

Title

Analytic sensitivity of the Abbott BinaxNOW™ lateral flow immunochromatographic assay for the SARS-CoV-2 Omicron variant

Authors

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Word count

2136

Abstract

The emergence of the SARS-CoV-2 Omicron variant has motivated a re-evaluation of the test characteristics for lateral flow immunochromatographic assays (LFIAs), commonly referred to as rapid antigen tests. To address this need, we evaluated the analytic sensitivity of one of the most widely used LFIAs in the US market, the Abbott BinaxNOW™ COVID-19 Ag At-Home Card using 32 samples of Omicron and 30 samples of the Delta variant. Samples were chosen to intentionally over-represent the range of viral loads where differences are most likely to appear. We found no changes in the analytic sensitivity of the BinaxNOW™ assay by variant even after controlling for variation in cycle threshold values in the two populations. Similar to prior studies, the sensitivity of the assay is highly dependent on the amount of virus present in the sample. While the analytic sensitivity of the BinaxNOW™ LFIA remains intact versus the Omicron variant, its clinical sensitivity is influenced by the interaction between viral replication, the dynamics of tissue tropism and the timing of sampling. Further research is necessary to optimally adapt current testing strategies to robustly detect early infection by the Omicron variant to prevent transmission.

1 Introduction

2 The identification of infection by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-
3 2), the virus that causes coronavirus disease 2019 (COVID-19), aids the individual by prompting
4 timely initiation of treatment and aids the public by allowing for separation of contagious hosts
5 from susceptible secondary contacts. Lateral flow immunochromatographic (LFIA) assays,
6 commonly referred to as rapid antigen tests, are an important element of pandemic containment
7 as they provide results within 15 minutes and can be performed by the public without supervision.
8 However, a drawback is their lower analytic sensitivity relative to nucleic acid amplification based
9 assays like the reverse transcriptase polymerase chain reaction (RT-PCR). In practice, the impact
10 of this drop in sensitivity is limited to the brief window during which a person is efficiently
11 transmitting live virus but still has viral loads below the limit of detection (LoD) of the LFIA. In prior
12 waves of COVID-19, this time period is estimated to be days 1 to 2 post-infection¹. Tests taken
13 during that time window have a risk of being falsely negative and can lead individuals to transmit
14 the virus to others unbeknownst to them.

15
16 The COVID-19 pandemic has spread in a series of waves due to the successive emergence of
17 variants of concern (VOCs) that contain sets of mutations that confer selective advantages over
18 prior lineages due to improved transmissibility, immune escape, and more efficient viral
19 replication. These mutations are concentrated in the spike protein as it is the target of greatest
20 immune pressure, but VOCs also contain single nucleotide polymorphisms in the nucleocapsid
21 (N) protein, which encapsidates the viral RNA. The recently emerged Omicron variant has a
22 mutation (P13L) and a deletion (d31-33) in the N-terminal domain, and two changes in the central
23 linker domain (R203K and G204R²) that have not yet been fully characterized.

24
25 The N protein is the target analyte for the majority of LFIA currently approved for emergency use
26 by the US Food and Drug Administration (FDA). These assays typically use monoclonal
27 antibodies coupled to nanoparticles to bind epitopes present on the SARS-CoV-2 N protein, which
28 are then carried by capillary action to a detection line where the complex is bound by a second
29 monoclonal antibody which binds elsewhere to the SARS-CoV-2 N protein. The resulting
30 concentrated nanoparticle can be visualized by eye. The regions of the protein to which antibodies
31 are raised are derived either from the wild type strain or early VOCs such as the Alpha variant.
32 The precise epitopes targeted by the commercially-available LFIA are proprietary, therefore
33 independent evaluation is necessary to evaluate whether changes to nucleocapsid protein in the
34 Omicron variant affect the analytic sensitivity of the test. Given the critical role that LFIA play in

35 early case detection and return to work assessments, quantifying changes in test performance is
36 of high value to the scientific community, policy makers and the public.

37

38 In this study we report the analytic sensitivity of the widely used Abbott BinaxNOW™ COVID-19
39 Ag Card against the Omicron variant, using the Delta variant as a reference. Our primary
40 hypothesis is that the analytic sensitivity of the Abbott BinaxNOW™ LFIA will be unaffected by
41 the nucleocapsid mutations specific to the Omicron variant.

42 **Methods**

43 We utilized 32 specimens positive for the Omicron variant and 30 positive for the Delta variant,
44 collected between December 16th 2021 and December 18th 2021 as part of Harvard University's
45 asymptomatic SARS-CoV-2 student and staff screening program. During the time period of
46 collection, >98% of the community was fully vaccinated. All samples were obtained from the
47 anterior nares of individuals using a RHINOstic nasal swab (Rhinostics, Boston, MA) and initially
48 placed into a sterile dry tube. Upon reaching the central testing laboratory, samples were eluted
49 into 300 μ l of phosphate buffered saline and inactivated at 65°C for 30 minutes. All samples were
50 received by the laboratory and eluted within 24 hours. Real-time RT-PCR was performed using
51 the Quaceris SARS-CoV-2 assay with fluorescence and cycle thresholds (Cts) for the N and RdRP
52 genes determined by the Applied Biosystems RT-PCR instrument running the QuantStudio 7
53 Design and Analysis Desktop Software, version 1.7. Details of the protocol can be found in the
54 FDA Emergency Use Authorization summary for the Harvard University Clinical Laboratory
55 (HUCL) assay³. The LoD of the assay is 2.5 RNA copies / μ l.

56
57 Samples were genotyped using a multiplex PCR assay with primers specific to both variants.
58 Based on the expected limit of detection (LoD) for the BinaxNOW™ LFIA⁴, we weighted our
59 samples to over-represent those with Ct values below 30 as this is where differences in analytic
60 sensitivity would most likely appear. The final analysis for the Delta variant samples consisted of
61 22 samples with Ct < 30 and 8 samples with Ct \geq 30. The final analysis for the Omicron variant
62 samples consisted of 24 samples with Ct < 30 and 8 samples with Ct \geq 30.

63
64 After RT-PCR analysis, the specimens selected for this analysis were frozen once at -20°C. For
65 LFIA testing, 50 μ l of each sample was placed in a 1.5ml microcentrifuge tube into which the kit-
66 supplied swab was placed and rotated for 15 seconds. This volume was chosen based on a prior
67 study that determined 50 μ l to be the optimal volume for balancing the ratio of analyte to kit-
68 supplied running buffer for *in vitro* testing of samples with the BinaxNOW™⁴. Based on our
69 observations, we noted that on average 5 μ l of sample remained in the tube after swab rotation.
70 All subsequent steps for sample evaluation were per the assay's Instructions For Use⁵. Briefly,
71 six drops of kit-supplied running buffer were placed into the top hole of the card and swabs were
72 inserted into the bottom hole and pushed upwards until the tip was in contact with the buffer. The
73 cards were then sealed and read twice after 15 minutes by two independent readers blinded to
74 the identity of the sample. A third reader was used for any discordant reads and a photo was
75 taken of the card after the completion of the incubation period. All samples were run in triplicate.

76

77 Descriptive statistics were performed using chi-squared tests or Fisher's exact test for categorical
78 variables and 2-sample t-tests for comparing Ct values by variant. Logistic regression was used
79 to estimate the odds ratio of a positive LFIA test given variant type after accounting for Ct value
80 differences. All analyses were performed in R, version 4.1.2. This study was deemed non-human
81 subjects research and approved by the Mass General Brigham Institutional Review Board
82 (protocol 2021P003604).

83 **Results**

84 A total of 62 samples were run in triplicate (30 Delta samples and 32 Omicron samples). Only 2
 85 samples differed within technical replicates and there were no differences by reader, therefore all
 86 results represent the data obtained from a single replicate and reader 1. Table 1 shows the
 87 distribution of Ct values for the samples chosen for this analysis did not differ by variant.

88

Variant	Sample size	Median Ct value (IQR)	p value
Delta	30	27 (5.88)	0.94
Omicron	32	27 (6.02)	

89 **Table 1: Baseline distribution of Ct values by SARS-CoV-2 variant.** IQR, interquartile range.

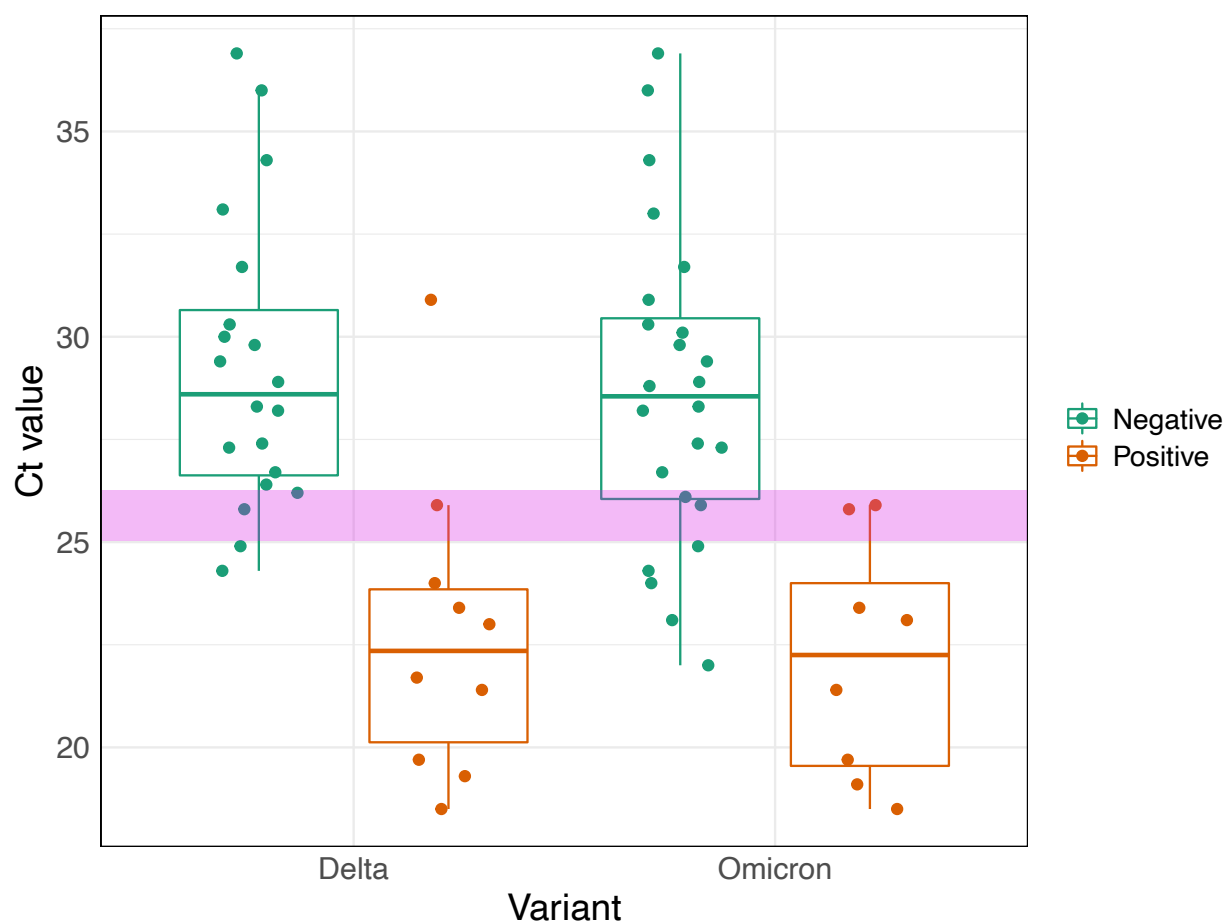
90

91 The BinaxNOW™ test was positive in 9 of 22 (41%) Delta samples and in 8 of 24 (33%) Omicron
 92 samples with Ct < 30. It was positive in 1 of 7 (12%) Delta samples and in 0 of 8 (0%) Omicron
 93 samples with Ct ≥ 30. There were no statistically significant differences between the variants with
 94 respect to test positivity for either Ct range (Table 2). The complete dataset for this study is
 95 available by request.

Ct range	Variant	BinaxNOW™ result	N (percent)	p value
<30	Delta	Negative	13 (59%)	0.82
		Positive	9 (41%)	
	Omicron	Negative	16 (67%)	
		Positive	8 (33%)	
≥30	Delta	Negative	7 (88%)	1.00
		Positive	1 (12%)	
	Omicron	Negative	8 (100%)	
		Positive	0 (0%)	

96 **Table 2: Distribution of BinaxNOW™ LFIA results stratified by Ct value ranges and SARS-**
 97 **CoV-2 variant.**

98 Figure 1 shows the distribution of positive and negative results by Ct value and variant. The
99 expected LoD for the BinaxNOW™ is estimated to be 40,000 to 80,000 RNA copies per swab,
100 which is derived from samples obtained prior to the emergence of the Delta variant⁴. This
101 corresponds approximately to a Ct of 25 on the HUCL assay based on previously performed
102 standard curve data normalized to the sample volume taken up by the swab. Two of 10 (20%)
103 Delta variant samples and 5 of 11 (45%) Omicron samples were negative despite having levels
104 of virus above the LoD.
105



106
107 **Figure 1: Distribution of BinaxNOW™ antigen test results by Ct value and SARS-CoV-2**
108 **variant.** The y-axis indicates the Ct value of the positive sample. The pink horizontal band
109 represents the expected LoD range of the BinaxNOW™, based on work from Perchetti et al⁴ and
110 standard curve data for the HUCL RT-PCR assay.
111
112 After adjustment for each sample's Ct value, there was no difference in the odds of test positivity
113 for the Omicron variant versus the Delta variant (odds ratio 0.41; 95% CI 0.07 - 2.0).

114 Discussion

115 The emergence of the Omicron variant in mid-November 2021 was followed by many anecdotal
116 reports of false negative LFIA tests. In this study, we found no significant difference in the analytic
117 sensitivity of the widely used Abbott BinaxNOW™ COVID-19 Ag Card LFIA for the N protein of
118 the SARS-CoV-2 Omicron variant with respect to the Delta variant. We noted a greater proportion
119 of negative samples containing viral loads above the assay's LoD for Omicron specimens than
120 for Delta specimens, but this finding did not reach statistical significance, possibly due to our
121 study's small sample size. Given that N protein mutations have been shown to impact LFIA
122 performance in the past⁶, these findings provide a degree of reassurance that the assay is
123 performing as expected.

124
125 Our results reinforce the findings of a smaller study that also examined the analytic sensitivity of
126 the BinaxNOW™ versus Omicron with respect to its ability to detect both variants and in the
127 estimated amount of nucleocapsid protein necessary to visualize a signal⁷. Our findings extend
128 that work by including a larger number of independently collected clinical specimens and widening
129 the range of viral loads in the sample set. Our results are also in agreement with the study by
130 Deerein et al, which utilized serial dilutions of Omicron and Delta samples derived from viral
131 cultures⁸. In that study, there was no difference in the analytic sensitivity for the Abbott Panbio™
132 COVID-19 Ag Rapid Test Device, which utilizes the same epitopes as the Abbott BinaxNOW™
133 COVID-19 Ag Card test marketed in the United States. Similarly, our findings agree with a recently
134 published study from Schrom et al evaluating the analytic sensitivity of the BinaxNOW™ using
135 paired RT-PCR anterior nares specimens obtained from a community testing center in San
136 Francisco with a high rate of test positivity⁹. Our study provides complementary information to
137 their findings by quantifying the threshold for detection in RNA copy numbers per swab and by
138 comparing results with Omicron to Delta variant samples, which enables us to determine the
139 degree of performance drift. Finally, our study is in line with announcements from the
140 manufacturer¹⁰ regarding the performance of the LFIA, though the data underlying their press
141 release are not publicly available.

142
143 Independent of whether the analytic sensitivity for the BinaxNOW™ remains intact, the real-world
144 performance of the test may still substantially differ from its use during prior waves of the COVID-
145 19 pandemic. This is because the clinical sensitivity of an assay is influenced by factors inherent
146 to the virus as well as host factors such as pre-existing immunity. For instance, Abramson et al
147 reported the propensity for the BinaxNOW™ and the Quidel Quickvue™ LFIAs to be falsely

148 negative early in infection despite very high viral loads detected concomitantly from saliva
149 samples¹¹. Antigen detection from nasal swab samples eventually turned positive several days
150 after symptom onset, suggesting that viral replication may initially localize to the oropharynx prior
151 to transitioning to the nasopharynx. A study by Marais et al also found higher viral loads in saliva
152 compared to paired mid-turbinate swabs but did not follow study participants over time to
153 document a transition¹². The BinaxNOW™ is currently only approved for use with anterior nares
154 swabs in the US⁵.

155
156 Our results may be affected by the use of heat-inactivated samples that underwent one freeze-
157 thaw cycle prior to analysis. However, this is not likely to have had a major impact on assay
158 performance, as samples would have undergone denaturation regardless and the period of time
159 they were frozen was short. The consistency of our data with prior studies defining the analytic
160 sensitivity of the BinaxNOW™ as well as the lack of variation within technical replicates and
161 across multiple blinded readers, further suggests that our findings are reproducible. Although our
162 sample modality was not identical to a nares swab, we utilized a volume of media (50 µl) that has
163 been shown to be approximately equal to the volume of mucus expected to be taken up by a
164 swab when used *in vivo*⁴.

165
166 In summary, we found no difference in the analytic sensitivity of the Abbott BinaxNOW™ COVID-
167 19 Ag Card for the Omicron variant relative to contemporaneously collected Delta variant
168 samples. If sufficient virus is present in a sample, the assay should be expected to turn positive.
169 The clinical (ie 'real-world') sensitivity of the assay is impacted by the viral load in the nares at the
170 time of sampling, which in turn is influenced by variant-specific replication kinetics and host
171 immunity. Increasing data suggests this may be the primary driver for the anecdotal reports of
172 false negative LFIA results soon after symptom onset. Larger studies performing serial sampling
173 from the oropharynx and nasopharynx with quantitative measures of viral loads in symptomatic
174 and asymptomatic people will provide critical data for informing case detection algorithms.

175
176 **Acknowledgements**

177 This study was funded by a grant from the Massachusetts Consortium for Pathogen Readiness.

178
179 **Conflicts of interest**

180 SK is the editor for the diagnostics section of the Infectious Diseases Society of America COVID-
181 19 Real-time Learning Network. No other authors have any financial conflicts of interest.

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