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Performance of rapid antigen tests in identifying Omicron BA.4 and BA.5 infections in South Africa

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ABSTRACT

Background: Concerns around accuracy and performance of rapid antigen tests continue to be raised with the emergence of new SARS-CoV-2 variants.
Objective: To evaluate the performance of two widely used SARS-CoV-2 rapid antigen tests during BA.4/BA.5 SARS-CoV-2 wave in South Africa (May – June 2022).
Study design: A prospective field evaluation compared the SARS-CoV-2 Antigen Rapid test from Hangzhou AllTest Biotech (nasal swab) and the Standard Q COVID-19 Rapid Antigen test from SD Biosensor (nasopharyngeal swab) to the Abbott RealTime SARS-CoV-2 assay (nasopharyngeal swab) on samples collected from 540 study participants.
Results: Overall 28.52% (154/540) were SARS-CoV-2 RT-PCR positive with median cycle number value of 12.30 (IQR 9.30–19.40). Out of the 99 successfully sequenced SARS-CoV-2 positive samples, 18 were classified as BA.4 and 56 were classified as BA.5. The overall sensitivities of the AllTest SARS-CoV-2 Ag test and Standard Q COVID-19 Ag test were 73.38% (95% CI 65.89–79.73) and 74.03% (95% CI 66.58–80.31) and their specificities were 97.41% (95% CI 95.30–98.59) and 99.22% (95% CI 97.74–99.74) respectively. Sensitivity was >90% when the cycle number value was <20. The sensitivity of both rapid tests was >90% in samples infected with Omicron sub-lineage BA.4 and BA.5.
Conclusion: Accuracy of tested rapid antigen tests that target the nucleocapsid SARS-CoV-2 protein, were not adversely affected by BA.4 and BA.5 Omicron sub-variants.

1. Introduction

Shortly after the identification of SARS-CoV-2 Omicron variant B1.1.529 on November 24th 2021 [1], several new sub-lineages have been identified, including BA.4 and BA.5 which were first identified in South Africa in February 2022 [2]. In addition to over 30 mutations in the S-gene, Omicron lineages are characterised by several mutations in the nucleocapsid, including P13L, Del31–33, R203K and G204R, that are characteristic to all Omicron sub-lineages and S413R found in BA.2 and BA.3. Additionally, BA.4 sub-lineage contains an additional nucleocapsid mutation P151S. Therefore, there were concerns about the performance of SARS-CoV-2 rapid antigen tests – majority of which target

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the nucleocapsid protein. While the SARS-CoV-2 testing is decreasing globally evolution of SARS-CoV-2 continues to challenge the existing prevention and diagnostic measures. In this study, we evaluated the performance of two widely used rapid antigen tests in comparison to the Abbott RealTime SARS-CoV-2 RT-PCR during the BA.4/BA.5 dominated wave of COVID-19 in KwaZulu-Natal, South Africa.

2. Methods

2.1. Study setting and specimen collection

Sample collection was performed in the province of KwaZulu-Natal in South Africa at 2 drive-through testing centers from 5 May 2022 until 26 June 2022 (spanning the 5th wave of SARS-CoV-2 infections dominated by BA.4 and BA.5 Omicron sub-variants) [2]. Residents of the selected communities falling under any of the following criteria were offered SARS-CoV-2 testing: tested SARS-CoV-2 positive in the previous seven days; presence of COVID-19 symptoms in the previous seven days; exposed to COVID-19 5–10 days ago; health care worker; or doctor referral for testing. After obtaining informed consent, study participants provided information on demographics, symptom type and onset date, and vaccination status. Three respiratory swabs were collected [1 nasal and 2 nasopharyngeal (NP)]. The collection of the nasal swab was prioritized to avoid cross contamination between sites and was followed by the collection of the 2 NP swabs. Trained medical staff performed the rapid antigen testing immediately after sample collection. The second NP swab was used for SARS-CoV-2 RT-PCR reference test and was shipped within 3 h of collections, at room temperature to the central laboratory for processing. The study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval No: BREC/00,001,195/2020 and BREC/00,003,106/2021).

2.2. SARS-CoV-2 testing and sequencing

We evaluated two lateral-flow rapid antigen kits: SARS-CoV-2 Ag rapid test from Hangzhou AllTest Biotech (nasal, Hangzhou, China), and Standard Q COVID-19 Ag test from SD Biosensor (NP, Republic of Korea). According to the manufacturers, the tests detect SARS-CoV-2 nucleocapsid protein with no cross-reaction with other common respiratory pathogens except SARS-CoV. All samples were collected, and assays performed by trained medical staff and as per manufacturer's instructions.

Upon arrival at the laboratory, NP swabs were resuspended in 2 ml of viral transport media (VTM). The Abbott RealTime SARS-CoV-2 assay (target sequences in the RdRp and N genes of the SARS-CoV-2 genome) was the comparator (Chicago, IL. USA). Positive results are reported with cycle number (CN) values [3] . SARS-CoV-2 positive samples were sequenced at KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP) as previously described [1]. Briefly, RNA was extracted on an automated Chemagic 360 instrument (Perkin Elmer, Hamburg, Germany). Libraries for whole genome sequencing were prepared using the Oxford Nanopore Midnight protocol with Rapid Barcoding as per the manufacturer's protocol and sequenced on the Gridion. Raw reads were assembled using Genome Detective version 1.132 (https://www.genomedetective.com) against the reference NC_045512.2 (numbering equivalent to MN908947.3). Mutations were confirmed visually with . bam files using Geneious version 2020.1.2 (Biomatters). Sequences with > 80% coverage were deposited on the GISAID sequence database. The sequencing information is provided in the **Sup. Table 1.**

2.3. Statistical analysis

Fully vaccinated participants were classified as any participant that received either one dose of Ad26.CoV2. S (Johnson & Johnson) or two doses of BNT162b2(Pfizer-BioNTech) two or more weeks prior to testing. Data was analysed and graphed using GraphPad Prism version 8.3.1 (GraphPad software, La Jolla, CA), and SPSS version 24. Test performance characteristics were calculated in reference to Abbott RealTime SARS-CoV-2 assay results. The 95% confidence intervals were calculated to assess the level of uncertainty induced by sample size, using the Wilson's score method. A *t*-test was used to assess differences in CN values between true and false positive results and vaccination status groups. Kruskal-Wallis with Dunn's corrections for multiple comparisons test was done to assess differences in CN values between symptom categories.

3. Results

3.1. Study sample characteristics

The evaluation was performed on 540 samples (Table 1.). The median age of study participants was 42 [interquartile range (IQR) 30–55], with 56.66% of participants being female. Majority of study participants (74.63%) presented within the first week of symptom onset. Fully vaccinated participants made up 50.74% of the study cohort with 67.15% (184/274) having received 2 doses of BNT162b2 vaccine and 32.84% (90/274) having received one dose of the Ad26.CoV2.S vaccine. Overall SARS-CoV-2 RT-PCR positivity was 28.52% (154/540) with median CN value of 12.30 (IQR 9.30–19.40). Out of the 99 successfully sequenced SARS-CoV-2 positive samples, 18 were classified as BA.4 and 56 were classified as BA.5.

3.2. Rapid antigen test performance

Overall performance of the tests is summarised in Fig. 1 **and Sup. Table 2**. Overall sensitivity and specificity of the AllTest SARS-CoV-2 Ag test were 73.38% (95% CI 65.89–79.73) and 97.41% (95% CI 95.30–98.59) respectively. The overall sensitivity and specificity of the Standard Q COVID-19 Ag test were 74.03% (95% CI 66.58–80.31) and 99.22% (95% CI 97.74–99.74). The sensitivity of both tests increased to >80% in samples with CN values <25 and above 90% in samples with CN value <20. Both tests performed worst in patients with no symptoms with sensitivity of 33.33% (95% CI 12.06–64.58). With respect to Omicron BA.4 sub-lineage, both tests performed well with sensitivity of 100.00% (95% CI 82.41–100.00) for AllTest SARS-CoV-2 Ag test and sensitivity of 94.44% (95% CI 74.24–99.01) for Standard Q COVID-19 Ag test. Similarly, both tests performed well in samples characterised as Omicron BA.5 with sensitivity of 89.29% (95% CI 78.53–95.00) for

Table 1
Study participants/sample characteristics.

Sample size (N)	540
Age, years (median, IQR)	42, 30–55
Gender (%, n/N Female)	56.66, 306/540
% PCR Positivity (n/N)	28.52, 154/540
Presence of symptoms	
Asymptomatic/Pre-symptomatic (%, n/N)	11.85, 64/540
<7 days PSO (%, n/N)	74.63, 403/540
>=7 days PSO (%, n/N)	13.52, 73/540
Vaccination status	
Fully vaccinated (%, n/N)	50.74, 274/540
Unvaccinated (%, n/N)	34.26, 185/540
Partially vaccinated (%, n/N)	15.00, 81/540
HIV positive (%, n/N)	0.56, 3/537
Oxygen saturation (median, IQR)	98.00, 98.00–99.00
CN value (median, IQR)	12.30, 9.30–19.40
Omicron lineage	
(% of SARS-CoV-2 positive, n/N)	
B.1.1.529/BA.2, 21L	23.23, 23/99
BA.4, 22A	18.18, 18/99
BA.5, 22B	56.56, 56/99
Recombinant	2.02, 2/99

*Missing data- SARS-CoV-2 RT-PCR results:1; HIV status (self-reported): 3; SARS-CoV-2 sequencing: 55;.

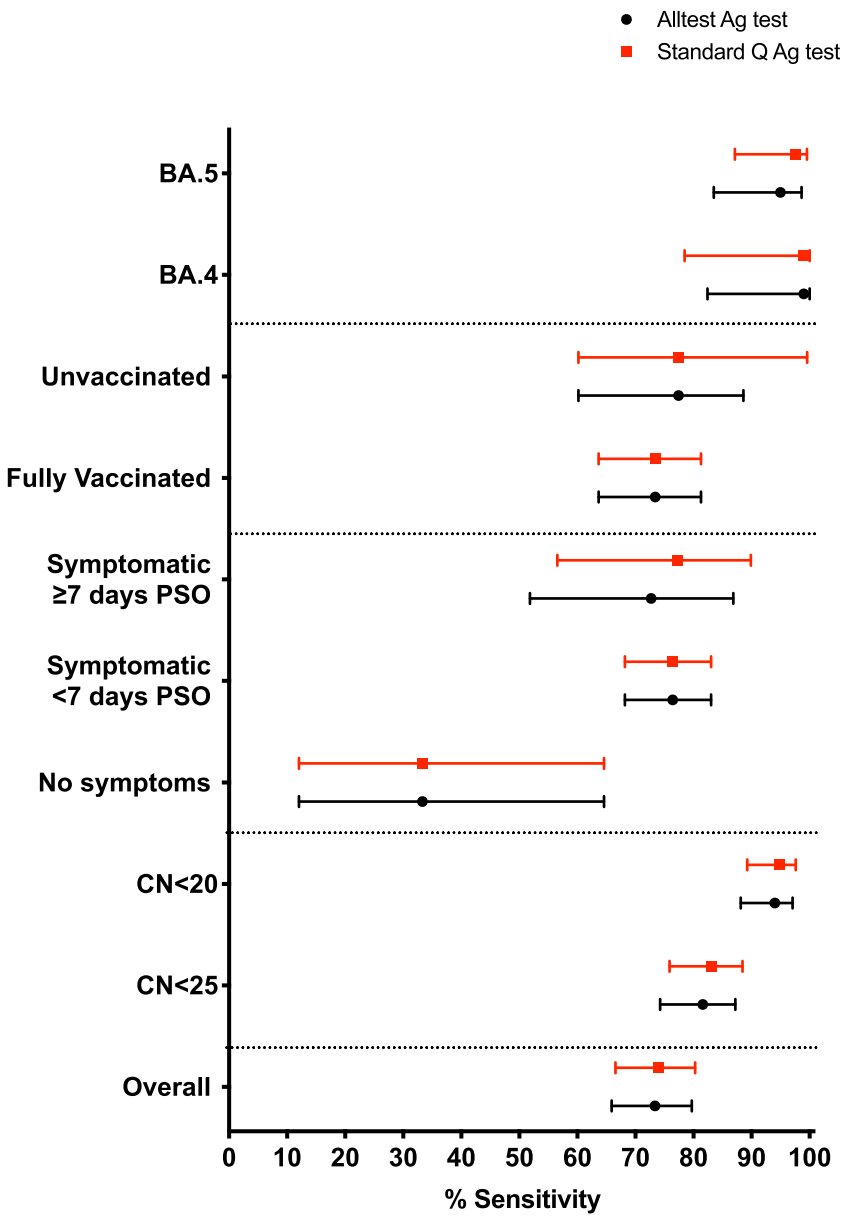

Fig. 1. Sensitivity of the AllTest SARS-CoV-2 Rapid Ag and the Standard Q COVID-19 Ag tests across different categories (CN value, presence and duration of symptoms, vaccination and SARS-CoV-2 lineage. The error bars represent 95% Confidence interval.

AllTest SARS-CoV-2 Ag test and sensitivity of 87.50% (95% CI 76.37–93.81) for Standard Q COVID-19 Ag test. This remained true in the analysis only including samples with >80% sequence coverage (**Sup. Table 2 C**). Sensitivity of both tests was slightly higher in unvaccinated (77.42% for AllTest and 77.41% for Standard Q) compared to fully vaccinated study participants (73.40% for AllTest and Standard Q), **Sup. Table 3**.

As expected, samples that resulted in false negative values for both tests had significantly higher SARS-CoV-2 CN values compared to true positive samples (**Sup. Figure 1**). We observed no difference in CN values between samples based on time post symptom onset and vaccination status (**Sup. Figure 2. A,B**).

4. Discussion

The performance of SARS-CoV-2 tests requires reassessment with the emergence of new variants carrying novel mutations that could potentially impact test performance.

Both AllTest and Standard Q Ag rapid tests performed well with an overall sensitivity >70%. Sensitivity increased to above 80% for both tests in samples with CN values < 25. As expected, both tests performed poorly in patients without symptoms [4–7]. With respect to detection of infections with the Omicron sub-lineage, sensitivity of both tests was >90% for Omicron sub-lineages BA.4 and BA.5 showing that the additional nucleocapsid mutations in these sub-variants do not impact the performance of the two evaluated rapid antigen tests. As we have previously reported [8], we observed slightly higher sensitivity in unvaccinated individuals compared to vaccinated individuals however this analysis is confounded by the lack of information on timing and presence of previous natural infections.

Our study has several limitations, including a bias towards samples with high viral load in which viral sequencing was successful. Irrespective, our data shows that both nasal AllTest and nasopharyngeal Standard Q Ag rapid test performance was not impacted by the mutations in the BA.4 and BA.5 Omicron sub-variants. The performance of rapid antigen tests for the currently dominant variant, XBB.1.5, a

recombinant of diversified BA.2 lineages, is likely to mirror the test performance previously reported for BA.2 variants [8].

CRediT authorship contribution statement

Conceptualization (AS, NS, CE), Data curation (AS, NS, SN, JG, DT, EJS, CC), Formal analysis (AS, GL, MdV, LL, JG, DT, EJS), Funding acquisition (AS, NS, CE, ABMK, KN, SAK, QAK), Methodology (AS, NS, CE, MdV), Project administration (AS, NS, CE, MdV, ABMK, KN), Resources (KN, CC, CE, TdO, SAK, QAK), Supervision (AS, NS, CC), Visualization (AS, NS, MdV), Writing - original draft (AS, NS), Writing - review & editing (AS, NS, CE, SN, JG, DT, EJS, MdV, LL, GL, CC, CE, KN, ANMK, TdO, SAK, QAK).

Data use agreement: De-identified patient-level data can be accessed by contacting the corresponding author with a detailed description of the research question.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antigen rapid diagnostic tests were provided by FIND, the global alliance for diagnostics, and FIND was involved in the study design development. CE and MdV are employees of FIND. The rest of the authors declare no conflict of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2023.105498.

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