Performance Characteristics of BinaxNOW COVID-19 Antigen Card for Screening Asymptomatic Individuals in a University Setting

Nkemakonam C Okoye[1,*], Adam P Barker[2,3,*], Kenneth Curtis[4], Richard R Orlandi[5], Emily A Snavely[1], Cameron Wright[6], Kimberly E Hanson[7, 2, 3], Lauren N Pearson[1, 3]

1 Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah, USA

2 Department of Pathology, Section of Clinical Microbiology, University of Utah School of Medicine, Salt Lake City, Utah, USA

3 ARUP Laboratories Institute for Clinical and Experimental Pathology, Salt Lake City, Utah, USA

4 ARUP Laboratories, Salt Lake City, Utah, USA

5 Department of Surgery, Division of Otolaryngology - Head and Neck Surgery, University of Utah School of Medicine, Salt Lake City, Utah, USA

6 University of Utah Medical Group, Salt Lake City, Utah, USA

7 Department of Medicine, Division of Infectious Diseases, University of Utah School of Medicine, Salt Lake City, Utah, USA

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Address correspondence to Lauren N Pearson, Department of Pathology, University of Utah, 15 N Medical Drive East, Salt Lake City, UT 84112; 801-587-1583; Lauren.pearson@aruplab.com

* Drs. Okoye and Barker contributed equally to this study and are listed as co-first authors. Author order was determined on the basis that Dr. Okoye provided original conceptualization for the study together with the senior author.

ABSTRACT

We compared the performance of the Abbott BinaxNOW COVID-19 Antigen Card to a standard RT-PCR assay (ThermoFisher TaqPath COVID-19 Combo Kit) for the detection of SARS-CoV-2 in 2,645 asymptomatic students presenting for screening at the University of Utah. SARS-CoV-2 RNA was detected in 1.7% of the study participants by RT-PCR. BinaxNOW identified 24 infections but missed 21 infections that were detected by RT-PCR. The analytical sensitivity (positive agreement) and analytical specificity (negative agreement) for the BinaxNOW was 53.3% and 100%, respectively when compared against the RT-PCR assay. The median cycle threshold (Ct) value in the specimens that had concordant positive BinaxNOW antigen result was significantly lower compared to those that were discordant (Ct 17.6 vs. 29.6; $p < 0.001$). In individuals with presumably high viral loads (Ct < 23.0), a 95.8% positive agreement was observed between the RT-PCR assay and BinaxNOW. Due to the possibility of false negative results, caution must be taken when utilizing rapid antigen testing for screening asymptomatic individuals.

INTRODUCTION

With its high degree of transmissibility, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative pathogen for the novel 2019 coronavirus disease (COVID-19), has undoubtedly led to one of the most remarkable global public health epidemics in recent history. Timely identification and isolation of infected individuals is crucial in mitigating rampant community spread of SARS-CoV-2. The gold standard method for COVID-19 diagnosis remains detection of SARS-CoV-2 ribonucleic acid (RNA) in respiratory tract specimens

using nucleic acid amplification techniques such as reverse transcription polymerase chain reaction (RT-PCR). However, SARS-CoV-2 nucleic acid amplification tests (NAAT) are generally more expensive than alternative methodologies and may have prolonged turnaround times due to limited test supplies, reagent allocation, and fixed laboratory capacity, which have been exacerbated by extremely high demand.

Efforts to expand testing capacity have led to the development of several rapid antigen tests designed to detect SARS-CoV-2 nucleocapsid antigen, primarily in symptomatic individuals (1). At the time of this writing, the United States Food and Drug Administration (FDA) has granted emergency use authorization (EUA) to eleven SARS-CoV-2 antigen tests (2). Although these antigen tests are intended to be utilized in symptomatic individuals (within the first five to seven days of symptom onset), the United States Department of Health and Human Services (HHS), through the Public Readiness and Emergency Preparedness Act (PREP Act), permits their use for screening asymptomatic individuals in congregate facilities, including schools (3). However, there is limited data on the performance characteristics of rapid antigen tests in asymptomatic or pre-symptomatic individuals. A recent meta-analysis of published literature on rapid, point-of-care antigen tests reported an average sensitivity and specificity of 56.2% and 99.5%, respectively, when compared to NAAT (1). However, these studies were not limited exclusively to asymptomatic individuals, the specimen type was primarily nasopharyngeal and/or oropharyngeal, and none of the antigen tests included have received EUA approval from the FDA.

In this study, we evaluated the diagnostic performance characteristics of the Abbott BinaxNOW COVID-19 Antigen Card (hereby referred to as BinaxNOW) in a population of college-

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age students who were asymptomatic at the time of testing. BinaxNOW is a rapid lateral flow immunoassay that qualitatively detects SARS-CoV-2 nucleocapsid antigen in direct nasal swab specimens. The package insert cites a positive agreement of 97.1% and a negative agreement of 98.5% when compared against an EUA RT-PCR assay (4). These data were based on a clinical study involving a total of 102 patients, of which 95 had symptoms consistent with COVID-19 and only 7 were asymptomatic. This was recently updated to a positive agreement of 84.6%, based on a larger study involving 460 symptomatic individuals. Of note, the United States federal government has distributed 150 million BinaxNOW Antigen Cards to states across the country (5). BinaxNOW also received EUA for at-home use under the supervision of a telehealth proctor (6). Therefore, characterizing the performance characteristics of BinaxNOW for off-label use in an asymptomatic population is essential given its potential widespread application for asymptomatic screening in a variety of settings.

MATERIALS AND METHODS

Study population and specimen collection.

The participants of this study were primarily college-age (undergraduate and graduate) students at the University of Utah in Salt Lake City, Utah, USA. At the time of specimen collection, the students were first queried to ensure that they were not experiencing any signs and/or symptoms of COVID-19. Specimen collection occurred at a temporary indoor testing site from November 13-20, 2020. Two nasal swabs were collected from each participant, following the technique recommended by the United States Center for Disease Control and Prevention (CDC) (7). The study participants were instructed to swab both nares at the level of the mid

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turbinate for each collection. Trained non-medical personnel observed the specimen collection process. The first swab collected from the participants was randomly assigned to be tested either with BinaxNOW or the RT-PCR assay in an effort to minimize sampling bias.

Detection of SARS-CoV-2 viral antigen

The BinaxNOW Antigen Cards utilized in this study were received from the Utah Department of Health as part of a United States federal government initiative to expand COVID-19 testing capacity. Testing was performed by trained non-medical personnel (University of Utah Hope Corps Interns) according to the manufacturer's instructions (4). Each testing personnel was trained on the test procedure (including appropriate use of personal protective equipment) and result interpretation using detailed step-by-step videos provided by the manufacturer. To evaluate for competence, each testing personnel was required to pass an assessment quiz and successfully perform external quality control using a positive control swab and a sterile swab (negative control). External quality control was also performed for each new kit of BinaxNOW Antigen Cards.

Results were interpreted visually after 15 minutes. A specimen was deemed positive for SARS-CoV-2 viral antigen if two pink/purple colored lines (control line on the top and sample line on the bottom) were observed on the test card, as illustrated in the assay product insert (4). A faint pink/purple colored line in the sample region of the test card (in addition to a pink/purple colored control line) was also interpreted as a positive result. A single pink/purple colored line in the control region of the test card was interpreted as a negative result. If no line was observed in the control region or if the line remains blue in color, then the result was interpreted as invalid.

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Participants were notified of their BinaxNOW result using the NAVICA Mobile App, which is a free mobile app provided by Abbott (8). Any participant that tested positive was contacted to return to the testing site within 24 hours and submit a saliva specimen for SARS-CoV-2 NAAT at ARUP Laboratories. These individuals were instructed to self-isolate while awaiting NAAT confirmation. Individuals that received an invalid BinaxNOW result were also contacted for repeat antigen testing. Participants receiving a negative antigen test were counseled that these results were "presumptive" and did not negate the need for mitigation behaviors designed to reduce the spread of SARS-CoV-2.

Detection of SARS-CoV-2 nucleic acid

The other nasal swab was placed into ARUP COVID-19 Transport Media™ (9) and tested at ARUP Laboratories using the ThermoFisher TaqPath COVID-19 Combo Kit, hereby referred to as the TaqPath COVID-19 Kit (10). These specimens were stored frozen (-20 °C) and tested within 10 days of receipt in the clinical laboratory. The TaqPath COVID-19 Kit targets regions of three coronavirus genes: ORF1ab, the gene for the S protein, and the gene for the N protein. 40 amplification cycles are performed by the assay. At least two genes have to be detected for the result to be reported as positive for SARS-CoV-2. The cycle threshold (Ct) value for each specimen was reported as the average of the Ct values of the detected coronavirus genes. An inconclusive result was reported when only one gene is detected after consecutive repeat testing. Detection of SARS-CoV-2 RNA in the confirmatory saliva specimens was performed in real-time using one of three FDA EUA assays (either Hologic Panther Fusion SARS-CoV-2 assay, Roche Cobas SARS-CoV-2 assay, or ThermoFisher TaqPath COVID-19 Combo Kit). All participants were notified of their NAAT results.

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Statistical analysis

The TaqPath COVID-19 Kit was used as the benchmark for assessing the diagnostic accuracy of BinaxNOW. The analytical performance characteristics (sensitivity, specificity, and predicative values) were calculated from a 2×2 contingency table using GraphPad Prism 8 software. Agreement between methods was assessed at various Ct cutoffs reported in the package insert for BinaxNOW (4) and published literature. The 95% confidence intervals are based on the Wilson-Brown method. A non-parametric t test (Mann-Whitney test) was performed using GraphPad Prism 8 software to evaluate for statistical significance (p values) between median Ct values. Kappa coefficient was calculated using the Microsoft Excel Analyse-it software package (version 5.20).

RESULTS

Positivity rate of the rapid antigen test and nucleic acid amplification test

Two nasal swab specimens were collected from 2,645 individuals. Among the study participants, 1369 (51.8%) identified as female, 1274 (48.2%) identified as male, while 2 (0.1%) identified as non-binary. The average age of the study participants was 24 years (range: 15 to 86 years). **Table 1** summarizes the results from BinaxNOW and the TaqPath COVID-19 Kit. A negative result with BinaxNOW was observed in 2,618 (99.0%) individuals, while a positive result was observed in 24 (0.9%) individuals. An invalid BinaxNOW result was initially observed in 3 (0.1%) individuals; however, repeat testing using a new nasal swab specimen from these individuals yielded a negative result. For the TaqPath COVID-19 Kit, SARS-CoV-2 RNA was not

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detected in 2,595 (98.1%) individuals, 46 (1.7%) individuals had detectable SARS-CoV-2 RNA, while 4 (0.2%) individuals had an inconclusive result.

Concordance between the rapid antigen test and the nucleic acid amplification test

The analytical sensitivity and specificity of BinaxNOW is summarized in **Table 2**. Of the 46 individuals that had detectable SARS-CoV-2 RNA, 24 had a concordant positive antigen result, indicating a positive agreement of 53.3% between the two tests. The kappa coefficient (κ 0.69; 95% CI: 0.57 – 0.82) indicates substantial agreement between methods. The median cycle threshold (Ct) value in the specimens that had concordant positive results was significantly lower (Ct 17.6) than those that were discordant (Ct 29.6) ($p < 0.001$), as illustrated in **Figure 1.** In specimens with presumably high viral loads (Ct < 23.0), a 95.8% positive agreement was observed (**Table 3**). A 0% positive agreement was observed in samples with both Ct ≥ 33 and Ct ≥ 30, as shown in **Table 3**.

Collection of two consecutive bilateral nasal swab specimens did not significantly affect the detection of SARS-CoV-2 using either NAAT or the rapid antigen test ($p = 0.5683$; Fisher's exact test). The rapid antigen test was performed using the first nasal swab specimen in 12 (50%) out of the 24 individuals with concordant positive results. No statistically significant difference in median Ct value was observed in concordant positive samples regardless of whether the rapid antigen test was performed using the first nasal swab versus the second nasal swab (**Figure 2**) ($p = 0.5800$). A discordant result between the rapid antigen test and NAAT (i.e., antigen negative/NAAT positive) was observed in 21 individuals. Discordant results between BinaxNOW and the RT-PCR assay were more likely at Ct values > 23.0, as shown **Figure 3**. The antigen test was performed using the first nasal swab specimen in 9 (40.9%) out of the

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21 individuals with discordant results. While a slightly higher median Ct value was observed when the antigen test was performed using the second nasal swab versus the first nasal swab, the difference was not statistically significant ($p = 0.1752$), as shown in **Figure 2**. In one individual with a discordant result, an invalid BinaxNOW antigen result was initially obtained, with a negative result observed upon repeat testing using a new nasal swab specimen. It is worth mentioning that for this individual, the initial invalid BinaxNOW was obtained using the second nasal swab specimen, while the negative result from the repeat test was obtained from a third nasal swab. Hence, the validity of the negative BinaxNOW result in this individual could be questionable due to sampling bias. Invalid results were excluded in the diagnostic performance characteristics calculations.

Twenty-two out of the 24 individuals (91.7%) with a positive antigen result returned to the testing site and submitted a follow-up saliva specimen. There was 100% agreement between these positive BinaxNOW specimens and saliva NAAT.

DISCUSSION

When compared to NAAT, the BinaxNow Antigen Card showed low analytical sensitivity (53.3%) for detecting SARS-CoV-2 infection in an asymptomatic or pre-symptomatic population. This observation is consistent with the findings of other recent studies conducted using different SARS-CoV-2 antigen assays in unselected populations (11-13). Collection of two consecutive bilateral nasal swab specimens did not statistically affect the detection of SARS-CoV-2 using either the RT-PCR assay or the rapid antigen test. However, there was a trend toward higher Ct values in the second swab indicating a lesser amount of virus present, which

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may have disproportionally affected the antigen positivity rate. One study found a difference of 6-7 Ct between the limit of detection of the BinaxNOW antigen test and RT-PCR tests, indicating an approximate 100-fold difference in sensitivity (14).

Our results indicate that a relatively high viral load (and corresponding low Ct value <23) must be present to generate a positive BinaxNOW result. At the onset of our study, the BinaxNOW product insert reported a positive agreement of 83.3% in specimens with Ct ≥ 33 (4). The manufacturer has recently updated this information to a positive agreement of 37.8%. Ct values are a relative approximation of virus load. Differences in assay design and other important pre-analytic variables (e.g., specimen source, collection method, volume of transport media, etc.) impact reported Ct values such that these measurements are not directly comparable across real-time NAAT platforms (15).

In contrast to analytical sensitivity, the specificity of BinaxNOW testing was excellent (100%). The test was able to be performed successfully at the point of care by non-medical personnel with a relatively low invalid rate (0.1%), supporting the findings of another recently published study (16). These observations raise the question of whether confirmation of positive BinaxNOW results is necessary, as cautioned in a recent warning by the FDA regarding the potential for false positive results from rapid SARS-CoV-2 antigen tests (17). It is important to note, however, that operators underwent comprehensive training and quality control testing was performed regularly on-site. This is especially important in the context of at home testing. Additional studies are needed to determine whether BinaxNOW test performance will be comparable in a telehealth-observed home setting.

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Despite its relatively low analytical sensitivity, BinaxNOW may still be beneficial for surveillance testing in selected settings where testing resources are limited, especially when weighed against the alternative of no screening testing. Rapid antigen testing identified 24 infections in asymptomatic individuals, with qualitatively high viral loads, who may be more likely to be infectious to others (18, 19). These infections were all confirmed by saliva NAAT and individuals were instructed to self-isolate. Given the relatively low prevalence (1.7%) in our student population, the negative predictive value of BinaxNow was excellent (99.2%).

A total of 21 asymptomatic students had false negative antigen tests. We do not know if these individuals developed symptoms in the days following the negative antigen result. We also cannot speculate as to how infectious these individuals were; presumably, the risk of viral transmission to others is not zero (18, 19) although the higher Ct values associated with these samples may indicate a low risk of transmission. However, it is well established that asymptomatic carriers of SARS-CoV-2 can efficiently transmit the infection (20, 21). Thus, all participants were counseled to continue with physical distancing, face masking, and proper hand hygiene despite a negative BinaxNOW result. The public health implications of a false negative screening result in an asymptomatic population will depend on the population to which the test is applied. For example, tolerance for false negatives may be greater in a congregate setting consisting of young, otherwise healthy individuals (e.g., college campus) with few risk factors for severe clinical outcome from COVID-19 versus a long-term care facility setting or other demographic with one or multiple risk factors for poor COVID-19 associated outcomes.

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The limitations of this study include the relatively small number of positive results and lack of serial repeat testing data for the asymptomatic student cohort to determine if the 21 false negatives result would eventually test positive after subsequent assessments. This would be useful for validating the effectiveness of the proposed strategy of repeat serial testing using less sensitive antigen tests as an infection prevention and control measure (22, 23).

To the best of our knowledge, this is the first study evaluating the performance of a rapid SARS-CoV-2 antigen test in an exclusively asymptomatic population. The analytical sensitivity of BinaxNOW for off-label use in an asymptomatic population is lower than the performance claims for symptomatic patients reported by the manufacturer. As recommended by the manufacturer, negative results should be interpreted as presumptive negative. Careful assessment of the impact of false negative results is warranted before a testing strategy utilizing rapid SARS-CoV-2 antigen tests is implemented. The specificity BinaxNOW, however, was excellent.

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Figure Legends

Figure 1: Distribution of the RT-PCR cycle threshold (Ct) values in specimens with positive and negative BinaxNOW results. p value is based on the Mann-Whitney test. The lines signify median and interquartile ranges.

Figure 2: Distribution of the RT-PCR cycle threshold (Ct) values in specimens with concordant positive BinaxNOW results (**A**) and discordant negative BinaxNOW results (**B**) sorted by order of nasal swab collection. p value is based on the Mann-Whitney test. The lines signify median and interquartile ranges.

Figure 3: Frequency distribution of RT-PCR cycle threshold (Ct) values in all specimens with detectable SARS-CoV-2 and specimens with discordant BinaxNOW results.

Table 1. Summary of results from the BinaxNOW Antigen Card and the TaqPath COVID-19

Kit

	BinaxNOW Antigen Card	TaqPath COVID-19 Kit
Positive	24	46
Negative	2618	2595
Inconclusive / Invalid	3*	4[#]
Total	2645	2645

*Repeat testing yielded a negative result

[#]Only the N protein gene was detected in these specimens (Ct value was > 30)

Table 2. Diagnostic performance of BinaxNOW Antigen Card compared to TaqPath COVID-19 Kit for detection of SARS-CoV-2

BinaxNOW Antigen Card	TaqPath COVID-19 Kit		
	Positive	**Negative**	**Total**
Positive	24	0	24
Negative	21	2593	2614
Total	45	2593	2638
Analytical sensitivity (positive agreement) = 53.3% (95% CI: 39.1% – 67.1%)			
Analytical specificity (negative agreement) = 100% (95% CI: 99.9% – 100%)			
Positive predictive value* = 100% (95% CI: 86.2% – 100%)			
Negative predictive value* = 99.2% (95% CI: 98.7% – 99.4%)			
Kappa coefficient = 0.69 (95% CI: 0.57 – 0.82)			
*Predictive values are assuming a disease prevalence of 1.7% Note: 4 inconclusive RT-PCR results and 3 invalid BinaxNOW results were excluded from the calculations above			

Table 3. BinaxNOW Antigen Card diagnostic performance against the comparator RT-PCR method by cycle threshold counts

BinaxNOW Antigen Card	TaqPath COVID-19 Kit (Positive Results by Ct Category)					
	Ct < 33.0	**Ct ≥ 33.0**	**Ct < 30.0**	**Ct ≥ 30.0**	**Ct < 23.0**	**Ct ≥ 23.0**
Positive	24	0	24	0	23	1
Negative	18	3	12	9	1	20
Total	42	3	36	9	24	21
Positive Agreement (95% CI)	57.1% (42.2 – 70.9)	0%	66.7% (50.3 – 79.8)	0%	95.8% (79.8 – 99.3)	4.8% (0.8 – 22.7)

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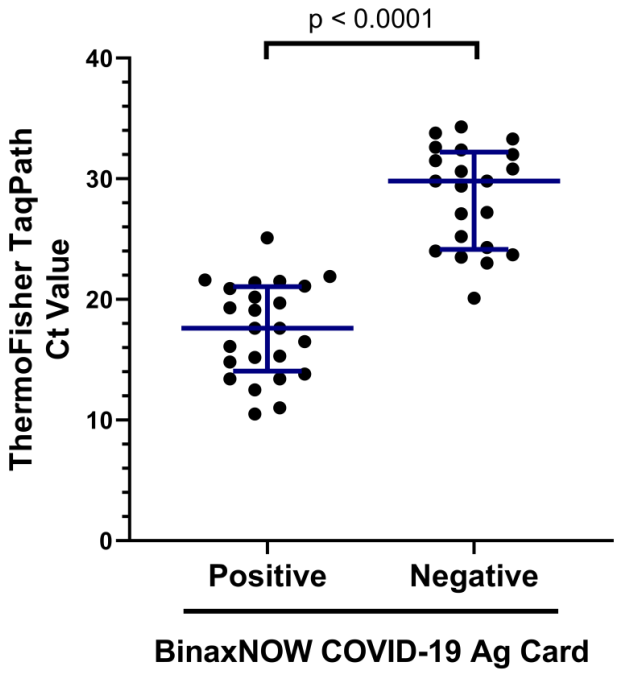

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382 **Figure 1**

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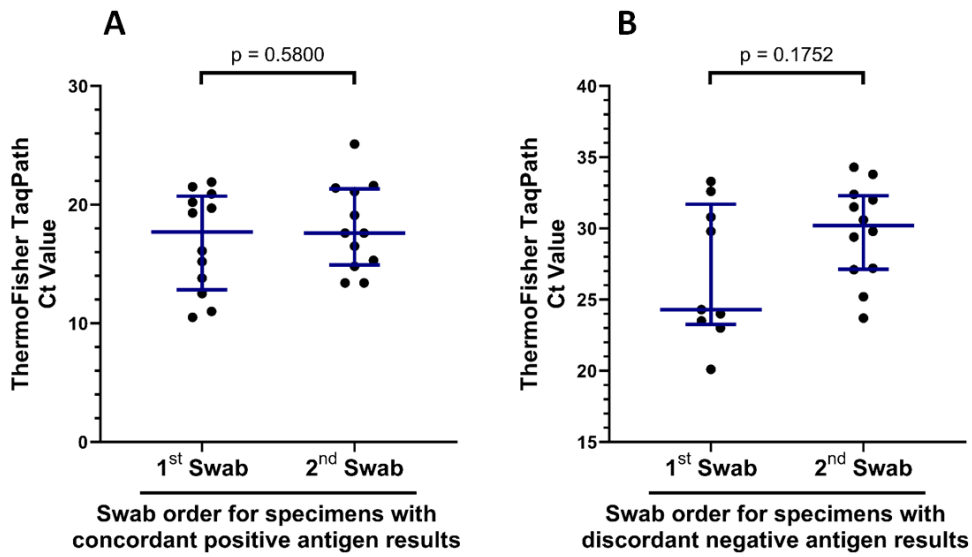

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385 **Figure 2**

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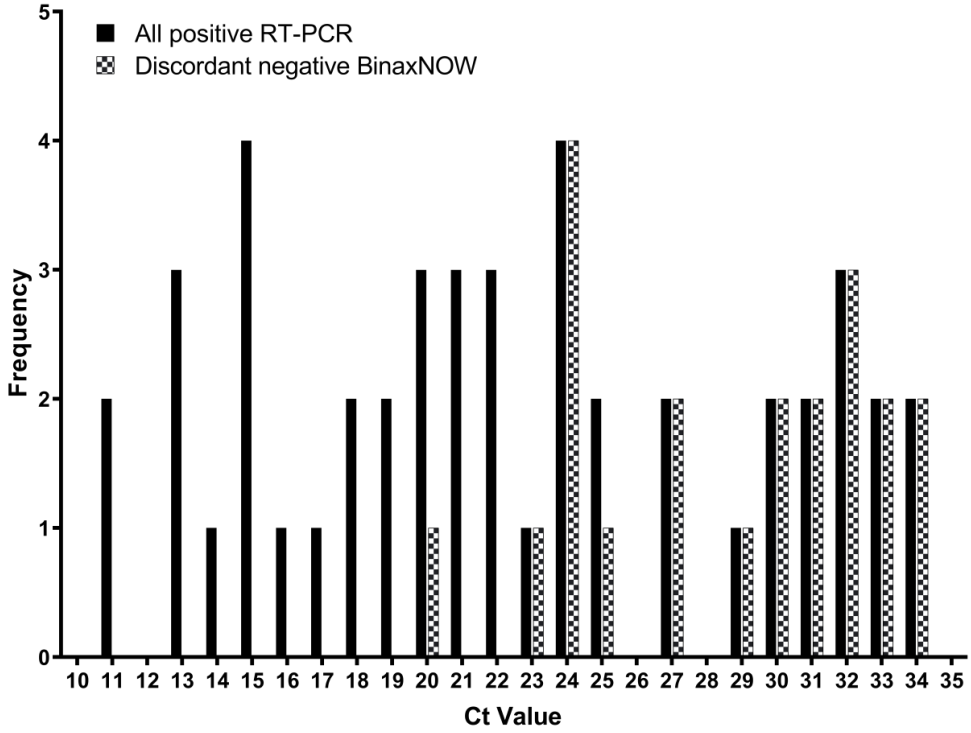

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389 **Figure 3**