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Performance characteristics of a rapid SARS-CoV-2 antigen detection assay at a public plaza testing site in San Francisco

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40 Word Summary:

This study examines the utility of Abbott BinaxNOW™ rapid direct antigen SARS-CoV-2 in the context of community screening at a public transit hub in comparison to RT-PCR.

Footnote Page

The authors report no commercial or other association that might pose a conflict of interest.

The content of this manuscript represents original work and has not been submitted or accepted for publication or presented at professional meetings.

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ABSTRACT

We evaluated the performance of the Abbott BinaxNOW™ Covid-19 rapid antigen test (Binax-CoV2) to detect virus among persons, regardless of symptoms, at a public plaza site of ongoing community transmission. Titration with cultured SARS-CoV-2 yielded a human observable threshold between $1.6x10^4$-$4.3x10^4$ viral RNA copies (cycle threshold (Ct) of 30.3-28.8). Among 878 subjects tested, 3% (26/878) were positive by RT-PCR, of which 15/26 had Ct<30, indicating high viral load. 40% (6/15) of Ct<30 were asymptomatic. Using this Ct<30 threshold for Binax-CoV2 evaluation, the sensitivity of Binax-CoV2 was 93.3% (14/15), 95% CI: 68.1-99.8%, and the specificity was 99.9% (855/856), 95% CI: 99.4-99.9%.

KEYWORDS

COVID-19; SARS-CoV-2; Rapid Antigen Test; Point of Care testing

INTRODUCTION

The global pandemic of SARS-CoV-2 infection has spread at an unprecedented pace [1] fueled by efficient transmission of infection by the respiratory route, including by asymptomatic and pre-symptomatic persons. Instances of successful control utilize masking, social distancing, and aggressive testing, tracing, and quarantine [2].

To date, the cornerstone of testing has been RT-PCR examination of respiratory secretions which has excellent sensitivity and specificity but is expensive and requires sophisticated equipment and highly trained personnel [3]. In practice, these features have often generated testing delays compromising their utility [4]. As a result, there is interest in rapid and economical assays that circumvent these limitations [5]. However, methods that do not include an amplification step are inherently less sensitive; their proper deployment will therefore require a rigorous evaluation of performance characteristics in different epidemiologic settings.

Lateral flow antigen detection diagnostics have been deployed for a variety of infectious diseases including malaria, RSV, and influenza. The Abbott BinaxNOW^{TM} COVID-19 Ag Card (hereafter referred to as Binax-CoV2) is one such assay that detects viral nucleocapsid (N) protein directly from nasal swabs. The test requires no instrumentation; results are scored visually and returned within 15 minutes. In August 2020, the FDA issued an emergency use authorization (EUA) for the diagnosis of SARS-CoV-2 infection in symptomatic patients within 7 days of symptom onset [6]. The US Department of Health and Human Services has distributed 150 million test kits. Given the value of a rapid assessment

of infectiousness, there is anticipated use in a broad range of subjects, including those who are asymptomatic.

Here we present a systematic examination of the performance characteristics of the Binax-CoV2 test in a community screening setting where testing was offered for symptomatic and asymptomatic subjects.

METHODS

Study Population and Specimen Collection

Over 3 days in September 2020, we offered testing in the Mission District, a Latinx-predominant neighborhood, known from prior surveys to have an elevated prevalence of SARS-CoV-2 infection [7,8]. Walk-up, free testing was conducted at a plaza located at an intersection of the Bay Area-wide subway system (BART) and the San Francisco city bus/streetcar system (MUNI). On the day of test, participants self-reported symptoms and date of onset, demographics, and contact information, as required by state and federal reporting guidelines. A laboratory technician performed sequential anterior swab (both nares) for the Binax-CoV2 assay followed by a second swab (both nares) for RT-PCR. Participants were notified of RT-PCR test results. For this study, Binax-CoV2 results were not reported back to study subjects.

Laboratory Testing for SARS-CoV-2

RT-PCR detection of SARS-CoV-2 was performed at the CLIA-certified lab operated by UCSF and the Chan Zuckerberg Biohub as described [9,10].

Field Testing using Binax-CoV2 assay

The Binax-CoV2 assay was performed by technicians on site as described by the manufacturer using the supplied swabs. Each assay was read by two independent observers, and a site supervisor served as a "tie-breaker". Beginning on day 2 of the study, each Binax-CoV2 assay card was scanned onsite using a color document scanner (CanoScan LIDE 400, Canon). Sample bands were retrospectively quantified from image data. Sample and background regions were localized by offset from the control band and relative mean pixel intensity decreases were calculated from blue and green channels averaged with respect to background.

Titration of in vitro cultured SARS-CoV-2 on Binax-CoV2 cards

SARS-CoV-2 from a UCSF clinical specimen was isolated, propagated and plaqued on Huh7.5.1 cells overexpressing ACE2 and TMPRSS2 [11]. Viral titers were determined using standard plaque assays [12]. For titration experiments, SARS-CoV-2 was diluted in Dulbecco's phosphate-buffered saline (DPBS) and 40 microliters of each dilution was absorbed onto the supplied swabs. Images of Binax-CoV2 cards were taken on an Apple iPhone6. All experiments using cultured SARS-CoV-2 were conducted in a biosafety level 3 laboratory.

N protein titration assay

SARS-CoV-2 N protein (1-419) was expressed in BL21(DE3) *E.coli* and purified by Ni-NTA chromatography, incorporating a 1M NaCl, 50 mM imidazole wash to remove bound RNA. Six concentrations of N protein were tested on ten lots of Binax-CoV2 kits. 40ul of N protein was absorbed onto the provided swab.

Ethics statement

The UCSF Committee on Human Research determined that the study met criteria for public health surveillance. All participants provided informed consent for dual testing.

RESULTS

Binax-CoV2 performance using a titration of in vitro cultured SARS-CoV-2

To explore the relationship of RT-PCR cycle threshold (Ct), viral load, and the corresponding visual Binax-CoV2 result, a dilution series of lab-cultured SARS-CoV-2 with known titers was assayed both by RT-PCR and by Binax-CoV2 (**Figure 1**). For this stock of virus, the threshold for detectability by human eye on the Binax-CoV2 assay was between 1.6-4.3x10^4 viral copies (100-250 pfu), corresponding to a Ct (average of N and E genes) of 30.3 and 28.8, respectively in this assay.

Community RT-PCR Testing Results

Of the 878 subjects tested, 54% were male, 77% were 18 to 50 years of age, 81% self-identified as Latinx, and 84% reported no symptoms in the 14 days before testing. Twenty-six persons (3%) were RT-PCR(+); of these, 15/26 (58%) had a Ct<30 and 6/15 (40%) were asymptomatic. Among asymptomatic individuals with Ct<30, 4/6 developed symptoms within 2 days after testing. Of the 11 persons RT-PCR-positive with Ct>30, 4 reported symptom onset $\geq$ 7 days before testing, 1 reported symptom onset 3 days prior to testing, and the remainder reported no symptoms.

Comparison of RT-PCR and Binax-CoV2 testing results from Community Testing

Because the readout of the Binax-CoV2 assay is by visual inspection, results may be subjective, especially when bands are faint or partial. The manufacturer's instructions suggest scoring any visible band as positive. On day 1 of testing, these reading instructions were used and 217 samples tested, of which 214 yielded valid Binax-CoV2 results: 7 of 214 (3.3%) were RT-PCR(+); using the manufacturer's proposed criteria, 5 of these 7 were Binax-CoV2(+). 207 of 214 were RT-PCR(-), 9 (4.3%) of which were Binax-CoV2(+). Thus, using the manufacturer's criteria, 9/14 Binax-CoV2(+) tests (64%) in this population of 217 tests were false positives (Binax-CoV2(+)/RT-PCR(-)). We felt that these initial criteria used on day 1 of testing were insufficient for classifying faint Binax-CoV2 assay bands, resulting in excessive false positive calls.

On subsequent testing days, we evaluated additional criteria for classifying a band as positive, in consultation with experts from the manufacturer's research staff. Optimal performance occurred when the bands were scored as positive *if they extended across the full width of the strip, irrespective of the intensity of the band.* Updated scoring criteria were implemented by the third day of testing on which a total of 292 tests were administered. 283 of 292 were RT-PCR(-), all of which scored Binax-CoV2(-), demonstrating these updated reading criteria markedly alleviated false positive readings. A total of 9 of 292 total day 3 tests were RT-PCR(+), of which 5/9 were Binax-CoV2(+) for antigen with these updated scoring criteria. The 4/9 RT-PCR(+) samples that were Binax-CoV2(-) had Ct>30, consistent with our laboratory-observed limit of detection for Binax-CoV2. We find that scoring a test as positive if bands extend across the full width of the strip, irrespective of band intensity, the least subjective and easiest method to implement in the field and have developed a training tool: https://unitedinhealth.org/binax-training.

The results of the 26 RT-PCR-positive individuals identified throughout the three-day study were stratified by RT-PCR test Ct value and categorized by Binax-CoV2 result (**Figure 2)**. The rapid antigen detection test performed well in samples with higher viral loads: 15 of 16 samples with Ct<32 were Binax-CoV2-positive (**Figure 2a**). By contrast, none of the 10 samples with Ct≥34 were positive by Binax-CoV2 antigen detection. Retrospective image quantification of Binax-CoV2 sample band intensity correlates with RT-PCR Ct values for those individuals (**Figure 2b**). In each case, the corresponding image is shown in order to demonstrate the correspondence between RT-PCR and the visual result (**Figure 2c**).

Sensitivity and Specificity

RT-PCR is considered a gold standard [3] and, in this assay, has a limit of detection of 100 viral RNA copies per mL. Direct antigen assays are inherently not as sensitive as RT-PCR. In the context of community based testing, we defined a threshold for high virus levels corresponding to the range of highest probability of transmissibility: a cycle threshold of 30, which corresponds to a viral RNA copy number of approximately $1.9x10^4$ in this assay [10,13]. Using this Ct<30 case definition and 95% confidence interval (CI), the sensitivity of the Binax-CoV2 was 93.3%, CI: 68.1-99.8% (14/15), and the specificity was 99.9%, CI: 99.4-99.9% (855/856). Adjusting the threshold to a more conservative Ct value of 33 ($2.6x10^3$ viral RNA copies), the sensitivity was 93.8%, CI: 69.8-99.8% (15/16) and the specificity was 100%, CI: 99.6-100% (855/855). Without a Ct threshold, the sensitivity of the Binax-CoV2 assay was 15/26 (57.7%, 95% CI: 36.9-76.6%) and specificity was 845/845 (100%: 95%CI: 99.6%-100%). Given that the Binax-CoV2 assay detects infected individuals with high levels of virus (>10^4), the sensitivity of the assay in the absence of a threshold will largely depend on the viral kinetics within the testing population. Sensitivity/specificity calculations were completed using retroactive Binax-CoV2 scores from images covering all three study days with the final scoring criteria.

Evaluation of Binax-CoV2 lot-to-lot variation

We quantified lot-to-lot variability in 10 different lots of Binax-CoV2 card tests using a dilution series of N protein. (**Supplementary Figure 1**). At protein concentrations of 17.2ng/ml and greater, sample band was detected in all lots, and thus would not affect the outcome of this binary assay (**Supplementary Figure 1A**).

DISCUSSION

These data describe the performance characteristics of the Binax-CoV2 antigen detection kit in the context of community testing including asymptomatic subjects. These results indicate a clear relationship between relative viral load and test positivity and provide a practical, real-world criterion to assist calling results in this setting. We found that small training modifications reduced the presence of false-positives, a legitimate concern for the roll-out of these tests.

The currently approved EUA for the Binax-CoV2 assay specifies use only in symptomatic individuals. The results presented here suggest that the Binax-CoV2 test should not be limited to symptomatic testing alone. Many asymptomatic individuals have high viral loads (corresponding to low Ct's) and, therefore, have a high probability of being infectious and transmitting the virus, a feature and likely driver of the pandemic that we and others have observed previously [7,14]. Limiting use of Binax-CoV2 to symptomatic individuals would have missed nearly half of the SARS-CoV-2 infections in this study.

Furthermore, the impact of testing on forward transmission is hampered by long wait times. We reported previously that in the community setting, by the time a person is tested, counseled and situated under isolation conditions, the effective isolation period is often nearly over [8]. This is particularly true for many communities of color, where reported delays in accessing tests and results are even longer [4,15]. Rapid tests could reduce these delays and maximize time of effective isolation. Limitations of our study include its cross-sectional design and overall small number of RT-PCR positive cases. Additional field performance of this assay is needed and will help inform optimal use strategies. We recommend evaluating

the Binax-CoV2 assay side by side with RT-PCR in each context it will be used prior to use of Binax-CoV2 without the use of RT-PCR.

During the early stages of infection, viral load may be too low to detect by direct antigen assays such as Binax-CoV2. This inherent lower sensitivity may be offset by faster turn-around and higher frequency of testing, with overall lower cost, relative to RT-PCR methods. That said, for persons who present with a high index of suspicion of COVID-19 and a negative Binax-CoV2 result, the test should be complemented with RT-PCR or a repeat Binax-CoV2 test at a later time to make sure case not missed.

In summary, under field conditions with supplementary technician training, the Binax-CoV2 assay accurately detected SARS-CoV-2 infection with high viral loads in both asymptomatic and symptomatic individuals. The Binax-CoV2 test could be a valuable asset in an arsenal of testing tools for the mitigation of SARS-CoV-2 spread, as rapid identification of highly infectious individuals is critical.

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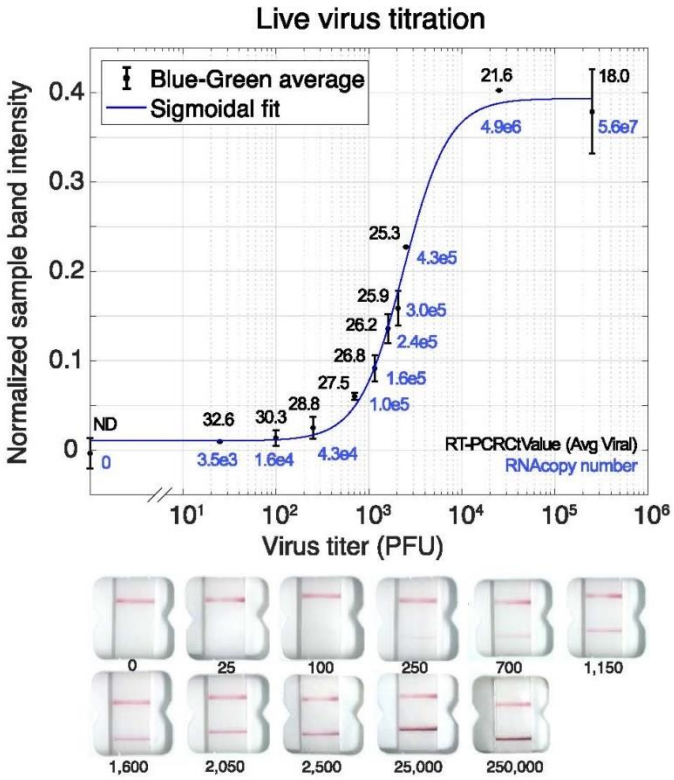
FIGURE LEGENDS

Figure 1. Titration of in vitro grown SARS-CoV-2 and detection on Binax-CoV2 assay. Normalized Binax-CoV2 sample band intensity (blue-green average) for cards loaded with a known amount of virus. Error bars represent standard deviation of sample band intensity of technical replicates. RT-PCR testing was performed at the CLIAHUB consortium [10]. Corresponding RT-PCR Ct values (average of N and E gene probes) are printed in black and the corresponding RNA copy number printed in blue. Note that Ct and genome copy number correlation varies by RT-PCR platform. Representative card images from each datapoint are shown below.

Figure 2. Comparison of Binax-CoV2 test with quantitative RT-PCR test.

(**A**) Average viral Ct values from all 26 RT-PCR-positive individuals from the community study are plotted in ascending order. Blue circles indicate Binax-CoV2-positive samples and yellow squares indicate Binax-CoV2-negative samples. Empty symbols represent individuals who were asymptomatic on day of test and filled symbols represent individuals who reported symptoms on day of test. (**B**) Normalized sample band signal from retrospective image analysis of Binax-CoV2 cards was plotted as a function of Ct value for all available scanner images (19/26 RT-PCR positives and a random subset of RT-PCR negatives). Binax-CoV2 True Positives are shown in blue with 'TP' labels, False Negatives in yellow with 'FN' labels, and True Negatives in red with 'TN' labels. (**C**) Corresponding Binax-CoV2 card images from the data in panel B.

Figure 1


Live virus titration
Blue-Green average
Sigmoidal fit
Normalized sample band intensity
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0.3
0.2
0.1
0
21.6
18.0
4.9e6
5.6e7
25.3
4.3e5
25.9
3.0e5
26.2
2.4e5
26.8
1.6e5
27.5
1.0e5
28.8
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30.3
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3.5e3
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4.3e4
RT-PCRCtValue (Avg Viral)
RNAcopy number
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10^2
10^3
10^4
10^5
10^6
Virus titer (PFU)
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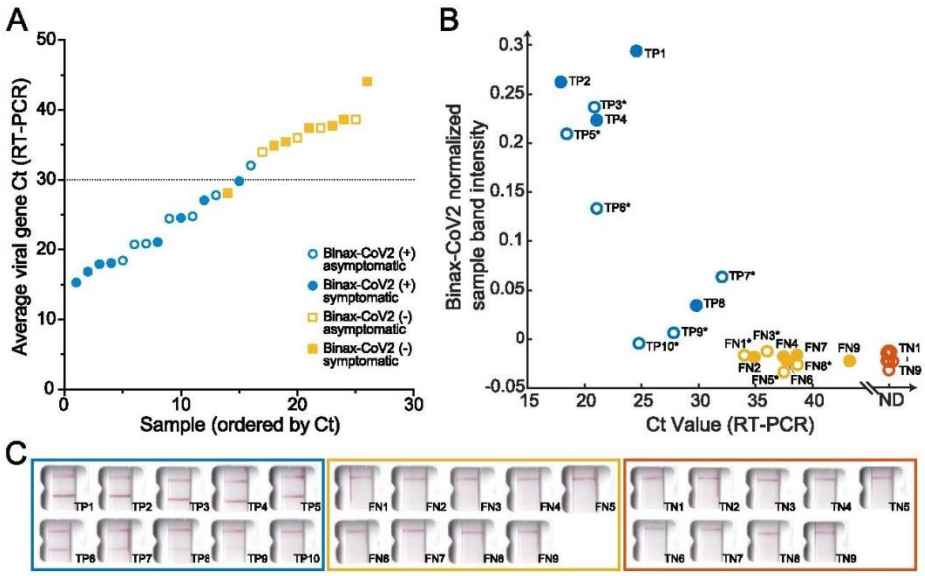
2,500
25,000
250,000

Figure 2


A
Average viral gene Ct (RT-PCR)
Sample (ordered by Ct)
Binax-CoV2 (+) asymptomatic
Binax-CoV2 (+) symptomatic
Binax-CoV2 (-) asymptomatic
Binax-CoV2 (-) symptomatic
B
Binax-CoV2 normalized sample band intensity
Ct Value (RT-PCR)
ND
C
TP1 TP2 TP3 TP4 TP5 TP6 TP7 TP8 TP9 TP10
FN1 FN2 FN3 FN4 FN5 FN6 FN7 FN8 FN9
TN1 TN2 TN3 TN4 TN5 TN6 TN7 TN8 TN9