sens	TGGATCACCGGTGGAATTGCTA	26744	1	22	61.75
SARSCoV_1200_27_ Anti-sens	TGTTCGTTTAGGCGTGACAAGT	27894	1	22	60.74
SARSCoV_1200_29_ Sens	TGAGGGAGCCTTGAATACACCA	28677	1	22	61.1
SARSCoV_1200_29_ Anti-sens	TAGGCAGCTCTCCCTAGCATTG	29790	1	22	61.61

Primer pool 2

Primer names	Nucleotide sequence	Start	Pool	Size	Tm
SARSCoV_1200_2_ Sens	CCATAATCAAGACTATTCAACCAAGGGT	1100	2	28	61.27
SARSCoV_1200_2_ Anti-sens	ACAGGTGACAATTTGTCCACCG	2266	2	22	61.33
SARSCoV_1200_4_ Sens	GGAATTTGGTGCCACTTCTGCT	3144	2	22	61.66
SARSCoV_1200_4_ Anti-sens	CCTGACCCGGGTAAGTGGTTAT	4262	2	22	61.49

SARSCoV_1200_6_ Sens	ACTTCTATTAAATGGGCAGATAACAACTG	5257	2	29	60.18
SARSCoV_1200_6_ Anti-sens	GATTATCCATTCCCTGCGCGTC	6380	2	22	61.75
SARSCoV_1200_8_ Sens	CAATCATGCAATTGTTTTTCAGCTATTTTG	7298	2	30	60.39
SARSCoV_1200_8_ Anti-sens	TGACTTTTTGCTACCTGCGCAT	8385	2	22	61.39
SARSCoV_1200_10_ Sens	TTTACCAGGAGTTTTCTGTGGTGT	9303	2	24	60.32
SARSCoV_1200_10_ Anti-sens	TGGGCCTCATAGCACATTGGTA	10451	2	22	61.5
SARSCoV_1200_12_ Sens	ATGGTGCTAGGAGAGTGTGGAC	11372	2	22	61.48
SARSCoV_1200_12_ Anti-sens	GGATTTCCCACAATGCTGATGC	12560	2	22	60.48
SARSCoV_1200_14_ Sens	ACAGGCACTAGTACTGATGTCGT	13509	2	23	61.12
SARSCoV_1200_14_ Anti-sens	GTGCAGCTACTGAAAAGCACGT	14641	2	22	61.94
SARSCoV_1200_16_ Sens	ACAACACAGACTTTATGAGTGTCTCT	15608	2	26	60.18
SARSCoV_1200_16_ Anti-sens	CTCTGTCAGACAGCACTTCACG	16720	2	22	61.17
SARSCoV_1200_18_ Sens	GCACATAAAGACAAATCAGCTCAATGC	17622	2	27	62.03
SARSCoV_1200_18_ Anti-sens	TGTCTGAAGCAGTGGAAAAGCA	18706	2	22	60.68
SARSCoV_1200_20_ Sens	ACAATTTGATACTTATAACCTCTGGAACAC	19574	2	30	60.15
SARSCoV_1200_20_ Anti-sens	GATTAGGCATAGCAACACCCGG	20698	2	22	61.39
SARSCoV_1200_22_ Sens	GTGATGTTCTTGTTAACAACTAAACGAACA	21532	2	30	61.44
SARSCoV_1200_22_ Anti-sens	AACAGATGCAAATCTGGTGGCG	22612	2	22	62.03
SARSCoV_1200_24_ Sens	GCTGAACATGTCAACAACTCATATGA	23518	2	26	60.13
SARSCoV_1200_24_ Anti-sens	ATGAGGTGCTGACTGAGGGAAG	24736	2	22	61.74
SARSCoV_1200_26_ Sens	GCCTTGAAGCCCCTTTTCTCTA	25690	2	22	60.29
SARSCoV_1200_26_ Anti-sens	AATGACCACATGGAACGCGTAC	26857	2	22	61.5
SARSCoV_1200_28_ Sens	TTTGTGCTTTTTAGCCTTTCTGCT	27784	2	24	60.14
SARSCoV_1200_28_ Anti-sens	GTTTGGCCTTGTTGTTGTTGGC	29007	2	22	61.82

S2 Table: Summary of relevant metadata associated with saliva samples sequenced.

Saliva samples	Pangolin lineage	Nextrain Clade name	Who Name	Diagnosis Ct value	Fast qc raw read pairs	Coverage (%)
M6COM01	BA.5.2.18	22B	Omicron	20.59	44814	95.40
M6COM02	BA.4.1	22A	Omicron	23.01	15803	93.81
M16COM04	BA.5	22B	Omicron	23.03	88.622	95.69
M37COM06	BA.5	22B	Omicron	28.0	55448	97.64
M37COM08	BA.5	22B	Omicron	27.7	31665	95.19
M60COM05	BA.4	22A	Omicron	22.30	11483	90.93
VAC452	AY.122	21J	Delta	17.5	131645	99.89