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Evaluation of Rapid Antigen Testing (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 Diagnosis in a Tertiary Hospital

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ABSTRACT

Background. The Panbio™ COVID-19 Ag Rapid Test is a Food and Drug Administration (FDA)-approved point-of-care test (POCT) used for SARS-CoV-2 detection which has met minimum sensitivity and specificity requirements by the World Health Organization (WHO).

Objective. The study aimed to compare the clinical performance of a commercial lateral flow assay (LFA) to reverse transcriptase polymerase reaction (RT-PCR) in SARS-CoV-2 infection diagnosis.

Methodology. Clinical data and simultaneous LFA and RT-PCR samples collected from June 2021 to June 2022 were obtained to analyze the diagnostic accuracy of LFA compared to RT-PCR.

Results. A total of 265 samples was obtained. 34.45% of RT-PCR positive samples were reliably detected by LFA. COVID-19 was reliably ruled out by LFA in 99.32% RT-PCR negative samples. LFA sensitivity among symptomatic patients with ≤7 days of illness was 51.61%, slightly higher than those with >7 days of illness (18.92%), and significantly higher than asymptomatic patients (16.67%). Asymptomatic subjects have a varied range of Ct-values, indicating different stages of infection or viral loads. Individuals with symptoms for more than 7 days have higher Ct-values, suggesting they are in later stages of infection or have lower viral loads. The probability of a positive LFA result decreases significantly when the Ct-value is beyond 28-30.

Conclusion. The LFA evaluated in this study did not show significant sensitivity and specificity during the early disease course wherein viral loads are suggestively high. However, its utility to accurately rule out COVID-19 is quite reliable in subjects with symptoms that are >7 days since Ct-values are suggestively beyond 28-30 which implies a significantly decreased probability of a positive LFA result.

Key words: COVID-19 Antigen Test, Ct-value, LFA, RT-PCR, SARS-CoV-2

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INTRODUCTION

To date, SARS-CoV-2, the etiologic agent of COVID-19, has infected over 143 million and caused more than 3 million deaths worldwide. Accurate and prompt diagnosis and lately mass vaccination have become key measures in limiting the spread, preventing severe infection, and timely clinical management.

RT-PCR testing is the current diagnostic gold standard for the detection of SARS-CoV-2.[1] However, specialized instruments, dedicated laboratory supplies, and trained personnel are required to conduct the assays. Although the shortages of RT-PCR accredited laboratories and reagent supply have already been addressed, the current turnaround time is still longer than available POCTs such as antigen testing. This hinders early identification of infected individuals which is essential in the containment of transmission.

The Panbio™ COVID-19 Ag Rapid Test (Abbott, USA) is a lateral flow assay (LFA)-based POCT used for SARS-CoV-2 nucleoprotein detection in nasopharyngeal specimens for the diagnosis of COVID-19. It is simple, affordable, can generate results within 15-20 minutes, has been approved by the FDA, and has met the WHO minimum requirements

of sensitivity (≥80%) and specificity (≥97-100%) as specified by the Health Technology Assessment Council (HTAC).[2,3] And among all the published studies showing the comparison of its performance to RT-PCR, the results proved to be promising.

To the knowledge of the authors, no study comparing the performance of LFA and RT-PCR for SARS-CoV-2 detection, particularly Panbio™ COVID-19 Ag Rapid Test, has been done locally at the time of conception of this research. As such, the data on its specificity and sensitivity is limited to internationally published studies and all studies currently available have similar recommendations of utilizing this test only among symptomatic individuals.

METHODOLOGY

Research design

This study employed a retrospective cross-sectional analytical review of the results of simultaneous antigen and RT-PCR testing of patients at a tertiary hospital from June 2021 to June 2022.

Study population

This study involved all patients regardless of age, sex, or symptomatology who underwent simultaneous antigen and RT-PCR testing from June 2021 to June 2022. Symptomatic subjects were those who presented with clinical manifestations of COVID-19 such as fever, cough, dyspnea, etc. while asymptomatic subjects were those who did not present with clinical manifestations. All subjects during the study's duration were initially seen in the emergency room and subsequently discharged or admitted depending on their conditions at that time.

Sample size

All subjects who underwent simultaneous antigen and RT-PCR testing from June 2021 to June 2022 were identified using logbooks and laboratory information systems (LIS). The data collected included the age, symptomatology at the time of testing, duration of symptoms, and risk of exposure to SARS-CoV-2. A target of 100 positive LFA results comprised the minimum sample size required as recommended by the National Institute for Public Health and the Environment. Purposive sampling method was utilized. The collected data variables were encoded and tabulated accordingly. Descriptive data (frequency and percentages) and graphs or figures were constructed using Microsoft Excel Sheet Software ver. 16.66.1 (volume license 2019). Population characteristics were reported as mean. Difference testing for comparisons of groups was performed by Chi-square testing for categorical variables, independent samples Student's t-tests with Welch's correction for continuous normally distributed variables, and by using Mann-Whitney U tests for not non-normally distributed variables. Specificity and sensitivity with 95% confidence intervals and positive and negative predictive values of the LFA were calculated using the RT-PCR results as a reference test. Factors associated with LFA results were determined using logistic regression, using Nagelkerke's pseudo R2 as a measure of goodness-of-fit. Data were analyzed using a free, open-source software environment.

Ethical considerations

This study entailed a review of antigen and RT-PCR testing results through access to logbooks and LIS and a review of pertinent demographic and clinical data through access to case investigation forms. Only the investigators had access to the personal data of the participants. Data collection, gathering, and analysis commenced upon approval of the Research Ethics Committee and the study was conducted in accordance with Good Clinical Practice (GCP) principles and guidelines. Safeguarding patient information was ensured during data collection and encoding. Patient identifiers were excluded from the study. No patient interaction occurred throughout the study.

RESULTS

Table 1 presents the clinicodemographic profile of subjects who underwent simultaneous antigen and RT-PCR testing for SARS-CoV-2 at NKTI from June 2021 to June 2022.

Table 2 gives the overall diagnostic accuracy of LFA-based Panbio. The rapid antigen test was able to identify only slightly over a third of SARS-CoV-2 infected subjects (Sn: 34.45%); the rest were missed cases (65.55%). However, its ability to rule out COVID-19 correctly was near perfect (Sp: 99.32%). In terms of reliability of the Panbio results, nearly all that returned positive on this test were confirmed with COVID-19 (PPV: 97.62%), with only 1 (2.38%) instance of a false alarm. On the other hand, 34.98% of negative

Table 1. Clinicodemographic profile (n=265)

	All	PCR + (n=119)	PCR - (n=146)	*P*
	Median (Range); Frequency (%)			
Age, years	49.50 (12-82)	55 (16-82)	45 (12-81)	**<.001**§
Sex				
Female	132 (49.81)	58 (48.74)	74 (50.68)	.805†
Male	133 (50.19)	61 (51.26)	72 (49.32)	.805†
Declared as symptomatic	187 (70.57)	100 (84.03)	87 (59.59)	**<.001**†
Symptom duration, days				
≤7	74 (39.57)	31 (31.00)	43 (49.43)	**<.001**†
>7	49 (26.20)	36 (36.00)	13 (14.94)	**<.001**†
Unknown	64 (34.22)	33 (33.00)	31 (35.63)	**<.001**†
Ct-value				
ORF-1ab gene (n=103)	33.73 (17.80-40.81)	33.73 (17.80-40.81)	-	
N gene (n=117)	33.03 (14.19-40.00)	33.03 (14.19-40.00)	-	
LFA (Panbio™) result				
Positive	42 (5.85)	41 (34.45)	1 (0.68)	**<.001**§
Negative	223 (84.15)	78 (65.55)	145 (99.32)	**<.001**§

Statistical analysis used: §–Mann-Whitney; †–Fisher's exact test.

Table 2. Overall Diagnostic accuracy of LFA (Panbio™) test for SARS-CoV-2 (n=265)

LFA (Panbio™)	RT-PCR		Total
	Positive	Negative	
Positive	41	1	42
Negative	78	145	223
Total	119	146	265
Sensitivity, % (95% CI)	34.45 (25.98–43.72)	Positive LR (95% CI)	50.30 (7.02–360.32)
Specificity, % (95% CI)	99.32 (96.24–99.98)	Negative LR (95% CI)	0.66 (0.58–0.75)
PPV, % (95% CI)	97.62 (87.43–99.94)	Accuracy, % (95% CI)	70.19 (64.29–75.63)
NPV, % (95% CI)	65.02 (58.37–71.27)		

LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.
Likelihood ratios were estimated using the substitution formula where 0.5 was added to all cell frequencies before calculation.

Table 3. Diagnostic accuracy of LFA (Panbio™) test for SARS-CoV-2 (n=265) according to symptomatology and/or duration of symptoms

	Asymptomatic			≤7 days			>7 days		
	PCR								
Antigen	+	-	Total	+	-	Total	+	-	Total
+	3	1	4	16	0	16	7	0	7
-	15	57	72	15	44	59	30	13	33
Total	18	58	76	31	44	75	37	13	40
Sensitivity, % (95% CI)	16.67 (3.58-41.42)			51.61 (33.06-69.85)			18.92 (7.96-35.16)		
Specificity, % (95% CI)	98.28 (90.76-99.96)			100 (91.96-100.00)			100 (75.29-100.00)		
PPV, % (95% CI)	75.00 (24.94-96.44)			100 (79.41-100.00)			100 (59.04-100.00)		
NPV, % (95% CI)	79.17 (75.50-82.41)			74.58 (67.10-80.84)			30.23 (27.05-33.61)		
Positive LR (95% CI)	9.67 (1.07 to 87.29)			n/a			n/a		
Negative LR (95% CI)	0.85 (0.69 to 1.05)			0.48 (0.34-0.70)			0.81 (0.69-0.95)		
Accuracy, % (95% CI)	78.95 (68.08% to 87.46%)			80 (69.17-88.35)			40 (26.41-54.82)		

LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.
Likelihood ratios were estimated using the substitution formula where 0.5 was added to all cell frequencies before calculation.

Panbio tests were false results (NPV: 65.02%). Confirmed COVID-19 cases were about 50 times as likely to get positive Panbio results as non-COVID subjects did (LR+: 50.30). The former also tested negative in Panbio at a frequency of about two-thirds as much as non-COVID subjects did (LR-: 0.66).

Table 3 gives the diagnostic accuracy of LFA-based Panbio according to symptomatology and/or duration of symptoms. Discussion on this is in the next three paragraphs.

Among asymptomatic subjects, the results are as follows: less than one-fifth of SARS-CoV-2 infected subjects (Sn: 16.67%) were identified by rapid antigen testing while missed cases were more than four-fifths (83.33%). Its ability to rule out COVID-19 correctly was near perfect (Sp: 98.28%). In terms of reliability, only two-thirds read as positive on this test were confirmed with COVID-19 (PPV: 75.00%), with 15 (25.00%) instances of false positives. On the other hand, the probability that the SARS-CoV-2 was not present when the test was negative is higher (NPV: 79.17%). Confirmed COVID-19 cases were about 10 times as likely to get positive Panbio results as non-COVID subjects did (LR+: 9.67). The former also tested negative in Panbio at a frequency of more than four-fifths as much as non-COVID subjects did (LR-: 0.85).

Among subjects with symptoms for ≤7 days, the results are as follows: A little over half (Sn: 51.61%) were identified and less than half (48.39%) were not. COVID-19 was correctly ruled out in all SARS-Cov-2-negative cases (Sp: 100%). Positivity for SARS-CoV-2 was confirmed in all true SARS-CoV-2-positive cases (PPV: 100%), showing no false positives, while negativity was confirmed in three out of four SARS-Cov-2-negative cases (NPV: 74.58%). Confirmed COVID-19 cases tested negative in Panbio at a frequency of about half as much as non-COVID subjects did (LR-: 0.48).

Among subjects with symptoms for >7 days results are as follows: Around one-fifth (Sn: 18.92%) were detected and more than four-fifths (81.08%) were not. SARS-Cov-2-negative cases were ruled out completely in true negative cases (Sp: 100%). SARS-Cov-2 positive cases were confirmed within all cases (PPV: 100%), showing no false positive. SARS-Cov-2-negative cases were confirmed only in one out of three instances (NPV: 30.23%). Confirmed COVID-19 cases tested negative in Panbio™ at a frequency of more than four-fifths as much as non-COVID subjects did (LR-: 0.48).

Figure 1 shows that for both the E and N genes, cases that were negative on LFA but positive on RT-PCR denote potential false negatives of the LFA. With 62 and 76 cases for the E and N genes, respectively, this highlights instances where the LFA might have missed detecting the virus, even when the RT-PCR indicated a positive result. On the other hand, the positive LFA and positive RT-PCR cases, 41 for both genes, suggest instances where the LFA correctly identified the presence of the virus. The distribution of LFA-positive cases in both genes is along lower Ct-values in contrast to the distribution of LFA-negative cases along higher Ct-values.

In Figures 2 and 3, lower Ct-values, situated on the left end of the spectrum, predominantly correspond with positive LFA results (LFA=1), indicating a higher likelihood or

risk of the condition under study. Conversely, as Ct-value increases, there is a pronounced shift towards negative LFA outcomes (LFA=0), signaling a reduced risk or absence of the condition. The inflection point in the middle of the graph denotes a Ct-threshold (30 and 28 for E and N genes, respectively), beyond which the probability of a positive LFA result decreases significantly.

In Figures 4 and 5, the link between symptom duration and Ct-values is shown. The higher the Ct-value, the lower the viral load, which can suggest less severity or later stages of an infection. Asymptomatic subjects seem to have a varied range of Ct-values, indicating different stages of infection or viral loads. On the other hand, individuals who've shown symptoms for more than 7 days tend to have higher Ct-values, suggesting they might be in a later stage of infection or have a lower viral load.

DISCUSSION

Based on the latest interim guidelines released by the United States Centers for Disease Control and Prevention (US CDC), a positive antigen test can be reliably used for symptomatic patients due to the high specificity of the test.[4] However, relative precaution is warranted in the interpretation of the results of asymptomatic patients hence an algorithm was formulated for this population. He et al. inferred that COVID-19 infectiousness begins at 2-3 days prior to symptom onset, peaks around symptom onset, and takes 9-10 days in total.[5]

The results in this study agree with previous literature when plotting cycle threshold (Ct) values of nasopharyngeal swab specimens against the time after the symptom onset. Lowest Ct-values, which studies propose to correspond to

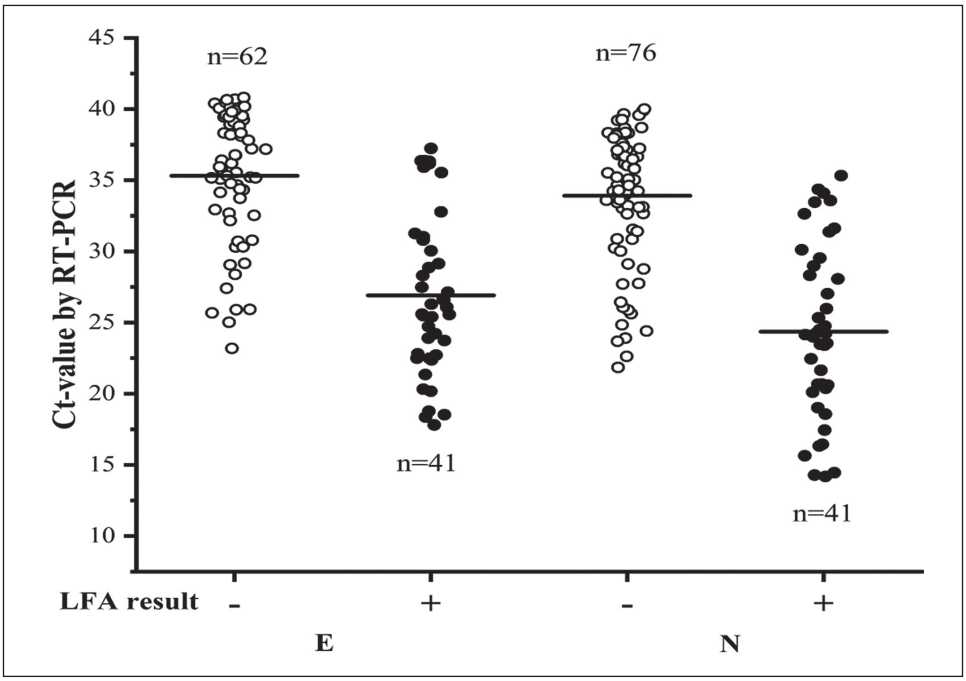

Figure 1. RT-PCR and LFA results of all participants.

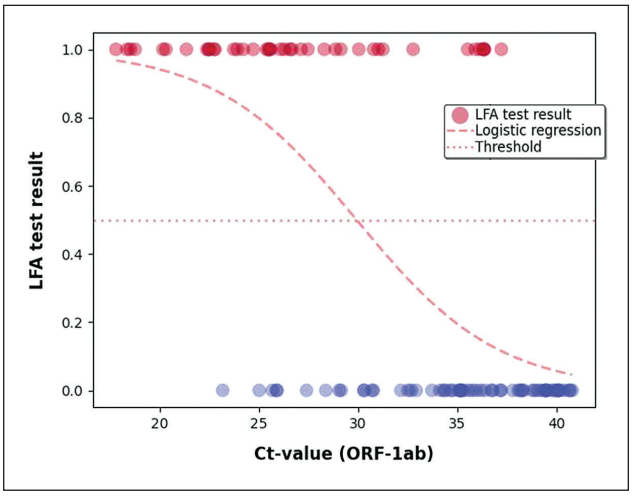

Figure 2. Association between LFA test results and E gene as the Ct-value.

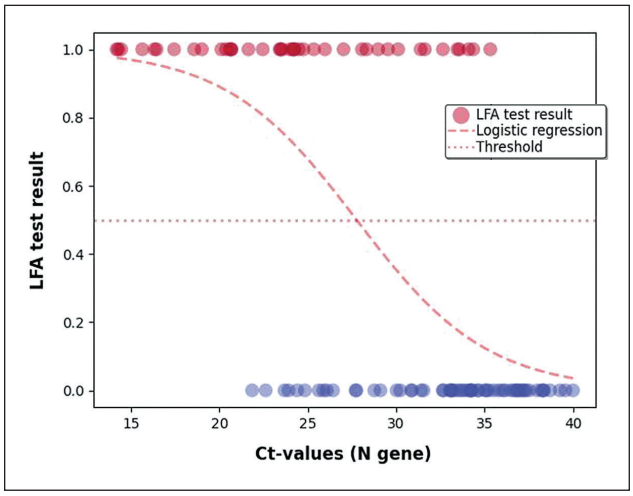

Figure 3. Association between LFA test results and N gene (N2 gene for GX) as the Ct-value.

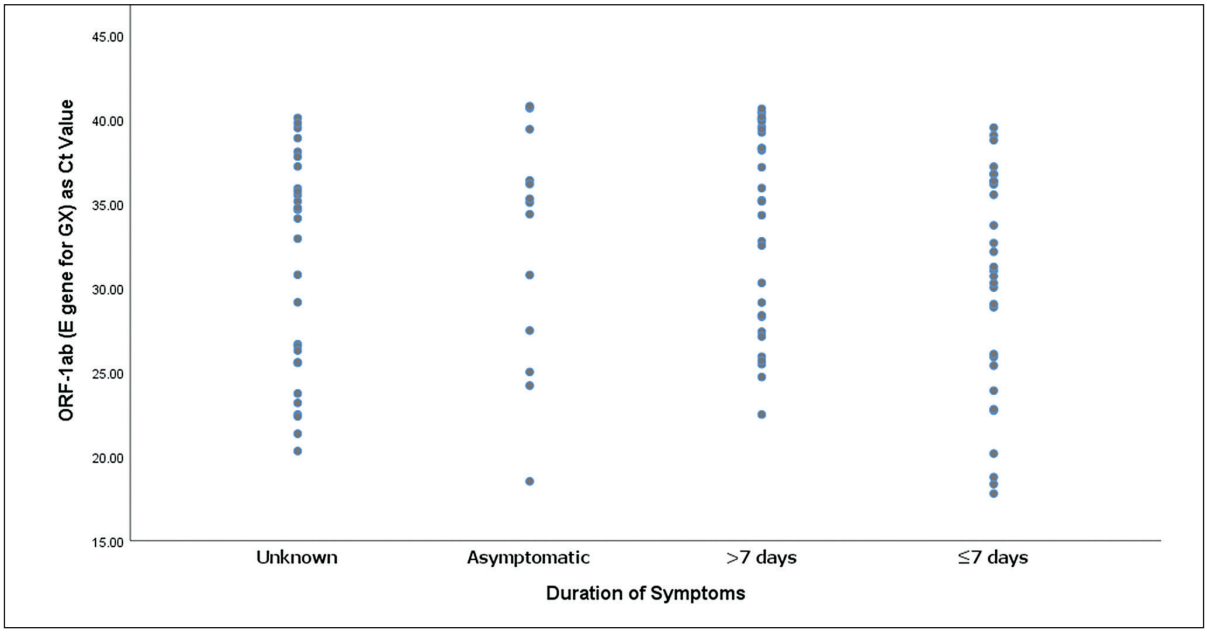

Figure 4. E gene as Ct-Value of RT-PCR positive subjects grouped by duration of symptoms.

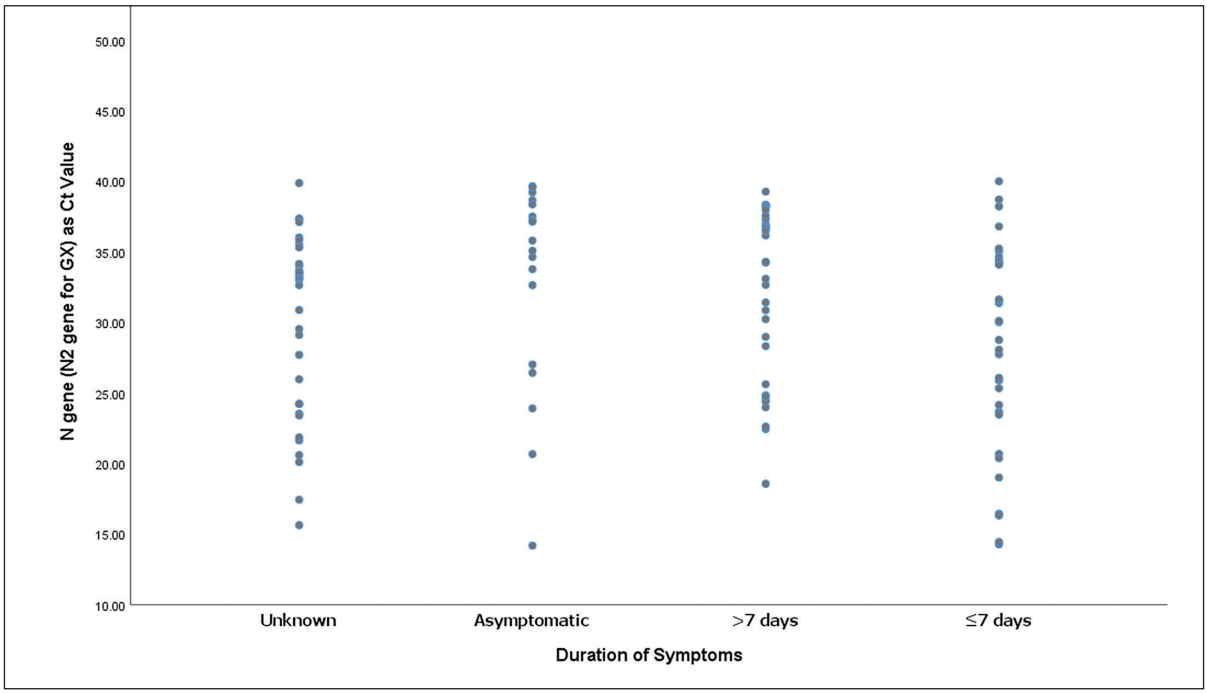

Figure 5. N gene as Ct-value of RT-PCR positive subjects grouped by duration of symptoms.

the highest virus loads, occurred early after the symptom onset, followed by a decline in virus load with increasing time after the symptom onset.[6-8] In addition, sensitivity is noted to decrease as duration of illness becomes more prolonged. This decreasing trend was not elucidated in previous studies although overall LFA sensitivity (34.45%) in this study is significantly lower than in previous studies which listed values over 70%.[8,9]

The reliability of LFA to detect SARS-CoV-2 positivity in truly infected patients (PPV: 97.62%) is comparable to previous literature showing that nearly all that tested positive on this test were truly positive for COVID-19.[8] On the other hand, the reliability of not detecting SARS-CoV-2 in truly uninfected patients is not as high (NPV: 65.02%).[8] These measures of reliability, as well as likelihood ratio, in relation to symptomatology, were not elucidated in previous studies.

As previously mentioned, studies propose that the lower the Ct-value, the higher the viral load, implying higher severity or earlier stages of an infection. However, this notion cannot be entirely supported since the association of the duration of symptoms and Ct-values was found to be weak.[9] In fact, this is supported by findings in the study showing symptomatic subjects of ≤7 days duration and

symptomatic subjects of >7 days duration both displaying a varied range of Ct-values. Although symptomatic subjects of >7 days duration tend to have higher Ct-values and during this period, Ct-values are suggestively beyond 28-30, which is near the range for clinically relevant levels of SARS-CoV-2 RNA,[9] thus probability of a positive LFA result decreases significantly. In addition, asymptomatic subjects also seem to have a varied range of Ct-values, indicating different stages of infection or viral loads, further weakening the association of duration of symptoms and Ct-values.

CONCLUSION

The LFA evaluated in this study did not show significant sensitivity and specificity in samples obtained during the early course of illness wherein viral loads are suggestively high. However, its utility to accurately rule out COVID-19 is quite reliable, particularly in subjects with symptoms that are >7 days since Ct-values are suggestively beyond 28-30 and the probability of a positive LFA result decreases significantly. Results show that LFA has a nearly perfect ability to rule out COVID-19 correctly in these situations.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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