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Research note

Real-life evaluation of a rapid antigen test (DPP SARS-CoV-2 Antigen) for COVID-19 diagnosis of primary healthcare patients, in the context of the Omicron-dominant wave in Brazil

Matheus Filgueira Bezerra 1, Lilian Caroliny Amorim Silva 2, Rômulo Pessoa-e-Silva 3, Gisele Lino Soares 4, Filipe Zimmer Dezordi 5, Gustavo Barbosa Lima 2, Raul Emídio Lima 2, Tulio L. Campos 6, Cassia Docena 2, Anderson Bruno Oliveira 4, Maira Galdino da Rocha Pitta 2, Francisco de Assis da Silva Santos 7, Michelly Pereira 3, 8, Gabriel Luz Wallau 5, 6, 9, Marcelo Henrique Santos Paiva 5, 7

1) Departamento de Microbiologia, Instituto Aggeu Magalhães, Fiocruz, Recife, Brazil
2) Núcleo de Plataforma Tecnológica, Instituto Aggeu Magalhães, Fiocruz, Recife, Brazil
3) Núcleo de Pesquisa em Inovação Terapêutica, Universidade Federal de Pernambuco, Recife, Brazil
4) Gerência de Vigilância Epidemiológica, Secretaria Municipal de Saúde, Caruaru, Brazil
5) Departamento de Entomologia, Instituto Aggeu Magalhães, Fiocruz, Recife, Brazil
6) Núcleo de Bioinformática, Instituto Aggeu Magalhães, Fiocruz, Recife, Brazil
7) Núcleo de Ciências da Vida, Centro Acadêmico do Agreste (CAA), Universidade Federal de Pernambuco, Caruaru, Brazil
8) Departamento de Fisiologia e Farmacologia - Universidade Federal de Pernambuco, Recife, Brazil
9) Department of Arbovirology, Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Center for Arbovirus and Hemorrhagic Fever Reference and Research, National Reference Center for Tropical Infectious Diseases, Hamburg, Germany

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ABSTRACT

Objectives: We aimed to investigate the real-life performance of the rapid antigen test in the context of a primary healthcare setting, including symptomatic and asymptomatic individuals that sought diagnosis during an Omicron infection wave.

Methods: We prospectively accessed the performance of the DPP SARS-CoV-2 Antigen test in the context of an Omicron-dominant real-life setting. We evaluated 347 unselected individuals (all-comers) from a public testing centre in Brazil, performing the rapid antigen test diagnosis at point-of-care with fresh samples. The combinatory result from two distinct real-time quantitative PCR (RT-qPCR) methods was employed as a reference and 13 samples with discordant PCR results were excluded.

Results: The assessment of the rapid test in 67 PCR-positive and 265 negative samples revealed an overall sensitivity of 80.5% (CI 95% = 69.1%–89.2%), specificity of 99.2% (CI 95% = 97.3%–99.1%) and positive/negative predictive values higher than 95%. However, we observed that the sensitivity was dependent on the viral load (sensitivity in Ct < 31 = 93.7%, CI = 82.8%–98.7%; Ct > 31 = 47.4%, CI = 24.4%–71.1%). The positive samples evaluated in the study were Omicron (BA.1/BA.1.1) by whole-genome sequencing (n = 40) and multiplex RT-qPCR (n = 17).

Conclusions: Altogether, the data obtained from a real-life prospective cohort supports that the rapid antigen test sensitivity for Omicron remains high and underscores the reliability of the test for COVID-19 diagnosis in settings with high disease prevalence and limited PCR testing capability. Matheus Filgueira Bezerra, Clin Microbiol Infect 2023;29:392.e1–392.e5

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Introduction

Rapid antigen tests (RAT) are a powerful tool for the mitigation of the COVID-19 pandemic since it does not require complex laboratory infrastructure, provides the timely result and increase the testing capacity of the healthcare system [1]. The currently available RATs are based on the nucleocapsid amino acid sequences from the early 2020 SARS-CoV-2 lineages [2], which have been fully displaced by multiple waves of variants of concern.

It is not clear whether the nucleocapsid mutations accumulated by the Omicron variant (P13L, del31/33, R203K and G204R) affect the binding affinity of antibodies employed in RATs [3]. To address this question, we prospectively accessed the performance of a RAT widely used in Brazilian public healthcare assistance, in the context of an Omicron-dominant wave real-life setting.

Methods

Samples

The study consisted of a prospective collection of 347 unselected individuals (all-comers) from the public COVID-19 Municipal Testing Center (Caruaru, Brazil). Sample collection was performed between 15 February 2022 and 29 March 2022. The patients enrolled included symptomatic individuals, contacts and asymptomatic individuals that needed testing for travelling. All patients were collected with two nasopharyngeal swabs: one for the PCR test and the other for immediate application of a RAT.

Ethics statement

The study was approved by the Ethics Committee (CEP-CCS/UFPE, CAAE31093420.4.0000.5208) and all the participants signed the written consent.

Rapid antigen test and molecular diagnosis of COVID-19

The DPP® SARS-CoV-2 Antigen test (Chembio) is registered in Brazil with the name TR-DPP® COVID-19 AG Bio-Manguinhos. The RATs were performed in loco, immediately after sample collection, following the manufacturer’s instructions.

All enrolled patients were simultaneously tested with two RT-qPCR diagnosis-validated assays: Duplex SARS-CoV-2 E/RP Molecular Kit (Bio-Manguinhos) and the in-house 2019-nCoV_N1/N2/RP protocol (CDC). The details are available in the supplementary material.

Investigation of SARS-CoV-2 variants

The assessment of SARS-CoV-2 lineages from the positive samples was performed with whole-genome sequencing or inference multiplex qPCR. For further details please see the supplementary material.

Statistical analysis

The combinatorial result of the two PCR methods was used as the reference standard to calculate the DPP COVID-19 AG performance rates. Mann–Whitney test was used to compare Ct values between groups. The Kappa test determined the agreement rate between the two PCR methods. Statistical tests were applied with a 95% confidence interval.

Results

Clinical-epidemiological characterization of the setting

Three hundred thirty-two (95.6%) out of 347 patients enrolled had valid PCR results and were included in the analysis (Fig. 1A). Of those, 67 were positive for SARS-CoV-2 (based on PCR results) and the disease prevalence in the setting was 20.2%. The epidemiological, clinical, demographic and vaccination features of the study can be found in Figure S1.

Validation of the PCR-combined reference method

Taking into consideration three possible outcomes (positive, inconclusive and negative), the weighted Kappa index comparing the results from the two PCR methods was 0.885 (CI95% = 0.786–0.910), which is classified as “almost perfect agreement” (Fig. S2). There was a Pearson correlation rate of 0.872 between the Ct values of the two PCR methods (Fig. 2A).

Evaluation of the TR-DPP COVID-19 Ag test performance

From the 67 PCR-positive patients, 54 also tested positive in the RAT test (overall sensitivity = 80.6%; CI 95% = 69.1%–89.2%) and two out of the 265 PCR-negative patients were false-positive in the RAT (specificity: 99.2%; CI 95% = 97.3%–99.1%). All RATs performed presented a valid result. Moreover, we observed high positive and negative predictive values (96.4% and 95.3%, respectively (Fig. 1B and Table S1).

We observed drastic differences in the test sensitivity across distinct PCR Ct values (Figs. 2B–D). Using a Ct value of 31 as a cut-off, the test sensitivity dropped from 93.7% to 47.4% (N1 target) and from 90.2% to 27.3% (E target) (Fig. 1B). The average N1 Ct values for true positives and false negatives were 24.1 (16–33.5) and 31 (22–37.1), and for the E target were 28.2 (22.2–38.2) and 34.1 (26.9–39.1), respectively (Fig. 2C).

Genomic characterization of SARS-CoV-2

We sequenced the SARS-CoV-2 genome of 40 out of 67 positive cases, with an average coverage breadth of 96.4% (87.7%–99.1%) and depth of 245.1 (72.7–948.5). Other 17 samples ineligible for sequencing were characterized with the qPCR inference protocol. All the 57 characterized samples (85% from all positives) were assigned as Omicron (Fig. 1A, Table S2). The phylogenetic analysis revealed that the genomes belong to BA.1 and BA.1-like lineage (Fig. S3).

Discussion

The current study investigated the performance of the DPP SARS-CoV-2 Antigen test in a primary healthcare setting during an Omicron wave. The overall test sensitivity and specificity were within the recommended by the World Health Organization [4] and the European Commission [5]. As previously reported for other RATs [6–9], the sensitivity was reduced in samples with lower viral loads. Of importance, the overall sensitivity observed in the current study was lower than the reported in the product specifications (sensitivity: 90.3 and specificity: 98.8%, www.bio.fiocruz.br) [10]. This divergence is probably due to study design differences, which resulted in distinct Ct values distribution (viral load). The manufacturer evaluated only symptomatic individuals sampled up to nine days from symptoms onset, and 79% of the
positive samples had a Ct value lower than 25, while only 48% of the positive cases were within this Ct range in our study. Nevertheless, when analyzing only samples within the Ct < 25 groups, we observed a test sensitivity of 96.6%, which is similar to the reported by the manufacturer (98%) for this subset. By prospectively analyzing patients in a real-life study (all-comers), we were able to avoid bias towards the selection of samples with a higher viral load, allowing a more realistic evaluation of the rapid test.

The current study brings two innovative aspects: (1) all samples were evaluated fresh at point-of-care for the RAT, which is more representative of the routine conditions than frozen retrospective samples, and (2) it evaluates the antigen test in a highly vaccinated population, which might influence the viral load. On the other hand, one important limitation of the study is the low number of positive patients, reducing the statistical resolution in the analysis of positive subgroups.

The RAT presented positive and negative predictive values higher than 95%, which supports its use for COVID-19 diagnosis in high-incidence settings without the need for confirmatory PCR results. However, it is important to stress that: (1) these data should not be extrapolated to other contexts (e.g. testing in hospital admissions) and (2) predictive values are largely influenced by the disease prevalence and the dynamics of the epidemiologic scenario must be taken into consideration for interpreting tests results [4].

Here, we confirmed the Omicron (BA.1/BA.1.1) lineage in all samples eligible for genome sequencing. In line with our findings, recent studies reported no impact of Omicron on the limit of detection [2,11] and the clinical performance [6,12] in other RAT models. Of interest, while BA.2 carries the same mutations in the nucleocapsid as the BA.1, further studies will be required to evaluate the BA.4 and BA.5 lineages, as they acquired the nucleocapsid S413R mutation.

Altogether, the data obtained here supports the maintenance of the rapid antigen test sensitivity for the BA.1 and BA.1.1 Omicron lineage and underscores the reliability of the test for COVID-19 diagnosis in high transmission settings.
Author contributions

This study was conceptualised by MFB, GLW and MHSP; Data curation, investigation and methodology were done by MFB, LCAS, RPS, GLS, GBL and REL. Formal analysis was done by MFB, FZD, and TLC. Resources were collected by MHSP, GLW, MGRP, and MP. The writing was done by MFB, LCAS, and RPS. Reviewing/editing was done by FASS, CD, MP, ABO, TLC, MGRP, GLW, and MHSP.

Transparency declaration

The authors have no competing financial interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.11.003.
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