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Highlights
- Pooled remnant clinical samples with variants of concern are tested using BinaxNOW
- BinaxNOW detects live and heat-inactivated virus in pooled and individual samples
- BinaxNOW sensitivity slightly lower against Omicron variant in laboratory tests
- BinaxNOW remains a useful public health tool to combat the COVID-19 pandemic
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SUMMARY
As the emergence of SARS-CoV-2 variants brings the global pandemic to new levels, the performance of current rapid antigen tests against variants of concern and interest (VOC/I) is of significant public health concern. Here, we report assessment of the Abbot BinaxNOW COVID-19 Antigen Self-Test. Using genetically sequenced remnant clinical samples collected from individuals positive for SARS-CoV-2, we assessed the performance of BinaxNOW against the variants that currently pose public health threats. We measured the limit of detection of BinaxNOW against various VOC/I in a blinded manner. BinaxNOW successfully detected the Omicron (B.1.1.529), Mu (B.1.621), Delta (B.1.617.2), Lambda (C.37), Gamma (P.1), Alpha (B.1.1.7), Beta (B.1.351), Eta (B.1.525), and P.2 variants and at low viral concentrations. BinaxNOW also detected the Omicron variant in individual remnant clinical samples. Overall, these data indicate that this inexpensive and simple-to-use, FDA-authorized and broadly distributed rapid test can reliably detect Omicron, Delta, and other VOC/I.

INTRODUCTION
The emergence of new SARS-CoV-2 variants has pushed the pandemic to new levels, raising concerns of increased infectivity, breakthrough cases among vaccinated individuals, and the viability of current test strategies. In 2021, new SARS-CoV-2 variants of concern and interest (VOC/I) such as the Delta (B.1.617.2), Lambda (C37), Mu (B.1.621), and Omicron (B.1.529) variants have caused multiple surges of COVID-19 cases worldwide and raised concerns about evasion of the currently available SARS-CoV-2 vaccines (Herlihy, 2021; Lopez Bernal et al., 2021; Shah et al., 2021b; Tchesnokova et al., 2021).

With the high prevalence of rapid diagnostic assays currently available to the public in point-of-care (POC) settings and as at-home over-the-counter (OTC) kits, an obvious question is whether these tests can even reliably detect Delta, Omicron, and other variants of concern/interest (VOC/I). The timing of detection is particularly important for Omicron, as it has been shown to have a shorter incubation period than previous variants, and thus may become transmissible faster after infection (Brandal et al., 2021; Burki, 2021; Jansen, 2021). If these home and community-based tests can indeed detect VOC/I, they can be implemented as part of broad public health strategies to help curtail the rapid spread of VOC/I. On the other hand, if these rapid tests cannot reliably detect the most prevalent VOC/I, their overall clinical utility at the current point of the pandemic should be called into question.

To that end, here, we report our objective assessment of Abbott’s BinaxNOW COVID-19 Antigen Self-Test, which has among the highest usage, availability, distribution, and production rates of rapid tests and was the first lateral flow assay (LFA)-based rapid antigen test to receive U.S. FDA Emergency Use Authorization (EUA) for the home OTC setting (Shah et al., 2021a; Prince-Guerra, 2021; Pollock et al., 2021; Hodges, 2021). BinaxNOW is a SARS-CoV-2 diagnostic assay that detects the viral nucleocapsid (N) protein in samples collected by anterior nasal swab and reports a qualitative positive, negative, or invalid result (BINAXNOW COVID-19 AG CARD, n.d). We previously assessed the usability of BinaxNOW...
as a self-administered test and conducted initial experiments assessing the test’s performance in detecting wild type virus and various variants (Frediani et al., 2021). Here, we report with a higher molecular resolution on the performance of BinaxNOW using a comprehensive panel of VOC/I that is currently of the highest public health significance.

In September 2021, the Mu (B.1.621) variant had been detected in every state in the US and was designated as a VOC by the World Health Organization (WHO), though it was later reclassified as a VOI (World Health Organization, 2022). Importantly, the Mu variant harbors a mutation, E484K, that likely enables the virus to blunt vaccine and infection-induced immunity (Wadman, 2021). Given the potential at the time for this variant to significantly worsen the outlook of the global pandemic, we tested the performance of BinaxNOW in duplicate and a blinded manner using remnant clinical samples (RCS) (Figure 1).

Most recently, the Omicron variant (B.1.1.529) has emerged and been designated a VOC by the WHO due to a high number of mutations in the spike protein (World Health Organization, 2021). The Omicron variant has 32 additional mutations in the spike protein, which may allow it to partially evade vaccine-elicted immunity by escaping neutralizing antibodies from previous strains of SARS-CoV-2 (Cao et al., 2021; Cele et al., 2021; Liu et al., 2021). Owing to its increased transmissibility, Omicron has quickly become the dominant variant in the United States and around the world (CDC, 2020). For these reasons, we assessed the performance of BinaxNOW using pooled, heat-inactivated RCS positive for Omicron (B.1.1.529) variant.

RESULTS

Overall, as detailed in Table 1, the BinaxNOW successfully detected the Mu (B.1.621) variant in individual RCS with Ct values ranging from 23 (highest viral concentration) to 30.3 (lowest viral concentration), which is
within the clinically relevant range reported by other groups (Faı´co-Filho et al., 2020; Jaafar et al., 2021; Mageley et al., 2020). BinaxNOW also successfully detected B.1.2 in individual RCS with Ct values ranging from 22 (highest viral concentration) to 34.8 (lowest viral concentration).

BinaxNOW successfully detected Mu (B.1.621), Delta (B.1.617.2), Lambda (C37), Gamma (P1), Alpha (B.1.1.7), Beta (1.351), Eta (B.1.525), and P2 viral variants in RCS pools consistently from samples with the lowest Ct values of 23.8, (highest viral concentrations) to those with the highest detectable Ct values of

Table 1. Assessment of the Abbot BinaxNOW COVID-19 Antigen Self-Test against the Mu (B.1.621) viral variant in genetically sequenced clinical nasal swab samples

| Sample ID | Lineage | Dilution factor | N2 Ct | N2 Ct Std Dev | Replicate 1 Result | Replicate 2 Result | Final Result |
|-----------|---------|-----------------|-------|---------------|--------------------|--------------------|-------------|
| Sample 1  | B.1.2   |                 | 22.0  | 0.01          | Positive           | Positive           | Positive     |
| Sample 2  | B.1.2   |                 | 22.7  | 0.16          | Positive           | Positive           | Positive     |
| Sample 3  | B.1.2   |                 | 24.3  | 0.10          | Positive           | Positive           | Positive     |
| Sample 4  | B.1.2   |                 | 20.1  | 0.13          | Positive           | Positive           | Positive     |
| Sample 5  | B.1.2   |                 | 27.7  | 0.08          | Positive           | Positive           | Positive     |
| Sample 6  | B.1.2   |                 | 22.9  | 0.05          | Positive           | Positive           | Positive     |
| Sample 7  | B.1.2   |                 | 32.4  | 0.12          | Positive           | Positive           | Positive     |
| Sample 8  | B.1.2   |                 | 34.8  | 0.91          | Positive           | Positive           | Positive     |
| Sample 8  | B.1.2   |                 | 4     | 35.4          | Negative           | Negative           | Negative     |
| Sample 8  | B.1.2   |                 | 16    | 36.5          | Negative           | Negative           | Negative     |
| Sample 8  | B.1.2   |                 | 64    | –             | Negative           | Negative           | Negative     |
| Sample 9  | B.1.621 |                 | 24.3  | 0.23          | Positive           | Positive           | Positive     |
| Sample 10 | B.1.621 |                 | 28.5  | 0.06          | Positive           | Positive           | Positive     |
| Sample 11 | B.1.621 |                 | 23    | 0.15          | Positive           | Positive           | Positive     |
| Sample 12 | B.1.621 |                 | 24    | 0.03          | Positive           | Positive           | Positive     |
| Sample 13 | B.1.621 |                 | 26.7  | 0.21          | Positive           | Positive           | Positive     |
| Sample 14 | B.1.621 |                 | 27    | 0.15          | Positive           | Positive           | Positive     |
| Sample 15 | B.1.621 |                 | 26.6  | 0.03          | Positive           | Positive           | Positive     |
| Sample 16 | B.1.621 |                 | 28.9  | 0.08          | Positive           | Positive           | Positive     |
| Sample 16 | B.1.621 |                 | 4     | 28.2          | Positive           | Positive           | Positive     |
| Sample 16 | B.1.621 |                 | 16    | 30.3          | Positive           | Positive           | Positive     |
| Sample 16 | B.1.621 |                 | 64    | 33.1          | Negative           | Negative           | Negative     |
| Sample 16 | B.1.621 |                 | 256   | 34.6          | Negative           | Negative           | Negative     |
| Sample 16 | B.1.621 |                 | 1024  | 37.3          | Negative           | Negative           | Negative     |
| Sample 16 | B.1.621 |                 | 4096  | 37.3          | Negative           | Negative           | Negative     |
| Sample 16 | B.1.621 |                 | 16,384| 0             | Negative           | Negative           | Negative     |
| Sample 17 | Negative control |                 | 0     | –             | Negative           | Negative           | Negative     |
| Sample 18 | Negative control |                 | 0     | –             | Negative           | Negative           | Negative     |

RT-qPCR for the SARS-CoV-2 N2 gene using CDC primers/probe set was also performed on each RCS sample and cycle threshold (Ct) values were used as estimates of viral load. For comparison, samples one to eight were obtained from individuals infected with B.1.2, whereas samples 9 to 16 were obtained from RCS positive for Mu (B.1.621) variant. Samples 8 and 16 were also serially diluted and again assessed with RT-qPCR, to assess the limit of detection of the assay.

within the clinically relevant range reported by other groups (Faı´co-Filho et al., 2020; Jaafar et al., 2021; Mageley et al., 2020). BinaxNOW also successfully detected B.1.2 in individual RCS with Ct values ranging from 22 (highest viral concentration) to 34.8 (lowest viral concentration).
28.9, (lowest viral concentrations) (Figure 2), which is within the clinically relevant range reported by other groups. Moreover, BinaxNOW also successfully detected subtypes of Delta (B.1.617.2), which harbor different N protein mutations. Our results also showed that BinaxNOW successfully detected all the aforementioned VOC/I with equivalent sensitivity, defined as within three Ct values of B.1.2.

BinaxNOW successfully and consistently detected the Omicron (B.1.1.529) variant in heat-inactivated pooled clinical samples with the highest detected Ct value of 24.6 (Figure 2). This value is lower than the highest detected value of other variants of concern, which may indicate that the BinaxNOW has lower sensitivity against the Omicron (B.1.1.529) variant compared to the previous VOC (Figure 2). BinaxNOW also demonstrated a lower sensitivity against pooled live Omicron (B.1.1.529) RCS compared to B.1.2 and Delta (B.1.617.2) RCS. The highest detected Ct value for Omicron (B.1.1.529) was 25, which is lower than that detected for B.1.2 (Ct 26.8) and Delta (B.1.617.2) (Ct 28.1) (Figure 3). In individual RCS, BinaxNOW detected samples with N2 Ct values as high as

Figure 2. Limit of detection of the Abbott BinaxNOW COVID-19 Antigen Self-Test against the current SARS-CoV-2 variants of concern and interest using pooled heat-inactivated samples

(A) Lowest viral load (highest RT-qPCR Ct value) for the indicated VOC/I detected by the BinaxNOW SARS-CoV-2 antigen assay. Green bars indicate successful detection (defined as within three Ct values) of the specific VOC/I compared to that of the control, the SARS-CoV-2 variant B.1.2 (top bar), which is not considered to be a VOC/I.

(B) Different nucleocapsid (N) protein mutations in the indicated Delta VOC pools.

Figure 3. Performance of the Abbott BinaxNOW COVID-19 Antigen Self-Test against live Omicron (B.1.1.529) variant

The highest detectable Ct value (lowest detectable viral load) of the BinaxNOW against the Omicron variant, Delta variant, and a control are shown. Performance was assessed against both live virus and heat-inactivated virus.
The highest detectable Ct value of the individual Omicron samples is lower than that of previously tested individual control (B.1.2) samples and samples containing the Mu (B.1.621) variant (Table 1).

DISCUSSION

Although the sensitivity of BinaxNOW appears to be slightly decreased against the Omicron (B.1.1.529) variant compared to previous VOC/I, the results still justify the continued use of this readily available, inexpensive, and simple-to-use rapid test kit as part of the community- and/or home-based testing strategies to combat the ongoing public health crisis. Ultimately, laboratory experiments cannot fully recapitulate the real-world application of a test kit, and the utility of the BinaxNOW will depend on a review of its clinical performance, which we are currently conducting.

Limitations of the study

We acknowledge the limitations of the current study are that we used RCS that may have some degradation and may not accurately reflect real-world testing conditions. In addition, the BinaxNOW kit lots used in this study varied from experiment to experiment and that could have generated slight differences in the test performance. Additional studies into the quantitative differences in the N antigen levels of Omicron variant patient samples will help to clarify the implications of the decreased performance. Future studies are also underway to compare BinaxNOW to other available rapid antigen tests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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| Sample  | Lineage   | Avg N2 Ct (3 replicates) | Std Dev N2 Ct | Replicate 1 | Replicate 2 | Final Result |
|---------|-----------|--------------------------|---------------|-------------|-------------|--------------|
| Sample 1 | B.1.1.529 | 21.2                     | 0.15          | Positive    | Positive    | Positive     |
| Sample 6 | B.1.1.529 | 24.7                     | 0.05          | Positive    | Positive    | Positive     |
| Sample 3 | B.1.1.529 | 25.8                     | 0.04          | Positive    | Positive    | Positive     |
| Sample 4 | B.1.1.529 | 26.2                     | 0.05          | Positive    | Positive    | Positive     |
| Sample 7 | B.1.1.529 | 26.3                     | 0.11          | Positive    | Positive    | Positive     |
| Sample 9 | B.1.1.529 | 26.3                     | 0.08          | Positive    | Positive    | Positive     |
| Sample 2 | B.1.1.529 | 27                      | 0.06          | Positive    | Positive    | Positive     |
| Sample 8 | B.1.1.529 | 28.2                     | 0.15          | Positive    | Positive    | Positive     |
| Sample 10| B.1.1.529 | 28.2                     | 0.14          | Positive    | Positive    | Positive     |
| Sample 12| B.1.1.529 | 28.2                     | 0.29          | Positive    | Positive    | Positive     |
| Sample 5 | B.1.1.529 | 28.3                     | 0.11          | Positive    | Positive    | Positive     |
| Sample 11| B.1.1.529 | 29.4                     | 0.16          | Negative    | Negative    | Negative     |

Genetically sequenced remnant clinical samples (N = 12) containing the N:D343G mutation were measured in triplicate using RT-qPCR, as a proxy for viral load. The samples were then tested in duplicate using the BinaxNOW COVID-19 Antigen Self-Test. The sample with the highest detectable Ct is highlighted in green.
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AUTHOR CONTRIBUTIONS
LB- Designed and performed experiments, supervised experiments, analyzed data, and edited paper and figures. AR- Designed and performed experiments, supervised experiments, analyzed data, and edited paper and figures. JL- writing and editing figures and manuscript and analyzed data, KV - Performed experiments, KB- Performed experiments, MG- Analyzed data and edited paper and figures, JS- Analyzed data and edited paper and figures, EL- Experimental sample procurement as part of NIH Variant Task Force. RC- Experimental sample procurement as part of NIH Variant Task Force. TP- Experimental sample procurement as part of NIH Variant Task Force. AP- Sample sequencing and sequence confirmation at Emory, RS- project planning and oversight, GM- project planning and oversight, WL- project planning and oversight, analyzed data, and edited paper and figures

DECLARATION OF INTERESTS
The authors declare no competing interests

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Biological samples  |        |            |
| Remnant Clinical Samples containing SARS-CoV-2 variants Mu (B.1.621) Delta (B.1.617.2), Lambda (C37), and Gamma (P1) and B.1.2 | Helix OpCo, LLC | N/A |
| Remnant clinical samples containing SARS-CoV-2 Omicron (B.1.529) | University of Washington | N/A |
| Critical commercial assays | | |
| CDC 2019-nCoV RT-PCR Diagnostic Panel  | IDT | Catalog # 10006770 |
| MagMax Viral RNA Isolation Kit  | Applied Biosystems | Catalog # AM1939 |
| qScript XLT 1-Step RT-qPCR ToughMix | QuantaBio | Catalog # 95133-100 |
| BinaxNOW COVID-19 Antigen Self-Test  | Abbott | N/A |
| Software and algorithms | | |
| Rosalind Diagnostic Monitoring (DxM) system | RadX | https://radx.rosalind.bio |
| GraphPad Prism  | GraphPad | https://www.graphpad.com/scientific-software/prism/ |
| NextClade | NextClade | https://clades.nextstrain.org/ |
| Other | | |
| Negative Nasal Wash | Lee BioSolutions | Catalog # 991-26-P |
| KingFisher Apex System | Thermo Fisher Scientific | Catalog # 5400910 |
| LightCycler 480II | Roche | Catalog # 05015278001 |

RESOURCE AVAILABILITY

Lead contact
Requests for further information and resources should be directed to the lead contact Wilbur Lam (wilbur.lam@emory.edu), or the co-corresponding author Greg Martin (greg.martin@emory.edu).

Materials availability
This study did not generate new materials.

Data and code availability
The genetic sequence data was analyzed the Nextclade (https://clades.nextstrain.org/) version 1.7.1 or higher. SARS-CoV-2 strains to be tested were identified using the Rosalind (https://radx.rosalind.bio) bioinformatics platform, version 3.33.1.0 or higher. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The study abides by the ethical guidelines of research at Emory University. The de-identified remnant clinical samples in this study were provided without any patient information. Sequencing of the SARS-CoV-2 to confirm the variant was done by the party from which we obtained the remnant clinical samples.

METHOD DETAILS

Heat-inactivated RCS (provided by Helix OpCo, LLC) from individuals positive for SARS-CoV-2 (N = 8) known to be infected with the Mu variant were selected based on genomic sequence quality (as determined by NextClade) and cycle threshold (Ct) value of N protein. We compared these results to samples
obtained from individuals (N = 8) infected with the B.1.2 strain of SARS-CoV-2, which is not considered to be a VOC/I by the WHO, and therefore served as our comparator. RT-qPCR for the SARS-CoV-2 N2 gene using CDC primers/probe set was performed on each RCS and N2 Ct values were used as estimates of viral load.

In addition, using genetically sequenced RCS collected from individuals positive for SARS-CoV-2 across the country, we created RCS “pools” of each of the VOC/I (Figure 1). The individual VOC/I pools were verified by repeating genetic sequencing to ensure quality control. Panels of the VOC/I pools of varying viral loads were then created by serial dilution using SARS-CoV-2 negative pooled human donor nasal wash (Lee Biosolutions, Catalog No. 991-26-P-1). Dilutions of every pool were then analyzed by RT-qPCR for the SARS-CoV-2 N2 gene using CDC primers/probe set (Figure 1). The performance of BinaxNOW was then assessed in a blinded manner in triplicate.

The pools for heat-inactivated Omicron variant were created using the same method as previously stated (Figure 1) and diluted to 10 dilutions that ranged from a Ct value of 21.2 to a Ct value of 31.7. The limit of detection was determined by testing each sample dilution 5 times with BinaxNOW. For testing, we used the direct spike method, where 20μL of sample was spiked onto the swab provided with the test and subsequent steps were according to the BinaxNOW instructions for use (IFU). This limit of detection was further confirmed by testing the highest detectable dilution as well as the two neighboring dilutions a further 20 times.

We then assessed BinaxNOW using non heat inactivated RCS obtained from the University of Washington that were confirmed to be Omicron (B.1.1.529). These samples were pooled and diluted to a range of Ct values between 19.3 and 28.8. The dilutions were tested according to the IFU of BinaxNOW rapid antigen test to determine the limit of detection. Finally, we obtained 12 RCS sequence confirmed to be Omicron from LabCorp and measured N2 Ct in triplicate by RT-qPCR (Table 2). These samples were tested in duplicate using BinaxNOW, following the IFU.

QUANTIFICATION AND STATISTICAL ANALYSIS
The mean and standard deviation of the Ct values in Tables 1 and 2 were calculated using Microsoft Excel.