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ORIGINAL ARTICLE

Validity and reliability of immunochromatographic IgM/IgG rapid tests for COVID-19 salivary diagnosis

Ana Carolina Serafim Vilela[1] | Camila Alves Costa[1] | Suzane Aparecida Oliveira[1] | Menira Borges Lima Dias Souza[2] | Fabiola Souza Fiaccadori[2] | Cláudio Rodrigues Leles[3] | Nádia Lago Costa[1]

[1]Laboratory of Immunohistochemistry and Saliva Analysis, School of Dentistry, Federal University of Goias, Goiania, Brazil

[2]Laboratory of Virology and Cell Culture, Institute of Tropical Pathology and Public Health, Federal University of Goias, Goiania, Brazil

[3]Department of Oral Rehabilitation, School of Dentistry, Federal University of Goias, Goiania, Brazil

Correspondence
Nádia Lago Costa, Faculdade de Odontologia, Universidade Federal de Goiás, Praça Universitária, s/n, Setor Universitário, 74605-220, Goiania, Goias, Brazil.
Email: nadia_costa@ufg.br

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Abstract

Objectives: To assess the accuracy of three immunochromatographic rapid tests for salivary detection of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens and the reliability of these tests comparing saliva with plasma samples.

Materials and Methods: Plasma and saliva samples from 62 patients diagnosed with coronavirus disease 2019 (COVID-19) and 20 healthy volunteers were assayed. IgM/IgG antibody against SARS-COV-2 was detected using three immunochromatographic rapid tests and compared with real-time reverse transcription-polymerase chain reaction (qRT-PCR).

Results: The tests' overall accuracy for detecting anti-SARS-CoV-2 antibodies ranged from 75.6 to 79.3 for saliva and 86.6–87.8 for plasma tests. The sensitivity of saliva and plasma tests increased with the severity of COVID-19 signs and symptoms. The chance of a positive plasma test in participants with a positive qRT-PCR test was 2.27 greater than a positive saliva test.

Conclusions: Although rapid immunochromatographic tests are more accurate using plasma than saliva, which was expected considering its original use, our findings support the use of saliva as a straightforward supplementary method to assess seroconversion in patients with COVID-19, with important sensitivity and sensibility, especially in severe and critical cases.

KEYWORDS
COVID-19, immunoglobulin G, immunoglobulin M, saliva, SARS-Cov-2

1 | INTRODUCTION

Viral genetic sequencing using real-time reverse transcription-polymerase chain reaction (qRT-PCR) is the most widely used technique for diagnosing coronavirus disease 2019 (COVID-19) due to its high sensitivity and specificity in cases of acute infection (Wang et al., 2020). However, qRT-PCR tests can be labor-intensive, requiring specialized equipment and a centralized laboratory, which increases the wait time for the results and the costs (Dinnes et al., 2021). In a systematic review, Mallet et al. (2020) demonstrated that the rate of qRT-PCR test false-negative results increases in nasopharyngeal samples collected 10 days after symptom onset. In this way, rapid immunochromatographic tests present a complementary diagnostic method, helping to identify infected patients (Dinnes et al., 2021; Zhao et al., 2020). Available rapid tests benefit from screening larger populations, with and without symptoms, in locations other than healthcare settings and would provide a faster diagnosis to allow early prevention of COVID-19 spread (Dinnes et al., 2021).

Rapid tests based on immunochromatographic analysis can detect immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in patients with current or past infection. The estimated median times for seroconversion were 11 days for total antibodies, 12 days for IgM, and 14 days for IgG. However, patients tested within a week after the onset of symptoms showed a high proportion of false-negative results in rapid antibody tests (Zhao et al., 2020).

All these diagnostic tests require close contact with the patient, increasing the risk of transmission to the healthcare professional during collection. Moreover, collecting blood samples and swabs from the nasopharynx or oropharynx are invasive and uncomfortable for the patient (To et al., 2020). Therefore, salivary tests have been proposed as an alternative biological fluid for diagnosing respiratory viral infections, including COVID-19; saliva is easily collected in sterile flasks or Salivette® kits (Fernandes et al., 2020; Khurshid et al., 2020). Saliva tests are simple to perform and more comfortable than nasal swabs. In the pandemic context, they are inexpensive, scalable, and sustainable strategies that allow easily repeatable and widely available testing over time (Fernandes et al., 2020; Tan et al., 2021).

The salivary glands are among the first organs in the body to be infected by the SARS-CoV-2 virus (Xu et al., 2020), and viral ribonucleic acid (RNA) can be detected in saliva before the appearance of lung lesions (Liu et al., 2011). The salivary glands are an essential viral reservoir, and the rates of identifying the SARS-CoV-2 virus in saliva can exceed 91% (To et al., 2020). The qRT-PCR test using saliva as a potential specimen had a substantial discriminative and diagnostic ability for SARS-CoV-2 detection, with high sensitivity and specificity (Atieh et al., 2021). Therefore, it was suggested that the spread of COVID-19 by asymptomatic infected subjects might be traced to contaminated saliva. The local immune system of the salivary glands produces immunoglobulin A (IgA), IgM, and IgG in oral fluids, and these antibodies are transferred from the circulation to the saliva by transcellular passive intracellular diffusion or active transport in the salivary glands or crevicular fluid (Khurshid et al., 2020).

There is little evidence of the utility of rapid tests using saliva samples based on immunochromatography to detect IgG and IgM. Because rapid tests are less costly, easier to perform, have better tolerability, and pose less risk to health professionals, we hypothesized that saliva could be an alternative to whole blood and serum samples to detect SARS-CoV-2 IgG/IgM antibodies using rapid tests. Therefore, we aimed to measure the accuracy of immunochromatographic rapid tests using salivary detection of IgM/IgG antibodies against SARS-CoV2 viral antigens and assess the reliability of rapid tests using saliva samples compared to plasma.

2 | METHODS

2.1 | Study location, design, and participants

This study was conducted in Goiânia, Goias, Brazil. A convenience sample of 82 participants was enrolled, including 62 subjects with infection confirmed by qRT-PCR (20 with asymptomatic-mild disease, 20 with moderate disease, and 22 with severe-critical disease), and 20 disease-free subjects with negative qRT-PCR tests (negative controls). Infected patients all experienced their first infection by SARS-CoV2, and they were classified according to COVID-19 disease severity, published in COVID-19 interim guidance of the World Health Organization (WHO, 2020). The participants were recruited at the School of Dentistry and the University Hospital of the Federal University of Goias. Participant recruitment and testing occurred between August 2020 and March 2021. At the time of the study, none of the participants had been vaccinated. The institutional ethical research committee approved the study (CAEE 30804220.2.0000.5078/38088920.9.3001.5078). Informed written consent was obtained from each included patient or responsible relative.

All participants were over 18 years of age and were tested for SARS-CoV-2 infection using a qRT-PCR test. Subjects who could not undergo blood collection due to hematologic problems and patients with xerostomia or lesions in the salivary glands that compromise salivary flow were excluded. Control individuals (disease-free subjects) were healthcare professionals responsible for treating COVID-19 patients at the University Hospital of the Federal University of Goias. These participants were monitored weekly with qRT-PCR tests or screened for signs and symptoms of the disease. Infected patients were invited to enroll in the study after having had a positive qRT-PCR test performed by professionals of the Laboratory of Virology and Cell Culture of the Institute of Tropical Pathology and Public Health at the Federal University of Goias.

2.2 | Nasopharyngeal swab, blood and saliva collection

A nasopharyngeal swab was collected from both nostrils for the qRT-PCR test. Saliva collection was performed using the Salivette® collector (Sarstedt) equipped with a tube containing collector cotton. The cotton was placed on the floor of the participant's mouth for approximately 2 min and then returned to the tube. After collection, the saliva sample was centrifuged for 10 min at 1903.67 *g* at 4°C.

The peripheral blood was harvested with ethylenediaminetetraacetic acid anticoagulant (2 ml, BD Vacutainer). The blood samples were centrifuged for 10 min at 349.65 *g* at 25°C to obtain the plasm and perform the rapid tests according to the manufacturers' guidelines.

The saliva and blood samples collection occurred from the 15th to the 30th day after positive findings on the qRT-PCR tests in infected patients and the same day after negative qRT-PCR test results in the healthy participants.

2.3 | qRT-PCR

Confirmation of SARS-CoV-2 was performed in the Laboratory of Virology and Cell Culture of the Institute of Tropical Pathology and Public Health at the Federal University of Goias. Nasopharyngeal

TABLE 1 Rapid tests for detection of IgG and IgM anti-SARS-CoV-2 antibodies that use the methodology by immunochromatography approved by ANVISA until March 2030

Test	Manufacturer	Sample	Volume	Sensitivity[a]	Specificity[a]	Time of lecture
Immupass VivaDiag™ SARS-CoV-2 Teste Rápido IgM/IgG (ANVISA Register: 81869080006; Lot number E2007001)	Vivachek Biotech (Hangzhou) Co.	Serum plasma blood	10 µl	95.04% (115/121, 95% CI, 89.60%–97.71%)	100% (431/431, 95% CI, 99.12%–100%)	Up to 15 min
Wama's Imuno-Rapid COVID-19 IgG/IgM (ANVISA Register: 10310030208; Lot number: 20L092)	Wama Produtos Para Laboratório Ltda	Serum plasma blood	10 µl	83.3% (CI[a] 95% = 77.1%–88.1%)	93.1% (CI[a] 95% = 88.3%–96.0%)	Up to 15 min
Leccurate SARS-CoV-2 Antibody Test—colloidal gold immunochromatography (ANVISA Register: 80638410090; Lot number 20CG2506Xs)	Beijing Lepu Medical Technology Co	Serum plasma blood	10 µl	92.8%	90.0%	Up to 15 min

Abbreviations: COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; IgM, immunoglobulin M; SARS-CoV2, severe acute respiratory syndrome coronavirus 2.

[a]Parameters provided by the manufactures in the package leaflet.

swabs were collected and processed in 24–48 h. Viral RNA was isolated using a commercial QIAamp Viral RNA Mini Kit (Qiagen). For SARS-CoV-2 RNA detection, we used qRT-PCR assay with probes and primers targeting two coronavirus regions (N1 and N2) and the human RNase P gene (internal control) (IDT) (Centers for Disease Control and Prevention. CDC-2019-Novel Coronavirus [2019-nCoV] Real-Time RT-PCR. 2020). The reaction system and amplification conditions were performed according to the manufacturer's specifications in a 7500 Fast Dx Real-Time PCR System (Life Technologies). The result was considered valid only when the reference gene's cycle threshold (Ct) value was 38 or less. The result was considered positive when the Ct value of the viral genes was 38 or less and negative when it was >38.

2.4 | Immunochromatography-based COVID-19 IgG/IgM rapid test (rapid tests)

Three lateral-flow immunochromatographic assay rapid tests for the qualitative detection of SARS-CoV-2 IgG/IgM antibodies were analyzed. COVID-19 specific rapid test kits were approved by the Brazilian health surveillance agency (ANVISA) using whole blood or serum, or plasma samples in the Instructions for Use or Product User Manual. The immunochromatographic tests detect the presence of IgG and IgM anti-SARS-CoV-2 antibodies in human whole blood or serum to identify current or past infection. Table 1 shows the characteristics of the rapid tests used in this study, as provided by the manufacturers. The sample volume adopted for serum was 10 µl, as recommended by the manufacturers. For saliva, a volume of 20 µl was used (Figure 1). Each test can give a result for IgG and IgM alone or in combination. Positive results were considered with only IgG, only IgM, or both, considering the entire evaluation period.

2.5 | Repeatability and reproducibility of rapid tests

Repeatability was assessed by taking replicates of samples known to be reagents and non-reagents for SARS-CoV-2 and testing simultaneously and independently for two research participants. Reproducibility (inter-test) was assessed by repeating the same protocol on different days. The evaluation was carried out for the three rapid test kits to detect IgG and IgM anti-SARS-CoV-2 in five serum and saliva samples.

2.6 | Data analysis

Descriptive analysis included the frequency of positive tests and the chi-square test to assess differences according to the level of symptoms of the participants. The Kappa coefficient was used to measure the level of agreement between tests using blood and

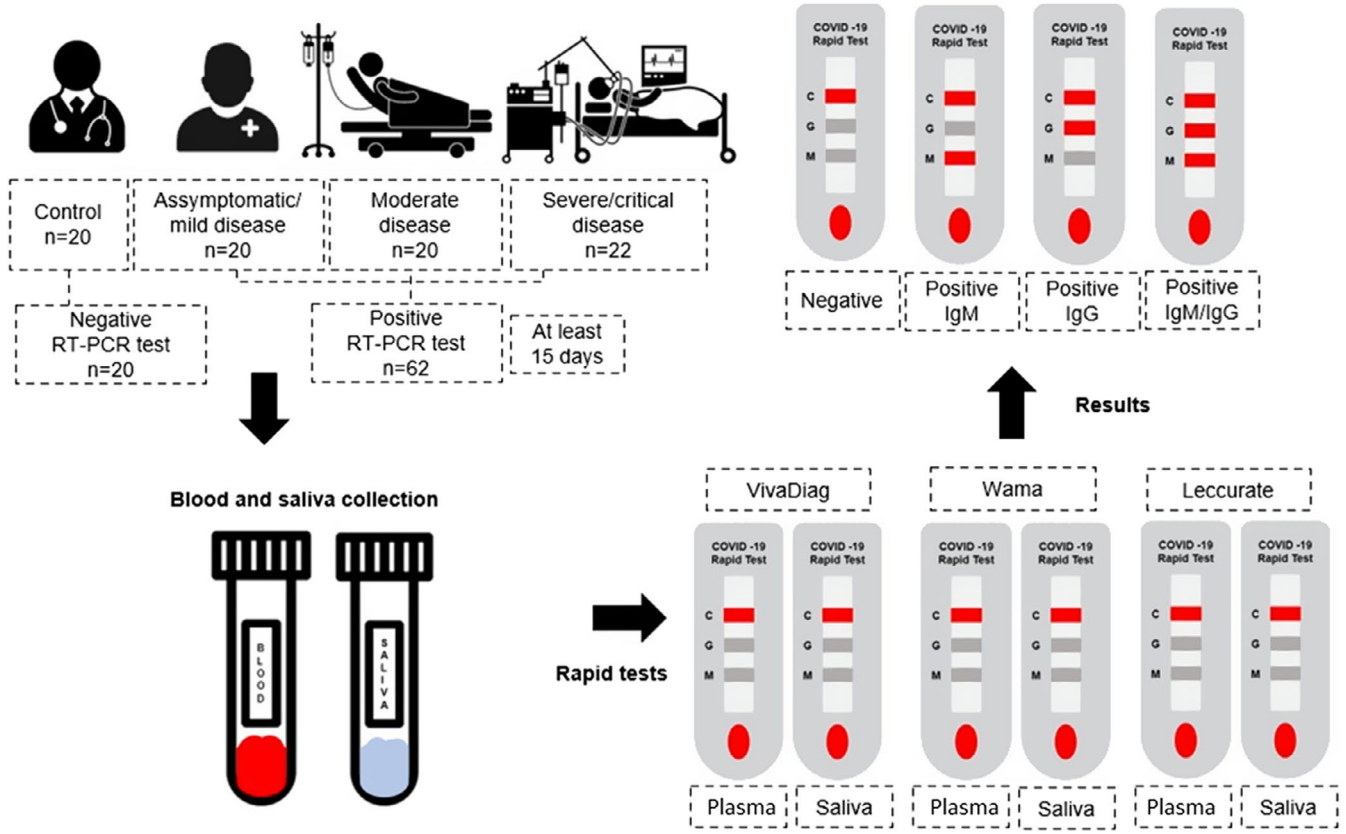

FIGURE 1 Schematic of our study methods

TABLE 2 Frequency of positive tests according to the level of symptoms (% in parenthesis)

	Plasma				Saliva			
Level of symptoms	T1	T2	T3	p^*	T1	T2	T3	p^*
Assymptomatic—mild ($n = 20$)	12 (60.0)	13 (65.0)	13 (65.0)	0.931	6 (30.0)	11 (55.0)	11 (55.0)	0.243
Moderate ($n = 20$)	18 (90.0)	18 (90.0)	18 (90.0)	1.000	16 (80.0)	15 (75.0)	15 (75.0)	0.911
Severe–critical ($n = 22$)	21 (95.5)	21 (95.5)	21 (95.5)	1.000	20 (90.9)	19 (86.4)	19 (86.4)	0.867
p^{**}	0.003	0.008	0.008		<0.001	0.025	0.025	

Abbreviations: T1, Immupass VivaDiagTM SARS-CoV-2 IgM/IgG Rapid; T2, Wama's Imuno-Rapid COVID-19 IgG/IgM; T3, Leccurate SARS-CoV-2 Antibody Test Colloidal Gold Immunochromatography.

*Pearson Chi-square test.; **Chi-square for trend.

saliva samples. Diagnostic test accuracy was assessed using the RT-PCR results as the reference method. Sensitivity, specificity, predictive values, and likelihood ratios were calculated using blood and saliva samples for the three kits of the rapid test. The chance of a correct diagnosis using the rapid tests was assessed when the type of fluid sample (blood or saliva), level of symptoms, the brand of the rapid test, and the time from the onset of symptoms were tested as independent variables. A generalized estimating equation regression was used, considering the non-independency of data as the measurements were repeated more than once for each participant (within-subject factor). Statistical significance was set at $p < 0.05$.

3 | RESULTS

During the 8-month recruitment and data collection period, 82 participants underwent rapid tests. A total of 148 participants were assessed; however, 66 were excluded due to missing information about the date of qRT-PCR ($n = 19$) or for being collected <15 days after the positive qRT-PCR test ($n = 47$).

The frequencies of positive tests according to the level of symptoms, fluid type, and rapid test used are detailed in Table 2. The rates of positive tests were similar for the three kits of rapid test using plasma or saliva samples ($p > 0.05$). Furthermore, the increase of antibody detection rates was directly proportional to the

severity of COVID-19 symptoms, with significant differences found for the three kits of rapid test and fluid samples (saliva and plasma) ($p < 0.05$).

Then, the results of the rapid tests were contrasted with the qRT-PCR (Table 3). High specificity values were observed for the three kits of rapid test. Sensitivity ranged from 67.7% (T1/Saliva) to 83.9% (T2 and T3/Plasma). Lower sensitivity values were observed for saliva (range = 67.7%–72.6%) than plasma tests (range = 82.2%–83.9%). Overall, rapid tests were excellent for the prediction of the disease in positive tests (positive predictive value >97.8%) and poor prediction of control cases in negative tests (negative predictive value <66.7%). As the specificity for most of the tests was 100%, the positive likelihood ratio (sensitivity/(1−specificity)) could not be calculated (except for T3/Saliva). By contrast, the negative likelihood ratios (−LRs) that provide the change in the odds of having a positive diagnosis in patients with a negative test (1−sensitivity/specificity) ranged from 0.32 to 0.16, which means a 68%–84% decrease in the odds of having the antibodies detected in a patient with a negative test result. Finally, the overall accuracy of the rapid tests ranged from 75.6% to 87.8% (95% confidence interval).

Table 4 shows the reliability analyses to measure the agreement among many plasma and saliva tests (inter-tests) and between plasma and saliva fluids for the same rapid test (intra-test). Kappa statistics showed almost perfect agreement between blood tests (>0.92) and moderate agreement ($\kappa = 0.46–0.58$) between saliva tests. Substantial agreements were observed for the intra-test analyses ($\kappa = 0.68–0.82$). When only COVID-19 subjects were considered ($n = 62$), there was a marked decrease in agreement measures, particularly for the saliva tests (Kappa inter-test = 0.19–0.34) and when plasma and saliva tests were compared (intra-test $\kappa = 0.46–0.67$). Intra-test agreement markedly improved when only moderate and severe-critical cases were considered (Figure 2).

The regression model in Table 5 describes the effects of clinical variables and rapid test features for the likelihood of a positive rapid test result (IgM/IgG antibody for SARS-COV-2 detected) in the study sample of COVID-19 participants ($n = 62$). Multivariate analysis showed that the change of a positive test increased for plasma tests (OR = 2.27; $p < 0.001$), moderate (OR = 4.63; $p = 0.015$), and severe-critical symptoms (OR = 10.3; $p < 0.001$). There were no effects of the type of the rapid test or time elapsed from the symptom onset.

TABLE 3 Accuracy analysis

Test kit	Biological sample/Test result	PCR		Sensitivity	Specificity	PPV	NPV	+LR	−LR (95% CI)	Accuracy (95% CI)
		Positive ($n = 62$)	Negative ($n = 20$)							
1	Plasma									
	Positive	51	0	82.2	100	100	64.5	–	0.18 (0.10–0.30)	86.6 (77.3–93.1)
	Negative	11	20							
	Saliva									
	Positive	42	0	67.7	100	100	50.0	–	0.32 (0.23–0.46)	75.6 (64.9–84.4)
	Negative	20	20							
2	Plasma									
	Positive	52	0	83.9	100	100	66.7	–	0.16 (0.09–0.29)	87.8 (78.7–94.0)
	Negative	10	20							
	Saliva									
	Positive	45	0	72.6	100	100	54.1	–	0.27 (0.18–0.41)	79.3 (68.9–87.4)
	Negative	17	20							
3	Plasma									
	Positive	52	0	83.9	100	100	66.7	–	0.16 (0.09–0.29)	87.8 (78.7–94.0)
	Negative	10	20							
	Saliva									
	Positive	45	1	72.6	95.0	97.8	52.8	14.5 (2.1–98.7)	0.29 (0.19–0.44)	78.0 (67.5–86.4)
	Negative	17	19							

Abbreviations: CI, confidence interval; LR, Likelihood-ratio Test; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; T1, Immupass VivaDiagTM SARS-CoV-2 IgM/IgG Rapid; T2, Wama's Imuno-Rapid COVID-19 IgG/IgM; T3, Leccurate SARS-CoV-2 Antibody Test Colloidal Gold Immunochromatography.

TABLE 4 Reliability analysis

Comparison		Crosstabulation				% overall agreement	Kappa coefficient	
		(+) (+)	(+) (−)	(−) (+)	(−) (−)		All cases	COVID-19 group
Test 1 plasma	Test 2 plasma	51 (62.2)	0	1 (1.2)	30 (36.6)	0.988	0.974 (0.92–1.00)	0.943 (0.83–1.00)
Test 1 plasma	Test 3 plasma	50 (61.0)	1 (1.2)	2 (2.4)	29 (35.4)	0.963	0.922 (0.83–1.00)	0.828 (0.64–1.00)
Test 2 plasma	Test 3 plasma	51 (62.2)	1 (1.2)	1 (1.2)	29 (35.4)	0.976	0.947 (0.88–1.00)	0.881 (0.72–1.00)
Test 1 saliva	Test 2 saliva	35 (42.7)	7 (8.5)	10 (12.2)	30 (36.6)	0.988	0.584 (0.41–0.76)	0.347 (0.10–0.60)
Test 1 saliva	Test 3 saliva	33 (40.2)	9 (11.0)	13 (15.9)	27 (32.9)	0.963	0.462 (0.27–0.65)	0.193 (−0.06–0.45)
Test 2 saliva	Test 3 saliva	36 (43.9)	9 (11.0)	10 (12.2)	27 (32.9)	0.963	0.531 (0.35–0.72)	0.271 (0.01–0.53)
Test 1 plasma	Test 1 saliva	40 (48.8)	11 (13.4)	2 (2.4)	29 (35.4)	0.841	0.681 (0.53–0.84)	0.456 (0.22–0.69)
Test 2 plasma	Test 2 saliva	45 (54.9)	7 (8.5)	0 (0)	30 (36.6)	0.915	0.825 (0.70–0.95)	0.675 (0.46–0.89)
Test 3 plasma	Test 3 saliva	44 (53.7)	8 (9.8)	2 (2.4)	28 (34.1)	0.878	0.748 (0.60–0.89)	0.582 (0.35–0.82)

Abbreviations: COVID-19, coronavirus disease 2019; Test 1, Immupass VivaDiagTM SARS-CoV-2 IgM/IgG Rapid; Test 2, Wama's Imuno-Rapid COVID-19 IgG/IgM; Test 3, Leccurate SARS-CoV-2 Antibody Test Colloidal Gold Immunochromatography.

4 | DISCUSSION

We compared the performance of three recently approved immunochromatographic IgM/IgG rapid tests using plasma and saliva and found accuracy values >75% for the three kits of rapid test using both biological samples. We also demonstrated that the sensitivity of the tests for both saliva and plasma increased with the worsening of COVID-19 symptoms. Although the values of accuracy and chance of positive diagnosis confirmed by qRT-PCR were higher using plasma than saliva, the values were close, especially in the most severe cases.

The molecular detection of SARS-CoV-2 RNA in nasopharyngeal swabs is the reference method for identifying symptomatic or asymptomatic individuals and is indicated for the primary stage of the disease when the virus is present in the lower or upper respiratory tract (Mallett et al., 2020). It has been demonstrated that samples collected 10 days after symptom onset significantly increased the chance of a negative test result (Mallett et al., 2020). In this context, anti-SARS-CoV-2 antibody tests are valuable for monitoring the population and can play an essential role in confirmation and late diagnostic of COVID-19 and managing viral infection (Ahn et al., 2020; Fujigaki et al., 2020; Guedez-López et al., 2020). In the present study, the overall accuracy ranged from 75.6 to 87.8 for all evaluated rapid tests, demonstrating the considerable capacity of plasma and saliva to detect true-positive cases confirmed by qRT-PCR. This finding highlights the significance of point-of-care testing, such as the rapid immunochromatographic test we used, as an alternative to laboratory tests such as enzyme-linked immunosorbent assay (ELISA) format and chemiluminescence (Pegoraro et al., 2021).

Immunochromatographic IgM/IgG rapid tests are rapid, simple, easy to perform, and practical as monitoring tools for COVID-19 infection (Khurshid et al., 2020). The sample indicated by the manufacturer should be plasma, serum, or whole blood, which provides higher specificity and sensitivity values (Table 1). Like the plasma, saliva contains a local immune system that includes IgA, IgM, and IgG production, predominant in oral fluid. These antibodies are transferred from the circulating blood for saliva by transcellular passive diffusion, active transport in the salivary glands, or crevicular fluid (Khurshid et al., 2020). We adapted rapid tests for use in saliva and obtained compelling results with overall sensitivities between 67.7% and 72.6% and specificity between 95% and 100%. A systematic review demonstrated excellent performance of saliva for detecting SARS-CoV-2 in qRT-PCR tests, with high sensitivity and specificity (Atieh et al., 2021). However, to date, few studies have analyzed the performance of rapid tests using saliva. Basso et al. (2021) compared salivary SARS-CoV-2 antigen detection by chemiluminescence immunoassay (CLIA) using two different immunochromatographic rapid tests and found limited sensitivity (13%). Similarly, Nagura-Ikeda et al. (2020) analyzed saliva samples collected on the day of hospital admission from 103 hospitalized patients with laboratory-confirmed COVID-19. They demonstrated that only 11.7% of patients tested positive using the rapid antigen test. Nevertheless, it is critical to mention that, in both studies, the time (days) range for

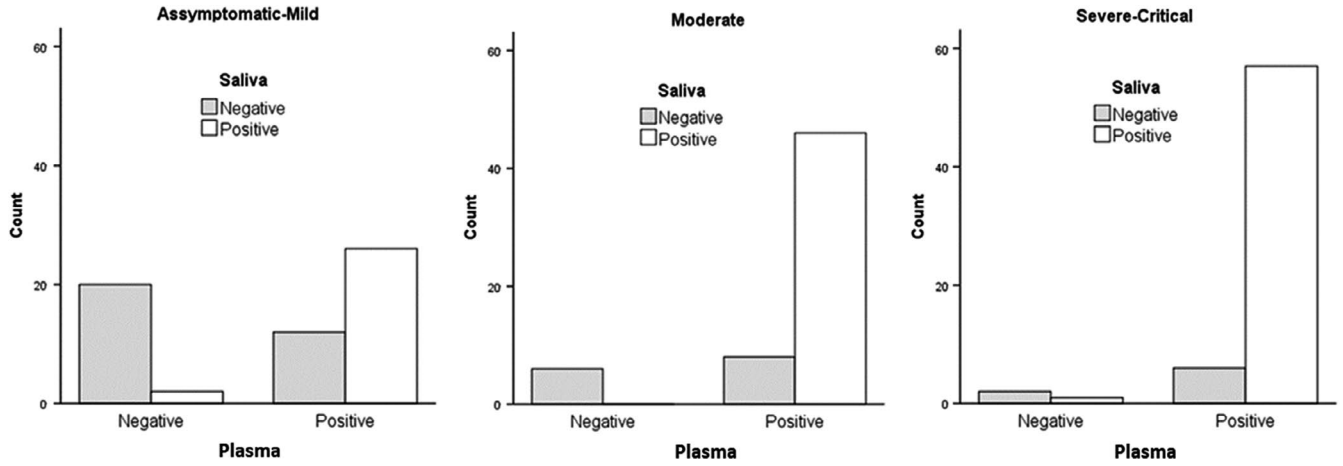

FIGURE 2 Overall agreement of the combined blood and salivary tests, according to the classification of symptoms. Data included the three kits of rapid tests performed: 60 tests in 20 participants with asymptomatic or mild symptoms; 60 tests in 20 participants with moderate symptoms; 66 tests in 22 participants with severe-critical symptoms

TABLE 5 Generalized estimating equation regression estimates for the chance of a positive test using different rapid test kits and fluid samples

Parameter	Categories	OR (95% CI)	*p*
(Intercept)		1.53 (0.53–4.37)	0.429
Sample	Blood	2.27 (1.48–3.48)	<0.001
	Saliva	1	
Level of symptoms	Severe-critical	10.3 (2.95–36.0)	<0.001
	Moderate	4.63 (1.35–15.8)	0.015
	Asymptomatic-mild	1	
Test kits	3	1.18 (0.67–2.07)	0.561
	2	1.18 (0.78–1.80)	0.437
	1	1	
Time from symptom onset (days)		1.00 (0.99–1.02)	0.953

Abbreviations: CI, confidence interval; OR, odds ratio.

salivary collection used was inadequate, which could explain the better accuracy of rapid tests with saliva samples found in the present study.

The estimated median time for seroconversion for COVID-19 infection is 15 days (Zhao et al., 2020). Patients tested within a week after the onset of symptoms showed a high proportion of false-negative results on rapid antibody tests (Zhao et al., 2020). Moreover, Ong et al. (2020) reported that the sensitivity of these tests improved after at least 7 days of symptoms in hospitalized patients. Corroborating these data, Zhao et al. (2020) analyzed the seroconversion by ELISA and demonstrated that only 38.3% of patients were positive for IgM antibodies within the first week after onset; however, the detection of IgM and IgG antibodies increased rapidly from day 15 after onset. Demey et al. (2020) analyzed four immunochromatographic tests in blood samples to detect antibodies to SARS-CoV-2 in 22 patients after they tested positive by qRT-PCR. The authors observed that the median of the antibody detection time was between 8 and 10 days, with a sensitivity of 60%–80% on day 10 and 100% on day 15. In contrast, Pegoraro et al. (2021) compared the diagnostic performances of three rapid immunochromatographic tests using automated ELISA and CLIA immunoassays in 159 hospitalized patients, demonstrating 41%–45% of diagnostic sensitivities and 91%–98% of specificities, with a substantial agreement (89.3%–91.2%). These studies explain our decision to wait at least 15 days after a positive qRT-PCR result to carry out the rapid tests.

Despite the satisfactory accomplishment of saliva compared to previous studies, we found that the chance of a positive patient being confirmed by qRT-PCR to be positive in a rapid test using plasma was 2.27-fold higher than using saliva. These results can be explained by the fact that these tests are developed to be performed with plasma samples (Demey et al., 2020; Pegoraro et al., 2021). Because saliva tests have lower specificity, the performance of the test is worse when disease-free individuals are excluded from the analysis. Although the blood tests were superior, the 95% confidence intervals of the accuracy values suggest that the saliva and blood tests could be used interchangeably, especially when the rapid test aims to confirm the presence of

the antibodies in severely ill patients. Therefore, our findings reinforce the importance of using saliva as an alternative sample for extensive population-level screening of COVID-19 due to its easy collection, handling (Fernandes et al., 2020), and antibody detection capacity. Nevertheless, adding a band to detect IgA in rapid tests indicated for using saliva would be interesting. Future studies testing saliva with rapid tests with IgA bands should be encouraged to verify the better performance of this biological medium, especially for early detection.

Another point to be highlighted is that the sensitivity of the tests with serum and saliva increased when COVID-19 symptoms worsened. Furthermore, the agreement between plasma and saliva was higher in patients with severe-critical symptoms than in patients with moderate and asymptomatic/mild symptoms. In the asymptomatic/mild symptoms group, the chance of false-negative tests was higher. Similar conclusions were presented by Hiki et al. (2021) using an anti-SARS-CoV-2 rapid test to determine IgG and IgM antibodies in symptomatic COVID-19 patients; they observed that, in several critical cases, the positive IgM and IgG detection was higher than in mild/moderate cases; mild cases were seronegative at least 10 days after symptoms, while severe/critical cases became positive at 0–6 days (40%) and 7–13 days (87.5%) after onset.

Finally, all tests we analyzed presented high specificity but sensitivity lower than those described by the manufacturer. However, the positive predictive values returned interesting values (ranging from 97.8% to 100%), suggesting a substantial capacity of rapid tests to detect truly positive cases. In addition to the high specificity, the negative predictive values were lower for the three rapid tests kits, ranging from 50% to 66.7%. These findings suggest that the high specificity of the tests in the present study must be carefully evaluated because it is not precisely known whether the failure of detection occurred due to the non-development of antibodies, or because the number of antibodies was already declining, or because there was an error in the test specificity (false-negative), explained by the low negative predictive values. These results reinforce the notion that anti-SARS-CoV-2 antibodies tests are valuable methods that cannot replace qRT-PCR tests but complement them and be an appropriate alternative for monitoring large populations.

This study has some limitations. The first is the small number of participants at a single hospital. More multicenter studies with a more significant number of saliva samples from different regions would be necessary to validate these rapid immunochromatographic tests. Second, confirmatory tools for the results of the rapid tests, including ELISA or CLIA, would be instruments that would add to the results obtained. However, in the pandemic context and given the available resources, their use was not possible. We suggest that comparative studies be carried out involving these three forms of antibody detection.

In summary, we adapted commercially available immunochromatographic IgM/IgG rapid tests (commercially available in Brazil) to be used with saliva samples collected at least 15 days after symptom onset in patients with COVID-19. Within the limitations, although rapid tests are more accurate using plasma, which was its original indication, our study findings provide evidence supporting the use of these tests for saliva to assess seroconversion in patients with COVID-19, especially those with severe presentations.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Ana Carolina Serafim Vilela: Conceptualization; Data curation; Investigation; Methodology; Writing-original draft. **Camila Alves Costa:** Data curation; Methodology; Project administration; Writing-original draft; Writing-review & editing. **Suzane Aparecida Oliveira:** Data curation; Investigation; Methodology; Writing-original draft. **Menira Borges Lima Dias Souza:** Investigation; Methodology; Validation; Writing-review & editing. **Fabiola Souza Fiaccadori:** Investigation; Methodology; Validation; Writing-review & editing. **Cláudio Rodrigues Leles:** Conceptualization; Data curation; Formal analysis; Writing-original draft; Writing-review & editing. **Nádia Costa:** Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Validation; Writing-original draft; Writing-review & editing.

ETHICS APPROVAL STATEMENT

The Ethics Committee approved this study of Universidade Federal de Goias (CAEE 30804220.2.0000.5078 / 38088920.9.3001.5078).

PATIENT CONSENT STATEMENT

All the participants were informed about this study's objectives, risks, and benefits, and those who agreed to participate signed the free, informed consent form.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/odi.14059.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID

Ana Carolina Serafim Vilela https://orcid.org/0000-0002-8123-3361
Camila Alves Costa https://orcid.org/0000-0002-8493-1835
Suzane Aparecida Oliveira https://orcid.org/0000-0002-6109-2493
Menira Borges Lima Dias Souza https://orcid.org/0000-0003-3145-1376
Fabiola Souza Fiaccadori https://orcid.org/0000-0002-4100-8872
Cláudio Rodrigues Leles https://orcid.org/0000-0002-6812-4849
Nádia Lago Costa https://orcid.org/0000-0003-0198-5828

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