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Independent Evaluation of Four SARS-CoV-2 Antigen Rapid Tests Against qRT-PCR in Niger

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Abstract

Background: Reliable, decentralised SARS-CoV-2 testing was a major challenge during the COVID-19 pandemic, particularly in low and middle-income countries where molecular capacity was limited. Antigen rapid diagnostic tests (Ag-RDTs) offered a practical alternative, but their field performance varied and locally generated data were needed to guide policy.

Objectives: We indepently evaluated four commercial Ag-RDTs, including an African manufactured test, under routine conditions in Niger to inform the national COVID-19 testing algorithm.

Methods: Between July 2021 and September 2023, four independent prospective evaluations were conducted among symptomatic patients (symptom onset ≤7 days) at two health facilities in Niamey. Each test namely STANDARD Q nasopharyngeal, Panbio nasopharyngeal and nasal and SAYTU nasal was assessed against qRT-PCR. Sensitivity and specificity were estimated with 95% confidence intervals.

Results: Across the four evaluations, 956 patients were tested and 422 (44.1%) were qRT-PCR positive. Sensitivity was 85.7% for the Panbio nasopharyngeal test, 80.1% for the SAYTU nasal test, 76.3% for the Panbio nasal test and 75.0% for the STANDARD Q nasopharyngeal test; specificity exceeded 95% for all four. Sensitivity was highest in high-viral-load specimens.

Conclusion: Only the Panbio nasopharyngeal test met both WHO criteria of sensitivity ≥80% and specificity ≥ 97%. However, the other three tests, including the Africa-manufactured SAYTU assay, may support screening where molecular capacity is limited, provided that negative results in symptomatic patients are confirmed by qRT-PCR.

Keywords: COVID-19 ; SARS-CoV-2 ; Rapid diagnostic test ; Sensitivity ; Specificity ; Niger

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the COVID-19 pandemic (Corman et al., 2020; Valencia, 2020). The virus is transmitted mainly by the respiratory route and causes a spectrum of disease ranging from asymptomatic infection to severe illness (Cucinotta & Vanelli, 2020; Rothan & Byrareddy, 2020). By 18 September 2022, more than 609 million confirmed cases and over 6.5 million deaths had been reported worldwide (World Health Organization, 2022). Testing suspected cases early in the course of illness is one of the most important measures for limiting household and community transmission (Ristic et al., 2021).

Real-time reverse-transcription polymerase chain reaction (qRT-PCR) remains the reference method for confirming SARS-CoV-2 infection because of its high accuracy, but it is laboratory-intensive and slow to return results (Corman et al., 2020; Sule & Oluwayelu, 2020; Younes et al., 2020). Antigen rapid diagnostic tests (Ag-RDTs) were developed to address these constraints, enabling point-of-care testing in decentralised and remote settings (Safiabadi Tali et al., 2021; Yamayoshi et al., 2020). Most Ag-RDTs

use nasopharyngeal swabs, which require trained staff and personal protective equipment (Lindner et al., 2021); tests using less invasive, self-collectable nasal specimens are now available and improve patient acceptability (Agulló et al., 2021; Patriquin et al., 2022). More than a thousand commercial SARS-CoV-2 Ag-RDTs exist, with widely varying sensitivity and specificity (Xu et al., 2022). The World Health Organization (WHO), in agreement with the European Centre for Disease Prevention and Control, has set minimum performance requirements of ≥80% sensitivity and ≥97% specificity for such assays (Al-Alawi et al., 2021).

Because Ag-RDT performance depends on the population tested, the circulating variants, the sampling method and operator conditions, in-country evaluation is recommended before a test is adopted into national policy. We therefore conducted four independent prospective evaluations of four SARS-CoV-2 Ag-RDTs against qRT-PCR among symptomatic patients in Niger. One of these tests, the SAYTU nasal assay (SAY-Na), is manufactured in Senegal and, to our knowledge, had not previously been independently evaluated; the others are used widely elsewhere but had not been assessed in the Niger context.

We here in aim to generate locally relevant performance data to inform the national COVID-19 diagnostic algorithm during the pandemic.

Methods

Ethical considerations:

The ethical agreement for this research was obtained from the Comité National d'Ethique pour la Recherche en Santé (CNERS) by decision reference N°063/2021/CNERS. Written informed consent was obtained from each enrolled patient or legal guardian before sampling.

Study design and settings

We conducted four independent, prospective, hospital-based diagnostic-accuracy evaluations between July 2021 and September 2023, including: the STANDARD Q COVID-19 Ag nasopharyngeal test (STQ-Np; SD Biosensor Inc., Republic of Korea) from July to October 2021, the Panbio COVID-19 Ag Rapid Test Device nasopharyngeal (PANB-Np) and nasal (PANB-Na) formats (Abbott Diagnostics GmbH, Jena, Germany) from February to September 2022 and the SAYTU COVID-19 Ag nasal test (SAY-Na; DIATROPIX, Institut Pasteur de Dakar, with support from FIND and Unitaid) from April to September 2023. All kits were donated to the Ministry of Public Health by international and regional partners, including the World Health Organization, the West African Health Organization, Africa CDC and non-governmental organisations. The evaluations were conducted at the Hôpital National de Niamey and the Clinique Gamkalle,

both involved in COVID-19 case management in Niamey, Niger. All laboratory tests were performed at the Centre de Recherche Médicale et Sanitaire (CERMES), the national COVID-19 reference laboratory. The study is reported in line with the STARD 2015 guidance for diagnostic-accuracy studies.

Study population and sampling

Participants were enrolled using non-probability sampling to the prevalence of COVID-19 in Niamey during each study period. We included symptomatic patients of any age or sex with symptom onset ≤7 days who presented with one or more of the following: fever, cough, sore throat, rhinorrhoea, headache, fatigue or loss of smell. Clinical and demographic information was collected with a standardised questionnaire before sampling.

Index tests

Each Ag-RDT is a lateral-flow immunoassay for the qualitative detection of SARS-CoV-2 antigen in nasopharyngeal or nasal specimens (Patriquin et al., 2022). Depending on the test, a nasopharyngeal or nasal swab provided in the kit was used. Results were read at 15 min by a qualified clinician and recorded as positive or negative. Operators read the Ag-RDT before the qRT-PCR result was available but were not blinded to clinical presentation.

Reference standard

qRT-PCR served as the reference standard. RNA was extracted with the RADI PREP Swab and Stool DNA/RNA Kit (Ref S0003895) and amplified on a QuantStudio 5 Real-Time PCR System using the RADI COVID-19. Detection Kit (Ref RV008; KH Medical), targeting the S and RdRp genes. A cycle-threshold (Ct) value >38 was considered negative.

Statistical analysis

Data were recorded in Microsoft Excel 2013 and analysed in SPSS v26.0. Sensitivity was calculated as the proportion of qRT-PCR–positive specimens correctly identified by the Ag-RDT, and specificity as the proportion of qRT-PCR–negative specimens correctly identified. Ninety-five per cent confidence intervals were calculated using the Wilson score method. As an exploratory analysis, Ag-RDT sensitivity was stratified by qRT-PCR Ct value as a proxy for viral load.

Results

Participants

Across the four evaluations, 956 symptomatic patients (mean age 41.6 years; 593 [62.0%] male) were enrolled between July 2021 and September 2023. The most frequent symptoms were fever (75.6%) and cough (61.7%). Overall, 422 patients (44.1%) were positive by qRT-PCR. Because the four tests were evaluated in separate patient groups and at different times, results are reported separately for each test and are not compared directly (Table I).

Table I: Diagnostic performance of four SARS-CoV-2 Ag-RDTs against qRT-PCR (each test evaluated in a separate patient sample).

	PANB-Na (N = 212)	**PANB-Np (N = 183)**	**STQ-Np (N = 151)**	**SAY-Na (N = 410)**
qRT-PCR positive / negative, n (%)	59 (27.8) / 153 (72.2)	77 (42.1) / 106 (57.9)	75 (49.7) / 76 (50.3)	211 (51.5) / 199 (48.5)
Ag-RDT positive / negative, n (%)	52 (24.5) / 160 (75.5)	69 (37.7) / 108 (59.0)	56 (37.1) / 95 (62.9)	176 (42.9) / 234 (57.1)
Sensitivity, % (95% CI)	76.3 (63.4–86.4)	85.7 (75.9–92.6)	75.0 (63.4–84.0)	80.1 (74.1–85.3)
Specificity, % (95% CI)	95.4 (90.8–98.1)	98.1 (92.5–100)	100 (95.3–100)	96.5 (92.9–98.6)

Ag-RDT, antigen rapid diagnostic test; CI, confidence interval; qRT-PCR, real-time reverse-transcription polymerase chain reaction.

STANDARD Q nasopharyngeal test (STQ-Np)

Of 151 patients tested, 75 (49.7%) were qRT-PCR positive and 56 (37.1%) were positive by STQ-Np. Sensitivity was 75.0% and specificity 100% (Table I). Among the 38 specimens with Ct ≤25, STQ-Np detected all positives, although this subgroup was small and the estimate imprecise. Concordance with qRT-PCR was 87.4%

Panbio nasal test (PANB-Na)

Of 212 patients tested, 59 (27.8%) were qRT-PCR positive and 52 (24.5%) were positive by PANB-Na. Sensitivity was 76.3% and specificity 95.4% ; concordance with qRT-PCR was 90.1% (Table I). In the small high-viral-load subgroup (Ct <20, n = 18), all PANB-Na results were positive, though the estimate is imprecise.

Panbio nasopharyngeal test (PANB-Np)

Of 183 patients tested, 77 (42.1%) were qRT-PCR positive and 69 were positive by PANB-Np. Sensitivity was 85.7% and specificity 98.1%; concordance with qRT-PCR was 92.9% (Table I). Sensitivity was high at low Ct (Ct <20, n = 8) and at intermediate Ct (25<Ct<30, n = 32), though the strata were small.

SAYTU nasal test (SAY-Na)

Of 410 patients tested, 211 (51.5%) were qRT-PCR positive and 176 (42.9%) were positive by SAY-Na. Sensitivity was 80.1% and specificity 96.5%; concordance with qRT-PCR was 88.1% (Table I). Among high-viral-load specimens (Ct <20, n = 33), sensitivity was 97.0%. To our knowledge this is the first independent field evaluation of this test. Sensitivity by Ct stratum for all four tests is shown in Figure 1.

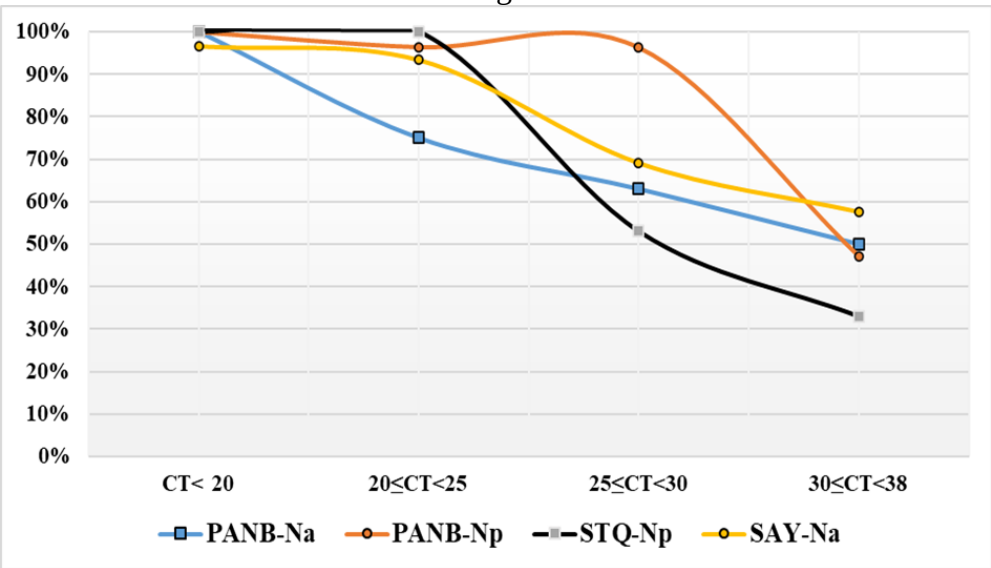

Figure 1: Sensitivity of the four antigen tests by Ct stratum

Discussion

We evaluated four commercial SARS-CoV-2 Ag-RDTs from three manufacturers against qRT-PCR under routine conditions in Niger, with the explicit aim of generating locally relevant data to inform national testing policy. All the four tests showed high specificity (>95%), but varied sensitivity and only one test met the WHO performance criteria.

For STQ-Np, our sensitivity (75.0%) was higher than reports from Serbia (58.1%) and Oman (65.8%) (Al-Alawi et al., 2021; Ristic et al., 2021) but lower than those from Thailand (98.3%) and the Republic of Korea (89.2%) (Chaimayo et al., 2020; Kim et al., 2022); such differences are driven partly by the number and viral-load distribution of positive samples. Our estimate was close to the FIND independent evaluation of the STANDARD Q assay (sensitivity 78.6%, specificity 99.4%).

For PANB-Np, our sensitivity (85.7%) was slightly below the manufacturer's figure and a Spanish study (about 90%–91%) (Merino et al., 2021) but higher than other reports from Spain and France (47%–80%) (Albert et al., 2021; Farfour et al., 2021; Linares et al., 2020), where the inclusion of asymptomatic cases or retrospective samples may explain lower

performance. For PANB-Na, our sensitivity (76.3%) was consistent with a Mozambican study (79.7%) (Sitoe et al., 2022); however exceeded reports from Spain, Canada and the Republic of Korea (44.7%–64.7%) (Agulló et al., 2021; Goodall et al., 2022; Oh et al., 2022). These differences plausibly related to larger samples and longer symptom-onset windows.

SAY-Na, the only assay in this evaluation manufactured in Africa, performed broadly in line with the manufacturer's evaluation in Brazil (sensitivity 89.2%, specificity 97.3%). Given the strategic importance of regional diagnostic manufacturing for pandemic preparedness in Africa, this first independent field evaluation is encouraging and supports further, larger assessment.

All the four tests performed best in high-viral-load specimens, consistent with the well-described association between antigen detection and viral load and the expected drop in sensitivity at Ct >30 (Dessalegn et al., 2022; Ristic et al., 2021; Scohy et al., 2020). Consistent with previous reports, nasal sampling offers a more acceptable, less invasive alternative to nasopharyngeal sampling and does not require specially trained staff (Dutta et al., 2022; Goodall et al., 2022; Père et al., 2020); some studies report modestly lower sensitivity with nasal swabs (Père et al., 2020; Wölfl-Duchek et al., 2022). Our data are compatible with this pattern, but because the nasal and nasopharyngeal Panbio formats were evaluated in different patients and at different times, we cannot make a direct within-study comparison between them.

Limitations

This study presents a number of limitations that warrant the discussions. First, each test was assessed in a different group of patients and during a different phase of the pandemic, so the four estimates are not directly comparable and should not be read as a ranking. Second, the evaluations spanned from July 2021 to September 2023, during which several SARS-CoV-2 variants circulated. For instance, variant-related differences in antigen expression may have influenced detectability and variant data were not available for individual specimens. Third, some subgroup analyses, including sensitivity by Ct stratum, were based on small numbers of samples mainly explained by the low COVID-19 prevalence in Niger.

Conclusion

Timely detection of SARS-CoV-2 is essential for case management and outbreak control. In these evaluations, all four Ag-RDTs showed high specificity, but only the PANB-Np met both WHO minimum criteria of sensitivity ≥80% and specificity ≥97% ; although the other three exhibit

appreciable sensitivities. These tests may nonetheless have a useful role in Niger's testing policy, particularly for rapid screening and point-of-care use where molecular capacity is limited. Importantly, the locally produced SAY-Na performed comparably to the imported nasal test and therefore, should be widely used in the continent for future emergencies.

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Authors' contributions

A.L., R.J., F.H. and M.I. conceived and designed the study. F.H., M.I, I.K.A., O.H., W.H., B.K.K., H.H.I., B.A., B.I., E.C.C. collected and analysed the data. F.H., M.I, I.K.A. and A.L. drafted the manuscript. A.L., S.M., R.J., M.I., E.C.C. and W.H. critically revized it.

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Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All data are included in the content of the paper.

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