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Highlights

- SARS-CoV-2 saliva antigen tests facilitate testing with poorly trained personnel
- Their diagnostic accuracy in clinical settings is essentially unclear
- We conducted a diagnostic accuracy study in a real-life clinical setting
- The diagnostic accuracy of the PCL saliva antigen test was 30,2%
- Application of the test might lead to a large number of false-negative test results

Diagnostic accuracy of SARS-CoV-2 saliva antigen testing in a real-life clinical setting

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Short title: Saliva antigen test in real-life clinical settings

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ABSTRACT

Background: SARS-CoV-2 antigen tests with saliva facilitate examination in settings that lack trained personnel. However, little is known on the diagnostic accuracy in real-life clinical settings. Therefore, we studied the diagnostic accuracy of a saliva antigen test to diagnose SARS-CoV-2 infection in a primary/ secondary care testing facility.

Methods: Individuals presented at a COVID-19 testing facility affiliated with a Swiss University Hospital were prospectively recruited (n=377). Saliva specimen was obtained, and the PCL Inc. COVID19 Gold antigen test was conducted in parallel with two real-time PCR.

Results: RT-PCR was positive in 53 individuals, corresponding to a prevalence of 14.1% (missing material in one individual). The PCL saliva antigen test was positive in 22 individuals (5.8%), and negative in 354 (93.9%). The sensitivity of the saliva antigen test was 30.2% (95% confidence interval, CI, 18.3 to 44.3), both overall and in symptomatic individididuals. The specificity was 98.1% (96.0, 99.3).

Conclusions: The diagnostic accuracy of a SARS-CoV-2 saliva antigen test in a primary/ secondary care testing facility was remarkably lower compared to the manufacturers' specifications.

BACKGROUND

Testing for SARS-CoV-2 is an essential component of the pandemic response. Rapid antigen tests using saliva were suggested as a quick, simple, comfortable, and non-invasive testing method. Only minimal training is required to conduct these tests, facilitating application in various primary care and even self-testing settings. Several studies suggested that saliva antigen tests might have an adequate performance to diagnose infection with SARS-CoV-2 (Mattiuzzi et al., 2020). However, little is known on the diagnostic accuracy *in real-life clinical settings*, which might be significantly different from manufacturers data (Jegerlehner et al., 2021, Mattiuzzi et al., 2020). Manufacturers claim often claim a sensitivity of around 95% (94.3% in case of the assay mentioned below).

With the present prospective cross-sectional study, we assessed the diagnostic accuracy of a SARS-CoV-2 saliva antigen test in a real-life primary/ secondary care setting.

PATIENTS AND METHODS

This study was conducted in line with an established prospective cross-sectional study; all methodological details were described previously (Jegerlehner et al., 2021). Consecutive individuals presenting at a COVID-19 testing facility affiliated to a Swiss University Hospital between September and December 2021 were included, in a period where the Delta variant was predominant at over 90%. The following inclusion criteria were applied: (a) suspected SARS-CoV-2 infection (including asymptomatic individuals following exposure), (b) age ≥ 18 years, and (c) signed informed consent. The flow of the individuals is given in Figure S1 of the supplementary material. The study protocol was approved by the appropriate ethical committee (Kantonale Ethikkommission Bern #2020-02729). All participants signed informed consent.

Clinical data were obtained using a detailed questionnaire (CDC, 2020, Health, 2021, Jegerlehner et al., 2021). The time point of last oral intake (food, or drink) was recorded. A specially trained nurse collected the saliva specimen in parallel with the nasopharyngeal swab collected for real-time polymerase chain reaction (RT-PCR). Sample material was processed within 15 minutes (saliva antigen test) or 12 hours (RT-PCR, stored at 4° C), respectively. Details of the RT-PCR determination have been reported previously (Brigger et al., 2021, Horn et al., 2022, Jegerlehner et al., 2021).

An immunochromatographic lateral-flow immunoassay (LFI) was used for the detection of SARS-CoV-2 (COVID19 Gold; PCL Inc., Seoul, Rep. of Korea; www.pclchip.com). SARS-CoV-2 antibodies are labeled with small gold particles and attached on a nitrocellulose membrane. The saliva antigen test was performed in parallel by a trained nurse. The instructions of the manufacturer were strictly followed (package leaflet); internal and external controls were applied. Participants were asked not to eat, drink or smoke for 30 minutes prior to sampling. After collecting saliva in the mouth, the participants spitted approximately 500 µL in the test tube filled with 500 µL of extraction buffer. The tubes were mixed and two drops were applied to the sample hole of the test card. The results were recorded after 10 minutes.

Statistical analyses were done using the Stata 14.2 statistical software (StataCorp. 2014. As measures of diagnostic accuracy, sensitivities, and specificities were calculated with the help of a 4×4 table, considering the saliva antigen test as index test and the RT-PCR as reference standard (Mallett et al., 2012). Stata statistical software: Release 14. College Station, Tx: StataCorp LP).

RESULTS

Overall, 377 participants were eventually included (Figure S1). Most individuals presented with symptoms consistent with SARS-CoV-2 infection (n=327; 86.7%). Fifty asymptomatic individuals were referred for workup upon exposure (13.3%). Detailed patient characteristics are given in Table 1. Fifty-three individuals tested positive with the RT-PCR done with the nasopharyngeal swab (prevalence 14.1%). Overall, the sensitivity of the saliva antigen test was 30.2% (95% confidence interval, CI, 18.3, 44.3), the specificity was 98.1% (95% CI 96.0, 99.3). Among symptomatic patients, the sensitivity was 30.2 % (95% CI 18.3, 44.3), specificity 97.8% (95.3, 99.2).

The number of false-negative test results was 37, and the number of false-positive test results was 6 (n=16 true positives; n=317 true negatives). The sensitivity of the saliva antigen test according to adapted cycle thresholds of RT-PCR is given in Figure 1. The sensitivity ranged from 30.2% (CT 40) to 33.3% (CT 26). Among 37 individuals with false-negative test results, the time point of last food or drink intake was shorter than 30 minutes in 2 individuals (25 minutes, 20 minutes).

DISCUSSION

In a prospective cross-sectional study conducted in the real-life clinical setting of a primary/ secondary care testing facility, the overall sensitivity of a saliva antigen test was 30.2%. Lower CT thresholds of the RT-PCR did not significantly change the sensitivity. This result is substantially lower than the manufacturer's specifications (sensitivity 94.3%).

Our results are consistent with previous studies that have shown low sensitivities of antigen tests in real-life clinical settings (De Marinis et al., 2021, Igloi et al., 2021, Jegerlehner et al., 2021, Kritikos et al., 2021). However, our results contrast with other studies and manufacturers' data investigating antigen tests with more restricted

study designs (Graham et al., 2021). A Cochrane Review pointed to the limitations of these studies, major methodological concerns, and a high risk of bias (Dinnes et al., 2020). As a limitation, our results were obtained using one particular antigen test in one particular setting. However, strikingly low sensitivities were observed in several studies assessing antigen tests in realistic settings (De Marinis et al., 2021, Igloi et al., 2021, Jegerlehner et al., 2021, Kritikos et al., 2021).

In conclusion, the diagnostic accuracy of the PCL saliva antigen test to diagnose a SARS-CoV-2 infection in a primary/ secondary care testing facility was considerably lower compared to manufacturers' data. This should be taken into consideration when setting up testing strategies.

DECLARATIONS

Ethics approval

The study was approved by the local ethical committee (Kantonale Ethikkommission Bern # 2020-02729).

Availability of data

The database is available on request at the corresponding author.

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Authorship contributions

SJ collected data, wrote the manuscript, and contributed to study design and interpretation of results. FSR and PB collected data, contributed to study design and interpretation of the results, and revised the manuscript. PJ contributed to study design, interpretation of the results, and revised the manuscript. MN designed the study, analyzed and interpreted the data, and wrote the manuscript.

COMPETING INTERESTS

MN received research support from Roche diagnostics outside of the present work.

All other authors declare that no conflict of interest exist.

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Figure legends

Figure 1: Diagnostic accuracy of a SARS-CoV-2 saliva antigen test in a real-life clinical setting. 377 individuals presented at a COVID-19 testing facility affiliated to a University Hospital were included. (A) Sensitivities and specificities are given according to RT-PCR, including 95% confidence intervals. (B) Sensitivities in relation to adapted cycle thresholds (CT) of RT-PCR. The manufacturer uses 40 cycles and if a signal is detected within these 40 cycle the sample is considered positive.

Table 1: Characteristics of 377 study participants presented at a COVID-19 testing facility affiliated to an Emergency department of a University Hospital. Abbreviations: RT-PCR, real-time PCR; SD, standard deviation.

Characteristic	All individuals	RT-PCR negative individuals	RT-PCR positive individuals	Missing values
Numbers of patients *(%)*	377 (100)	323 (85.7)	53 (14.1)	1 (0.3)
Age, *mean (SD)*	31.4 (10.4)	31.5 (10.5)	30.3 (10.2)	0
Female *(numbers, %)*	221 (58.6)	191 (59.1)	29 (54.7)	0
Reason for testing *(numbers, %)*				
Symptoms*	327 (86.7)	273 (84.5)	53 (100)	0
Exposure#	50 (13.3)	50 (15.5)	0	0
Presence of symptoms *(numbers, %)*				
Any symptom	327 (86.7)	273 (84.5)	53 (100)	0
Acute respiratory symptoms	172 (45.7)	144 (44.6)	28 (52.8)	0
Fever	81 (21.5)	62 (19.2)	19 (35.9)	0
Loss of smell and taste	27 (7.2)	19 (5.9)	8 (15.1)	0

All numbers and percentages refer to the subset of patients indicated in the respective column (all individuals, RT-PCR positives, or RT-PCR negatives). The percentages in the rows with the categories "reason for examination" or "presence of symptoms" sum within these categories. * Individuals presenting at a COVID-19 testing facility due to symptoms consistent with COVID-19; # individuals presenting because of exposure to individuals with SARS-CoV-2 infection.