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Diagnostic accuracy of non-invasive SARS-CoV-2 screening tests: a national prospective analysis

Thomas Althaus[1*], Alexandre Blake[2], Alizée Costantini[3], Christian Lavagna[3], Eva Jacquesson[3], Guillaume Groshenry[3], Alexa Troel[4], Bertrand Vanzo[4], Olivier Dejoux[3], Hervé Raps[3], Patrick Rampal[3] and Eric J. Voiglio[1]

Abstract

Background Providing non-invasive, accurate and affordable SARS-CoV-2 tests represents a public health priority, to better control the spread of the virus while protecting healthcare workers. Saliva is a robust alternative to nasopharyngeal (NP) swabs, but there is heterogeneity in collection and pre-analytical methods.

Methods Relying on a national COVID-19 Public Health Programme, we prospectively recruited 3,488 symptomatic and asymptomatic adults attending the Monaco community centre for NP RT-PCR testing from February 2021–2023. Saliva was concomitantly obtained with either a buccal swab or an oral sponge (OS) and analysed by an RT-PCR assay and a fully automated electrochemiluminescence enzyme immunoassay (ECLIA) rapid antigen test (RAT).

Results The sensitivity of the buccal RT-PCR varied according to previous SARS-CoV-2 infection, vaccination, and the presence of symptoms, while it remained around 95% for the OS RT-PCR. The specificity of the buccal RT-PCR approached 100% and was around 95% for the OS-RT PCR. The RAT sensitivity was 66.9% and 69.1% compared to NP and OS RT-PCR assays and increased to 71% and 97% in case of a high viral load (Ct < 25), respectively.

Conclusions RT-PCR assay using OS saliva showed high accuracy in symptomatic and non-symptomatic adults, including the identification of negative nasopharyngeal swabs. This method allows self-collection without any prior conditions for the patient nor laboratory pre-analytical steps. The ECLIA RAT offers high throughput but is only useful in individuals with high viral loads. Our findings encompassed the latest SARS-CoV-2 omicron subvariants, such as BA.4&5 and the XBB series.

Trial registration Registry: ClinicalTrials.gov. Registration number: NCT05074745. Registration date: 12-10-2021.

Keywords SARS-CoV-2, RT-PCR, Saliva, Oral sponge, Diagnostic accuracy, Rapid antigen test

*Correspondence:
Thomas Althaus
talthaus@gouv.mc
[1]Directorate of Health Affairs, 48 boulevard d'Italie, Monaco 98000, Monaco
[2]Independent Researcher, Durham, UK
[3]Centre Scientifique de Monaco, 8 Quai Antoine 1er, Monaco 98000, Monaco
[4]Interministerial Delegation for the Digital Transition, 2 rue du Gabian, Monaco 98000, Monaco

Background

The COVID-19 pandemic had a devastating impact on frontline health workers (HW), most of whom were nurses in close contact with SARS-CoV-2 patients [1]. The World Health Organisation (WHO) promotes the protection of HW testing for highly infectious pathogens such as SARS-CoV-2 as a priority [2]. Diagnostic tests are performed using nasopharyngeal (NP) swabs as a reference [3]. However, this invasive sampling strategy exposes HW to a high risk of contamination, in contrast to non-invasive methods such as saliva sampling, which reduces exposure to respiratory pathogens, with fewer constraints on training and biosafety measures [4, 5]. Regarding diagnostic qualities, there is increasing evidence that saliva provides comparable results to NP swabs when tested with reverse-transcriptase polymerase chain reaction (RT-PCR) assay, the gold-standard, however, there is a lack of consensus on sample collection, and analysis protocol [6]. Additional evidence on non-invasive sampling strategies reports inconsistent estimates regarding diagnostic quality and high heterogeneity of sampling protocols. A 2022 systematic review of 44 studies found an agreement of 89.7%, while a 2023 meta-analysis gathering 23 studies calculated a pooled sensitivity of 87% and specificity of 99% [7, 8]. Both reported different sampling conditions with no eating, drinking or smoking; a broad diversity of sampling methods; and frequent additional laboratory processing to reduce saliva viscosity. A single study simplified saliva self-sampling and processing using an oral sponge, but only included 147 symptomatic adults [9]. This indicates a lack of comparability in diagnostic accuracy evaluations using molecular assays.

This heterogeneity in saliva sampling and processing also applies to rapid antigenic tests (RATs), while relying on small sample sizes and only showing utility in case of high viral load [10–12]. A 2022 meta-analysis evaluating several diagnostic assays based on passive drool methods reported inconsistent head-to-head comparisons with NP RT-PCR testing [13]. The highest diagnostic accuracy was found when using chemiluminescent enzyme immunoassays, with a sensitivity varying between 82 and 93%. For saliva RATs, self-sampling methods have shown high concordance with professional sampling [14]. However, they also suffer from high heterogeneity, which prevents generalisation for routine use.

Furthermore, both RT-PCR assays and RATs evaluations tend to omit certain factors influencing viral detection and thus test utility, such as previous SARS-CoV-2 infections or vaccination status, emphasizing the potential lack of comparability across studies. Diagnostic evaluations also tend to cover a limited time period, with results only valid for specific variants. Lastly, studies gathered a limited number of confirmed SARS-CoV-2 cases, while relying on small sample sizes without a priori sample size calculation, which is a frequent bias in COVID-19 clinical research [15–17]. We here evaluated saliva RT-PCR assay and RATs using an oral swab and an oral sponge (OS) without any pre-analytical step, in both symptomatic and non-symptomatic adults in Monaco, concomitant to NP RT-PCR testing.

Methods

Study setting

Monaco is the most densely populated country in the world with 36,297 inhabitants living on a 2.02 square kilometre territory, with a median age of 46.4 years [18]. Demographic and health system features are comparable to neighbouring larger western European countries. Non-resident workers daily commute to Monaco, mostly from France and Italy, doubling the local population [19]. The circulation of SARS-CoV-2 was high in Monaco during the COVID-19 pandemic, particularly in households [20]. SARS-CoV-2 screening and vaccination services were freely provided to national residents and non-residents *via* the Monaco COVID-19 Public Health Programme (MCPHP), in a single primary care setting. Screening consisted of nasopharyngeal (NP) RT-PCR-testing. Additional SARS-CoV-2 screening included rapid antigen tests (RATs) validated by the French National Authority for Health (https://covid-19.sante.gouv.fr/tests), in Monaco pharmacies. Vaccination followed the WHO recommendations, and exclusively relied on the Pfizer mRNA vaccines, starting on the 31st of December 2020 [21]. All SARS-CoV-2 data (testing and vaccination) was automatically registered in the MCPHP national dataset. Any SARS-CoV-2 vaccine injection(s) and/or test result(s) carried out outside of the Principality of Monaco was only registered if providing corresponding evidence (e.g., vaccine injection report).

Study design and population

We designed a prospective cohort using simple random sampling: any individual aged of 18 years-old or more attending the primary care setting for SARS-CoV-2 NP RT-PCR testing was offered to participate to the CORDAGES study, regardless of the cause of attendance. Participants were asked to sign a consent form, while demographics were recorded on the MCPHP dataset by a HW. Participants were asked about their lifestyle, risk exposure to SARS-CoV-2, comorbidities, presence of symptoms, and contact with a confirmed SARS-CoV-2 case. Four tests were evaluated in two distinct periods of time: (1) buccal swab (BS) using an RT-PCR test and a RAT, sampling saliva from the inner side of the cheek from February 2021 to November 2021; (2) OS using an RT-PCR test and a RAT from December 2021 to February 2023. Only NP RT-PCR tests were communicated to

the participants *via* an automatic message system. The CORDAGES study flowchart is presented on Fig. 1.

Biological sampling management

For NP and BS, HWs used the cobas® PCR Media Dual Swab Sample Kit (Ref. 07958021190, Roche Diagnostics, Switzerland). For OS, participants used the Merocel® Standard Dressing (ref 400400; Medtronic, Dublin, Ireland), as previously published, and illustrated in Supplementary Fig. 1 [9]. Then, HWs intervened to exert a light pressure on the barrel of the syringe, in order to extract a minimum of 10 drops of saliva into the tube:

- For OS RT-PCR test: the tube (Ref. 07958048190, Roche Diagnostics, Switzerland) was previously filled with 1,8mL of lysis buffer (Ref. 06997538190, Roche Diagnostics, Switzerland).
- For OS RAT, the tube (Ref. 07958048190, Roche Diagnostics, Switzerland) was previously filled with 800µL of the SARS-CoV-2 Extraction Solution (Ref. 09370064190, Roche Diagnostics, Switzerland).

All tubes (NP, BS and OS) were then labelled with the participant's identifier (last name/name/date of birth/file number) by the HW and placed in a fridge at a controlled temperature between 4 and 8 °C, before being shipped to the Centre Scientifique de Monaco (CSM), the national laboratory, every two hours. Results from the BS RAT (chemo-colorimetric reaction based on an enzyme-linked immune-assay, targeting the nucleocapsid, PORTABLE COVID-19 ANTIGEN LAB®) were manually entered in the MCPHP dataset 15 min after testing [22].

All other tests were automatically uploaded in the MCPHP dataset from the laboratory. RT-PCR tests (NP, BS, and OS) were carried out the using the cobas6800 (Roche, Switzerland), targeting the ORF1a and E genes in the CSM laboratory. The OS RAT relied on a electrochemiluminescence enzyme immunoassay (ECLIA) targeting the nucleocapsid antigen, *via* a fully automated analysis using the cobas e801 (Elecsys® SARS-CoV-2 Antigen test, Roche, Switzerland) [23].

Statistical methods

In the initial part of the study starting in February 2021, we evaluated the RT-PCR test and RAT in the saliva from the inner side of the cheek, using a BS. With a viro-prevalence of 5% on Monaco at that time (https://www.worldometers.info/coronavirus/country/monaco/), we calculated the a priori sample size using the following formula [24] :

- For sensitivity, $n_{Se} = Z_{\alpha/2}{}^2 * Se * (1 - Se) / d^2 *$ Prevalence.
- For specificity, $n_{Sp} = Z_{\alpha/2}{}^2 * Sp * (1 - Sp) / d^2 * (1 -$ Prevalence).

Considering $\alpha = 0.05$, $Z_{\alpha/2}{}^2$ is equal to 1.96; Se, Sp and prevalence are predetermined values for sensitivity,

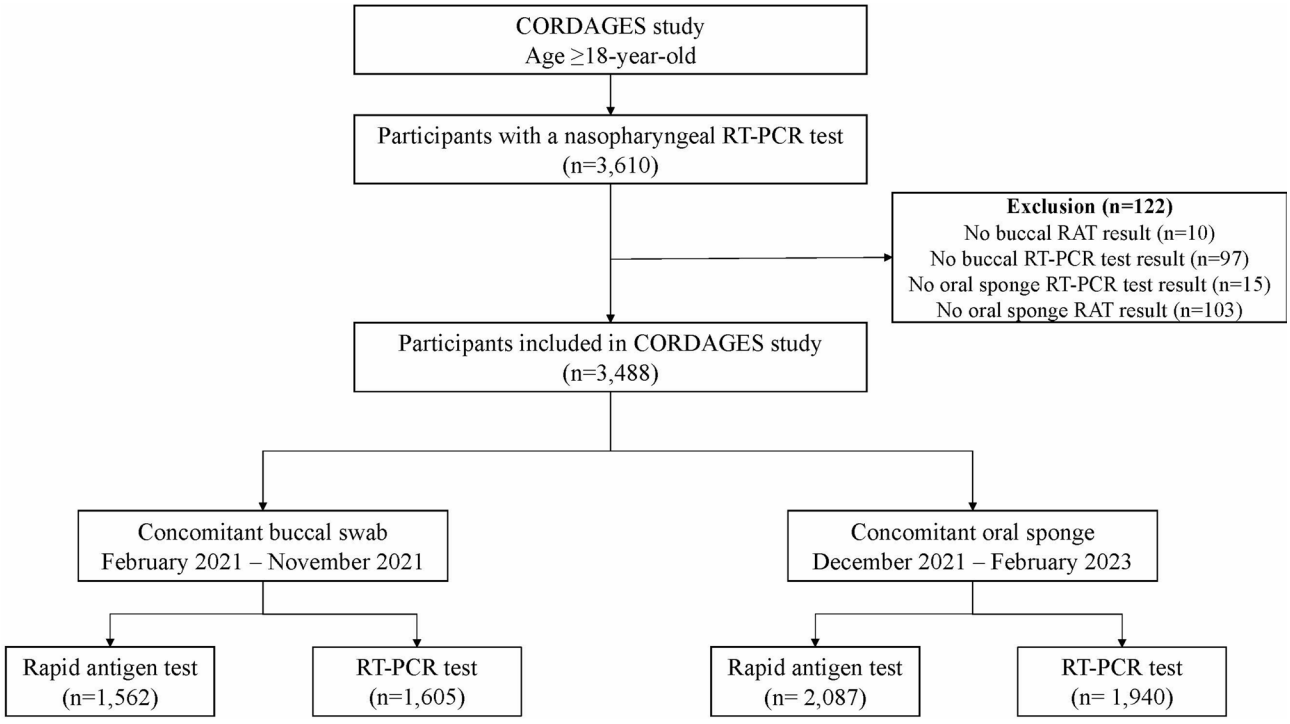

Fig. 1 CORDAGES study flowchart between February 2021 to February 2023 in Monaco

specificity and prevalence of SARS-CoV-2, respectively. The term « d » corresponds to the estimate accuracy (i.e., maximum marginal error). Following manufacturer's recommendations both for the RT-PCR test and chemo-colorimetric RAT in BS, we expected a sensitivity and specificity above 95% compared to the gold standard, which is the NP RT-PCR test [25, 26]. In order to limit the maximum marginal error to 5% with a 95% confidence, sample size was determined by inserting the following values in the formula: for sensitivity, the minimum number of participants was 1460 participants, and 77 for specificity. Measurement of sensitivity and specificity is summarised as follows:

	Reference positive	Reference negative
Index positive	True positive (TP)	False positive (FP)
Index negative	False negative (FN)	True negative (TN)

Sensitivity (Se) = TP / TP + FN

Specificity (Sp) = TN / TN + FP

Positive predictive value (PPV) = TP / TP + FP

Negative predictive value (NPV) = TN / TN + FN

In the OS RT-PCR assay, we estimated our a priori sample size based on a study using the same protocol, with a sensitivity varying between 93.2 and 94.2% compared to the NP RT-PCR test [9]. We rounded our sample size to 1,750 participants using the same formula. Regarding the automated OS ECLIA RAT (Elecsys® SARS-CoV-2 Antigen test, Roche, Switzerland), we used the manufacturer statement for nasal swab sensitivity (around 94%) to calculate a minimal a priori sample size of 1,750 participants [23].

The primary outcome was the diagnostic accuracy of saliva from either BS or OS using RT-PCR tests and ECLIA RATs, with a concomitant head-to-head comparison with the NP RT-PCR assay. We further analysed our data by subgroup (symptomatic *versus* asymptomatic; previously infected by a documented SARS-CoV-2 infection *versus* naïve from a previous SARS-CoV-2 infection; vaccination against COVID-19 *versus* no vaccination against COVID-19). Sensitivity and true positives were defined as the proportion of participants with a positive NP RT-PCR test for SARS-CoV-2. Specificity and true negatives were defined as the proportion of participants with a negative NP RT-PCR test for SARS-CoV-2. The clinical accuracies of the saliva tests from BS and OS were analysed using Receiver Operator Characteristic (ROC) plots [27]. ROC area under the curve (AUC) were calculated as the fraction "correctly identified to be positive" and the fraction "falsely identified to be positive". An AUC between 0.9 and 1 was defined as excellent accuracy, 0.8 to 0.9 as very good, 0.7 to 0.8 as good, 0.6 to 0.7 as sufficient, 0.5 to 0.6 as bad, and < 0.5 as poor.

Results

A total of 3,488 adults participated to the CORDAGES study: saliva from buccal swab (BS) was analysed between February and November 2021, including 1,605 by RT-PCR tests and 1,562 chemo-colorimetric RATs, while saliva from oral sponge (OS) was analysed between December 2021 and February 2023, including 1,940 RT-PCR tests and 2,087 ECLIA RATs (Fig. 1), for a total of 7,194 tests evaluated. Characteristics of participants are described in Supplementary Table 1. In brief, around half of our participants were females (median age between 44-51-year-old across test groups), and less than half had symptoms. BS and OS RT-PCR tests are detailed in Supplementary Table 2 according to NP RT-PCR tests.

Buccal swab: sensitivity of the RT-PCR test showed an overall sensitivity of around 85% and varied according to the presence of a SARS-CoV-2 previous infection (Table 1). Specificity of the BS RT-PCR test was approaching 100%, irrespective of a previous SARS-CoV-2 infection, vaccination, or presence of symptoms. The area under the curve (AUC) was calculated at 0.924 (95% confidence interval 0.895–0.953). BS RAT diagnostic performance showed 26.5% sensitivity and 78.0% specificity (Supplementary Tables 3 and 4).

Oral sponge: RT-PCR test showed a sensitivity of around 95%, irrespective of vaccination or presence of symptoms. In case of a SARS-CoV-2 previous infection, the sensitivity dropped to 89.8% (95% CI 87.3–92.3) and rose to 96.2% (95% CI 95.2–97.2) in the absence of it. Specificity remained around 95%, irrespective of a previous SARS-CoV-2 infection, vaccination or presence of symptoms. The AUC was measured at 0.948 (95%CI 0.936–0.959). Comparing the viral load of the RT-PCR assay according to the sampling method, the nasopharyngeal sphere was significantly greater than saliva from OS (p-value < 0.001), itself higher than saliva from BS(p-value 0.024), with a median Ct of 20.6 (95% CI [18.2–24.7]), 28.8 (95% CI [26.4–31.4] and 30.0 (95% CI [27.2–31.6]), respectively (Fig. 2). Considering OS RAT (Table 2), the most suitable threshold was 0.67 and 0.71 when comparing with NP and OS RT-PCR assays, respectively (sensitivity 66.9%, specificity 93.7%, AUC 0.814; and sensitivity 69.1%, specificity of 97.5%, AUC 0.849, respectively). For the analyses presented below, we used a threshold of 0.7. Details of the most suitable threshold evaluation is provided in Supplementary Table 5. The highest sensitivity for OS RAT was obtained amongst participants with the highest viral load (i.e., Ct < 25): 71% and 97% comparing to NP and OS RT-PCR assays, respectively. Clinical performance of the buccal and OS RT-PCR tests, and OS RAT are illustrated using ROC curves on Fig. 3.

Violin plots and overlayed boxplots are comparing SARS-CoV-2 viral load (in cycle thresholds) between nasopharyngeal, saliva from buccal and oral sponge

Table 1 Comparison of SARS-CoV-2 diagnostic accuracy using RT-PCR tests in buccal and saliva compared to nasopharyngeal swabs overall, and according to previous SARS-CoV-2 infection, vaccination status, and presence of symptoms in Monaco

	Nasopharyngeal						
	Overall	**Previously infected**	**Not previously infected**	**Vaccinated**	**Not vaccinated**	**Presence of symptoms**	**Absence of symptoms**
Buccal							
Sensitivity (95% CI)	85.3 (83.6–87.1)	30.8 (21.0–40.5)	90.8 (89.3–92.2)	100 (100–100)	83.6 (81.6–85.6)	85.7 (83.0–88.4)	84.9 (82.6–87.1)
Specificity (95% CI)	99.7 (99.4–99.9)	100 (100–100)	99.6 (99.3–99.9)	99.6 (98.9–100)	99.7 (99.4–100)	99.6 (99.2–100)	99.7 (99.3–100)
PPV (95% CI)	96.1 (95.1–97.0)	100 (100–100)	95.9 (94.9–96.9)	93.8 (90.9–96.7)	96.4 (95.4–97.4)	97.1 (95.7–98.4)	94.9 (93.5–96.3)
NPV (95% CI)	98.6 (98.0–99.2)	89.0 (82.4–95.6)	99.1 (98.7–99.6)	100 (100–100)	98.3 (97.6–99.0)	98.1 (97.0–99.1)	98.9 (98.3–99.6)
Saliva							
Sensitivity (95% CI)	95.0 (94.1–96.0)	89.8 (87.3–92.3)	96.2 (95.2–97.2)	95.5 (94.4–96.6)	94.0 (92.1–95.9)	95.2 (93.7–96.6)	94.9 (93.6–96.2)
Specificity (95% CI)	94.8 (93.8–95.8)	95.2 (93.4–97.0)	94.6 (93.4–95.8)	95.1 (93.9–96.3)	94.2 (92.3–96.0)	95.7 (94.3–97.1)	94.3 (92.9–95.7)
PPV (95% CI)	93.1 (92.0–94.2)	86.8 (84.1–89.6)	94.5 (93.3–95.7)	93.4 (92.0–94.7)	92.6 (90.5–94.7)	96.6 (94.3–97.0)	90.7 (89.0–92.4)
NPV (95% CI)	96.3 (95.4–97.1)	96.3 (94.8–97.9)	96.2 (95.2–97.2)	96.7 (95.8–97.7)	95.3 (93.6–97.0)	95.2 (93.8–96.7)	96.9 (95.9–97.9)

PPV – Positive predictive value

NPV – Negative predictive value

95% CI – 95% Confidence interval

sampling methods. Box edges represent the first and third quartiles, with the inner line showing the median Ct value. Whiskers extend to 1.5 times the interquartile range.

Discussion

Using a real-life SARS-CoV-2 testing community service, RT-PCR on saliva from oral sponge (OS) showed high diagnostic accuracy while being non-invasive. It has high potential as an alternative testing tool to NP RT-PCR. Beyond a 95% sensitivity, the OS RT-PCR assay presented additional advantages: around 5% of participants tested negative in NP swabs were detected in saliva; self-collection was likely to reduce HW's exposure and thus risk contamination; a low cost; and the absence of any pre-analytical step. This latter point refers to a lack of standardised and simple protocols for saliva sampling, while previous evaluations did not rely on such a large sample size analysis [7–9].

We also brought evidence on a fully automated ECLIA RAT in saliva, which was initially designed for NP swabbing, and measured similar performance [28]. This ECLIA RAT failed to meet the WHO recommendations, except among participants with a high viral load (Ct < 25), achieving a sensitivity of 97% when compared with the OS RT-PCR assay. Considering the throughput (300 samples in 120 min) at a moderate cost (USD 5 per test), this automated RAT may be relevant in large gathering events or health facilities where diagnostic capacity is limited, mostly to identify patients with high viral loads. Furthermore, each SARS-CoV-2 strain is defined by specific respiratory tropism, potentially affecting tests' diagnostic accuracy [29]. Our OS evaluation was carried out from December 2021 to February 2023, and thus updated evidence on diagnostic accuracy by encompassing SARS-CoV-2 omicron recombinant subvariants such as BA.4, BA.5 and the XBB series (Supplementary Fig. 2) [30]. This extended recruitment period should be seen in the context of the rarity of studies that have included such a large number of circulating variants in their analyses, including for RATs [31]. From a public health perspective, self-collection (and not self-testing) is encouraged by WHO [32]. As an example, Japan used a similar automated method for mass screening SARS-CoV-2 antigen in several airports and community health centre across the country [33]. Studies have reported contrasted results when comparing saliva self-collection and NP swabs by a HW [34–36]. A literature review and meta-analysis comparing self-sampling HW-collected RATs reported that deviations in the sampling procedure were the main cause of sensitivity loss, while highlighting the inadequacy of both quantity and quality of eligible studies [14]. We here bring evidence that saliva self-collection with OS could be similarly sensitive, while not requiring

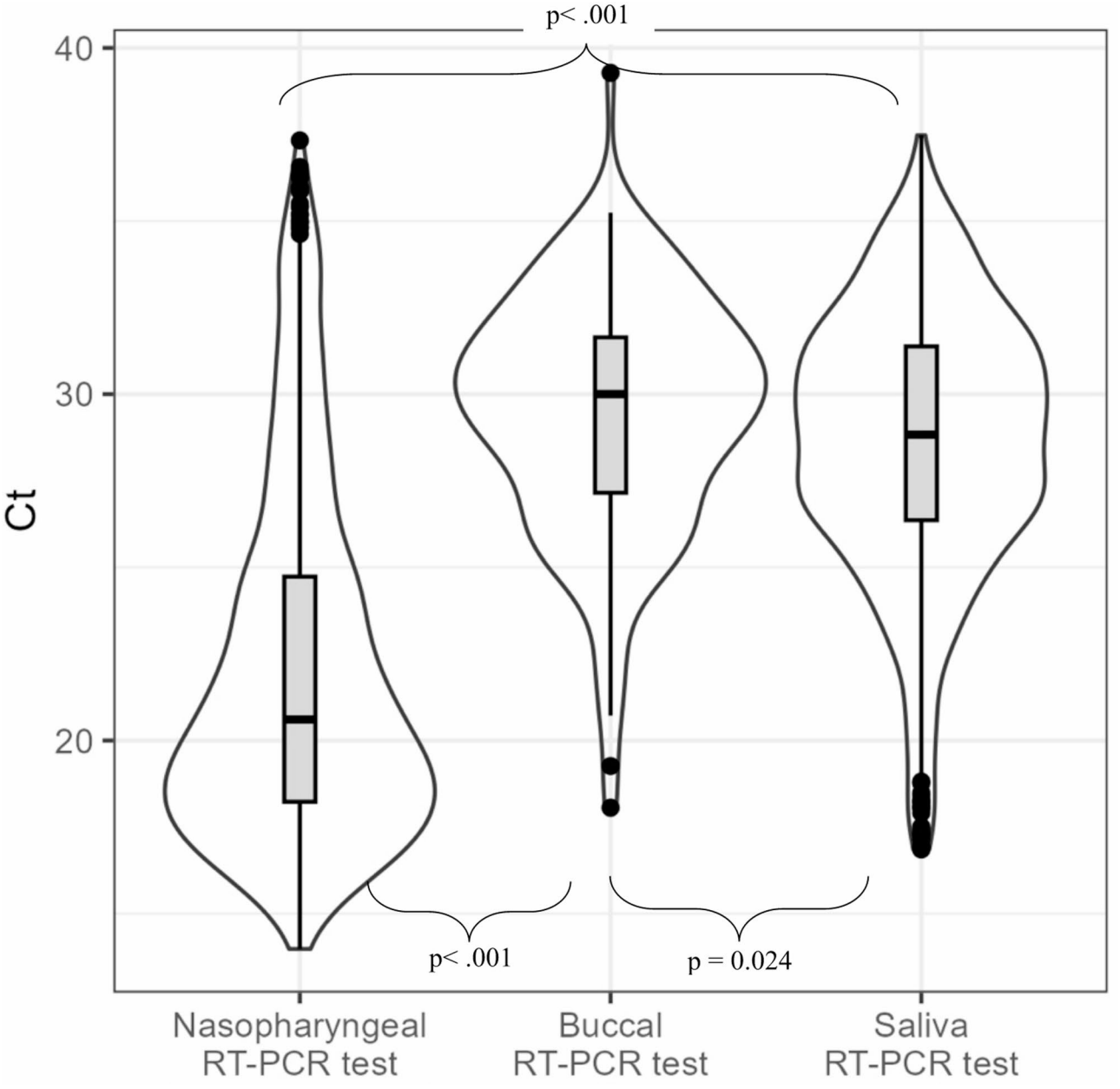

Fig. 2 Comparison of SARS-CoV-2 viral load (in cycle thresholds, Ct) using the RT-PCR assay in nasopharyngeal, saliva from either buccal swab or oral sponge

Table 2 Diagnostic accuracy of oral sponge (OS) rapid antigen test using a cut-off index of 0.7 according to cycle threshold (Ct) values compared with the RT-PCR assay in the nasopharynx (NP) and saliva from oral sponge (OS)

Test of reference	Ct values	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
NP RT-PCR	All samples	0.64 (0.61–0.67)	0.96 (0.95–0.97)	0.814 (0.794–0.834)
	< 30	0.68 (0.64–0.70)	0.97 (0.95–0.98)	
	< 25	0.71 (0.68–0.74)	0.97 (0.95–0.98)	
OS RT-PCR	All samples	0.70 (0.67–0.73)	0.96 (0.95–0.97)	0.849 (0.829–0.870)
	< 30	0.96 (0.94–0.98)	0.97 (0.96–0.98)	
	< 25	0.97 (0.94-1.00)	0.97 (0.96–0.98)	

AUC – Area under the curve; 95% CI – 95% confidence interval; Ct – Cycle threshold

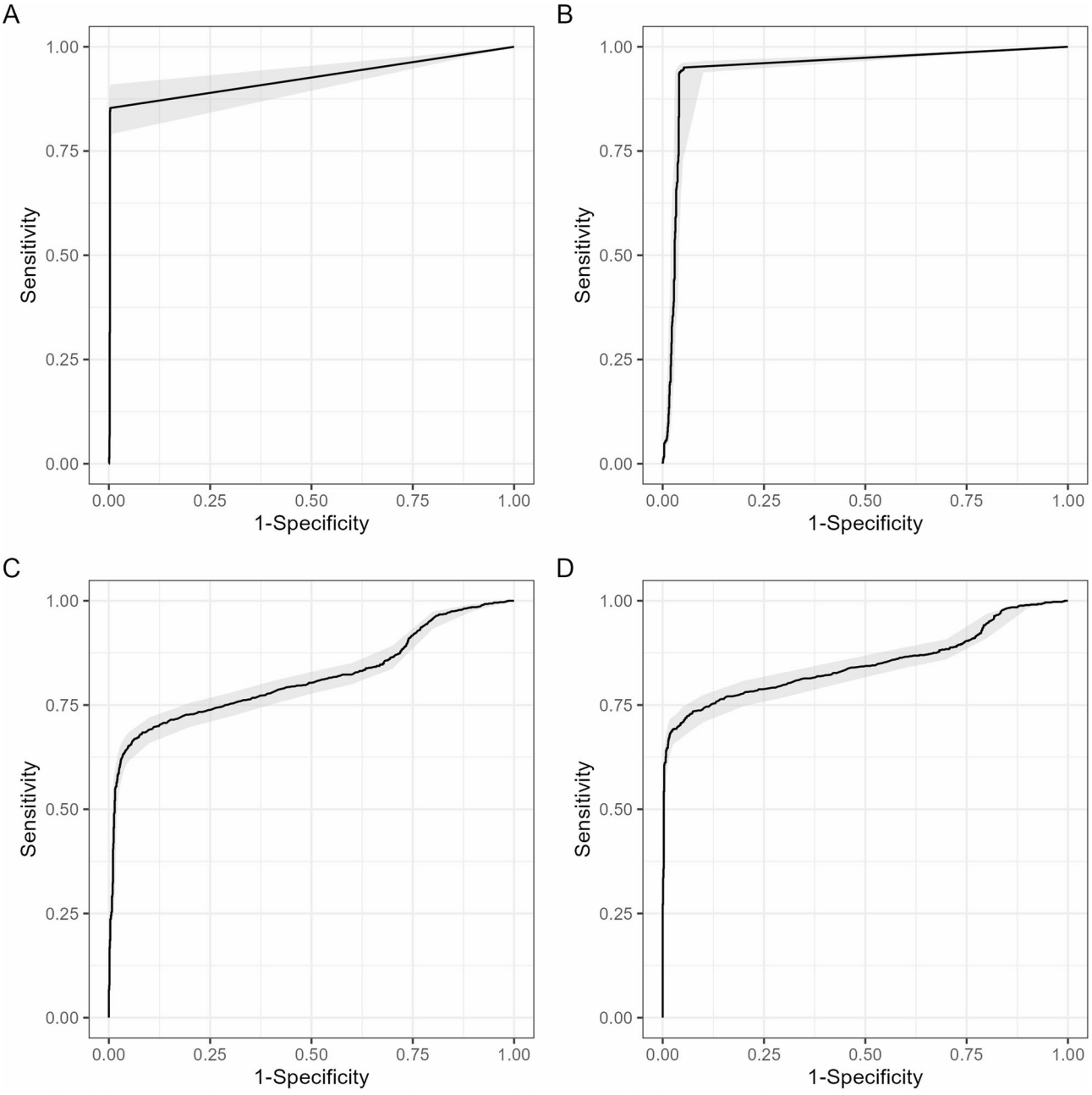

Fig. 3 Receiver operating characteristics (ROC) curves of buccal RT-PCR test (**A**), oral sponge (OS) RT-PCR test (**B**) against nasopharyngeal (NP) RT-PCR test, and OS RAT against NP RT PCR test (**C**) and OS RT-PCR test (**D**). The grey zone corresponds to the 95% confidence interval

any particular preparation from the patient nor any laboratory pre-analytical step. This indicates the possibility to screen a large number of individuals, while increasing acceptability amongst the population [37]. Another strength of our findings relates to a prospective recruitment with an a priori sample size and head-to-head concomitant testing with the gold standard method, namely the NP RT-PCR assay. This makes our findings reliable and potentially reproducible in other contexts.

Our findings suffer from several limitations: first, our analysis did not include children, while this population could also beneficiate from non-invasive SARS-CoV-2 testing methods. Paediatric assessments on such tests already exist, but usually rely on small sample size and/or do not include more recent SARS-CoV-2 variants and subvariants. Second, our two saliva sampling methods were not carried out concomitantly, with different circulating SARS-CoV-2 strains and prevalences, potentially explaining why saliva from BS presented lower accuracy

than from OS. In the literature, RT-PCR assays using saliva from passive drool methods or OS showed greater diagnostic performance than buccal swabbing, making unlikely this temporal difference to change our findings [38]. We considered the 5% discordant participants between NP and OS using RT-PCR tests to be a failure of NP to detect the virus rather than false positives from the OS RT-PCR assay. Although positive OS RT-PCR tests can be false, our assumption was based on several arguments: (1) PCR assays produce a limited number of false positives: surface contamination is a common source but mainly in hospital settings [39]; (2) False-positive SARS-CoV-2 RT-PCR tests are described in low prevalence-settings where the prior probability of a positive test is low [40]. This was not the case in Monaco where the prevalence was over 5%; (3) All our participants were symptomatic or contacts of a confirmed SARS-CoV-2 infection, indicating a high pre-test probability of infection; (4) RT-PCR assays based on NP sampling have shown a significant proportion of false negatives, mainly due to the sample collection process [41]. Inadequate NP collection has been reported despite extensive training, mainly due to patient discomfort with such an invasive method, which alters the sensitivity of the PCR assay; (5) SARS-CoV-2 tends to persist longer in saliva than in the nasopharynx [42]. Finally, we did not account for the time of test since symptom onset, if any: it is possible that our findings using OS are of a lesser relevance in case of late testing [8].

Conclusion

Based on a national SARS-CoV-2-testing programme, we demonstrated the simplicity and utility of an oral sponge to sample saliva using a RT-PCR assay and a fully automated ECLIA RAT in symptomatic and non-symptomatic adults. This method allows self-collection without any condition, reduces health worker exposure, and requires no pre-analytical step. Our findings encompassed the latest SARS-CoV-2 variants and subvariants such as omicron BA.4, BA.5 and the XBB series. The fully automated ECLIA RAT provides high throughput at a moderate cost and could be considered to identify individuals with high viral loads in a mass screening strategy for large events.

Abbreviations

AUC	Area Under the Curve
COVID-19	Coronavirus Disease-19
ECLIA	ElectroChemiLuminescent enzyme ImmunoAssay
HW	Health Worker
MCPHP	Monaco COVID-19 Public Health Programme
NP	Nasopharyngeal
OS	Oral Sponge
RAT	Rapid Antigen Test
ROC	Receiver Operator Characteristic (ROC)
RT-PCR	Reverse-Transcriptase Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11088-x.

Supplementary Material 1

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Author contributions

TA and EV had the idea for the study and contributed to study design. TA, AC, EJ, CL, GG, HR, OD, and PR contributed to the epidemiological investigation and data collection. TA, AB, AT, BV and EV contributed to analysis and interpretation of epidemiological data. TA and AB wrote the report, and all listed authors reviewed the manuscript.

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Data availability

Anonymised and analysed data can be obtained upon reasonable request from the corresponding author if approval from ethics boards has been granted.

Declarations

Ethics approval and consent to participate

This study complied with the Declaration of Helsinki for research involving human subjects. An institutional review board (The Consultative Ethics Committee of the Principality of Monaco agreed for this study, reference Approval Number: AP/jv 2021.2015) reviewed and approved the study protocol and the consent form prior to study initiation. All participants provided informed consent. Collected data was systematically anonymised for any statistical analysis purpose.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Disclaimer

The authors affiliated with the Government of Monaco are alone responsible for the views expressed in this publication and do not necessarily represent the decisions or policies of the Government of Monaco.

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