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COVID-Ag Test Use in the Setting of Emergency Department -Practical Issues

*Shaden S**

Department of Emergency Medicine, Hadassah Mount Scopus, Israel

Abstract

Background: During the COVID-19 pandemic the RT-PCR test was the gold standard. Due its costs, accessibility and time required for RT-PCR testing a demand for rapid point of care tests arose.

Objective: To evaluate the sensitivity and specificity of COVID-19 Ag test and to identify predictive factors for a false negative test in an emergency department setting.

Methods: COVID-19 Ag test was used in the emergency department during the Alpha variant outbreak in Israel. These test results were compared with those obtained by a RT-PCR SARS-CoV-2 assay. Clinical data was collected from all participants.

Results: Sensitivity was 63%, specificity was 100%, positive predictive value was 100% and negative predictive value was 93%. There was a statistically significant difference in the time span from the onset of symptoms and the time of testing between the positive antigen group and negative antigen group, 3.9 days with a SD of 5.6 and 5.6 days with a SD of 4 respectively (OR: 0.93, CI 0.87-0.99, P value 0.025). Statistical difference was noted in patients who presented without gastrointestinal symptoms (51, 32.3% and 107, 67.7% respectively, OR: 0.48, CI 0.25-0.92, P value 0.027). Mean cycle time in subjects with negative antigen test was 29.1 while it was 20.9 in subjects with positive antigen tests (OR: 0.71, CI 0.65-0.78, P<0.001).

Conclusion: This rapid antigen test is highly specific but lacks sensitivity. Positive tests correlate with earlier presentation, absence of gastrointestinal symptoms and lower cycle time which correlates with a higher viral load in the individual. Thus, we do recommend the use of rapid COVID-Ag test up to 2 weeks.

Keywords: COVID; Rapid Ag test; Cycle Time; Emergency department

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Correspondence:
Salameh Shaden, Department of Emergency Medicine, Hadassah Mount Scopus, Jerusalem, Israel

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Introduction

The clinical spectrum of Coronavirus Disease-2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection, varies from mild upper respiratory symptoms to severe pneumonia with acute respiratory distress syndrome and systemic complications. In addition, a large proportion of people infected with SARS-CoV-2 remain asymptomatic.

At present, the primary, gold-standard diagnostic method for SARS-CoV-2 infection is Reverse Transcription-Polymerase Chain Reaction (RT-PCR), which is employed in samples including nasopharyngeal swabs, saliva, sputum, and lower respiratory tract secretions. Rapid point of care testing for COVID-19, based on virus antigen detection has gained worldwide interest due to the costs and time required for the traditional RT-PCR tests. Rapid point of care testing is even more practical for peripheral or small medical facilities, where RT-PCR testing may not be available in house and where the need to transport the samples to a central facility, may results in higher costs and time delay.

Currently, there are multiple available tests from various companies (more than 300 to date). The majority are based on lateral flow sandwich immunoassays, microfluidic immunofluorescence assays and chromatographic digital immunoassays. These tests are not considered highly accurate as their sensitivity (compared to RT-PCR) ranges from 0% to 95% [1-4] with an average reported sensitivity of 56%, yet their overall specificity is high (about 97%) [5]. To date only 33 (about 10%) of these tests are FDA approved- all *via* an Emergency Use Authorization (EUA) procedure [6].

To date not much is known about the false negative rate of the rapid antigen tests in the

emergency department patient population, or whether there are factors that can predict false negative tests.

In the present study our aim was to evaluate the sensitivity and specificity of the Bionote. Now check COVID-19 Ag test and to identify predictive factors for a false negative test in our 40-bed Emergency Department (ED), which is part of a 330-bed university urban hospital, mainly during the Alpha variant outbreak [7].

Materials and Methods

Between October 2020 to February 2021, when the Alpha SARS-CoV-2 Variant of Concern (VOC) was the dominant circulating variant in the country, all patients who were evaluated for suspected SARS-CoV-2 infection in our ED were tested in parallel by RT-PCR and by rapid antigen test. Patients were enrolled for testing *via* a questionnaire done by the triage nurse, and if any one of the answers was positive the patient was tested. The questionnaire included 7 sections- difficult breathing, fever, cough, loss of taste or smell sensation, supposed to be quarantined, close contact with a positive COVID-19 patient or came from abroad in the last 2 weeks. All clinical and laboratory data was collected from the electronic medical records.

The rapid antigen test used was the Bionote. Now check COVID-19 Ag test which is a rapid chromatographic immunoassay for the qualitative detection of specific SARS-CoV-2 antigens in nasopharyngeal swabs. Results from the testing are received in about 15 min.

The following RT-PCR tests were used, according to the manufacturers' instructions, for the detection of SARS-CoV-2 RNA in ED patients:

A. GeneXpert DX system (Cepheid, California, USA) that detects the N2 and E viral genes.

B. DiaSorin Simplexa system (DiaSorin Molecular LLC, Italy) that detects the ORF1ab and the S viral genes.

C. NeuMoDx system (QIAGEN, Germany) that detects the N and Nsp2 viral genes.

Statistical analysis was done using the IBM SPSS STATISTICS program, version 25.0 and R statistical software version 3.5.0 (R Project for Statistical Computing). Descriptive statistics are given as Means and Standard Deviations (M&SD) or as frequency (n) with percentage (%), according to the scale of the variable. Associations between clinical presentation and false negative results of the antigen test were assessed by simple logistic regression models, and associations with CT results were assessed by simple linear regression models. Results of regression models were summarized by measures of associations, Odds Ratio (OR) and regression coefficient (B) together with 95% confidence intervals and p-value. Differences in CT results by chest X-ray findings were tested using one-way Analysis of Variance (ANOVA) model, followed by post-hoc analysis by Benjamin-Hochberg method, in order to adjust for multiple comparisons and to control Type I error (alpha). All significant variables at alpha level of 0.2 were entered to multivariable logistic regression model. A p-value <0.05 was considered statistically significant. All reported P-values are two-tailed.

This study was approved by the ethics committee of the Hadassah Medical Center, the research number is HMO-0726-21.

Results

In the time span of the study 1,226 patients that were screened positive according to the questionnaire were tested in our ED for COVID-19 test, 207 (16.9%) of them were found to be positive by RT-PCR test. Of the patients positive by RT-PCR, 131 (64%) were found to be positive by the Bionote. Now check COVID-19 Ag test. A negative result by both RT-PCR and antigen tests was obtained from 1,019 patients (83.1%), and none of the patients had both a positive antigen test and a negative RT-PCR test. The calculated sensitivity for the antigen test was 63%, the calculated specificity was 100%, assuming the prevalence was around 10%. The positive predictive value was 100% and the negative predictive value was 98%. The total accuracy of the test was 93.8%.

The mean age of subjects in both the true positive antigen group and the false negative antigen group was similar - 54.5 with a SD of 22.3 and 54.0 with a SD 21 respectively (OR 1 with a CI of 0.99-1.01, P-value of 0.872). Forty-one (37.6%) of patients who were discharged from the ED had a negative antigen as opposed to 68 (62.4%) of those with a positive antigen, while 35 (35.7%) of patients who were admitted to COVID-19 ("Corona") wards had a negative antigen and 63 (54.3%) had a positive antigen test (OR 1.09 with a CI of 0.62-1.91, P-value of 0.777). Of the patients who reported no known contact with a positive COVID-19 subject prior to their ED visit, 69 (37.3%) had a negative antigen test and 116 (62.7%) had a positive antigen test, as opposed to patients who reported a known contact with a positive COVID-19 subject, where 6 (28.6%) had a negative antigen and 15 (71.4%) had a positive antigen test (OR: 1.5 with a CI of 0.56-4.05, P-value of 0.423). There was a statistically significant difference in the time span from the onset of symptoms and the time of testing between the positive antigen group and negative antigen group, 3.9 days with a SD of 5.6 and 5.6 days with a SD of 4 respectively (with a OR of 0.93 with a CI of 0.87-0.99 and a P-value of 0.025). No significant difference was seen between the groups in their vital signs (fever, heart rate or oxygen saturation) as seen in Table 1. No difference was seen between

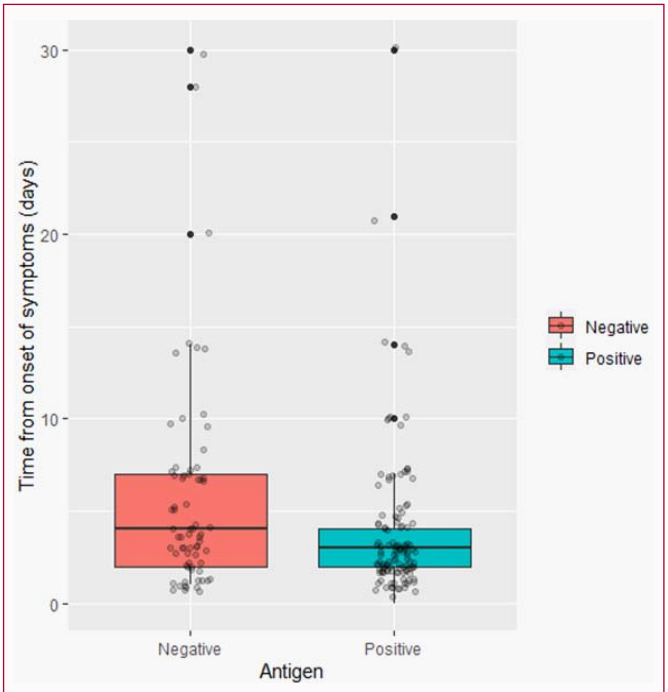

Figure 1: Rapid Ag tests results and time from onset of symptoms.

Table 1: Patients characteristics &clinical data.

Variables	Antigen		OR (95% CI)	p
	Negative N (%)	Positive N (%)		
Age (years)	54.0 (21.0)	54.5 (22.3)	1.00 (0.99-1.01)	0.872
ED decision				
Discharge	41 (37.6)	68 (62.4)	1.09 (0.62-1.91)	0.777
Admission	35 (35.7)	63 (54.3)	Ref.	
Exposure to verified COVID-19 patient				
No	69 (37.3)	116 (62.7)	1.50 (0.56-4.05)	0.423
Yes	6 (28.6)	15 (71.4)	Ref.	
Time from onset of symptoms	5.6 (5.6)	3.9 (4.0)	0.93 (0.87-0.99)	0.025
Chest X-ray findings				
Pneumonia	30 (44.8)	37 (55.2)	0.47 (0.24-0.90)	0.065
Interstitial changes	11 (40.7)	16 (59.3)	0.55 (0.23-1.34)	
Within normal limits	27 (27.6)	71 (72.4)	Ref.	
Fever				
<38.0ºC	39 (35.7)	71 (64.5)	0.89 (0.51-1.57)	0.689
≥ 38.0ºC	37 (37.8)	61 (62.2)	Ref.	
Pulse	98.1 (24.0)	94.7 (22.8)	0.99 (0.98-1.01)	0.314
Oxygen Saturation	94.5 (5.8)	95.1 (5.1)	1.02 (0.97-1.08)	0.439
General				
No	33 (39.8)	50 (60.2)	1.28 (0.72-2.28)	0.394
Yes	43 (34.4)	82 (65.6)	Ref.	
Upper Respiratory Symptoms				
No	69 (37.7)	114 (62.3)	1.57 (0.62-3.95)	0.338
Yes	7 (28.0)	18 (72.0)	Ref.	
Respiratory Symptoms Lower				
No	24 (32.4)	50 (67.6)	0.77 (0.42-1.41)	0.398
Yes	52 (38.8)	82 (61.2)	Ref.	
Gastrointestinal Symptoms				
No	51 (32.3)	107 (67.7)	0.48 (0.25-0.92)	0.027
Yes	25 (50.0)	25 (50.0)	Ref.	

ŝ Age, Time from onset of symptoms, fever and pulse were given in terms of number of means and standard deviations

the groups in the findings on their chest X-rays (within normal limits "WNL", infiltrates or interstitial changes as seen in Table 1). In regard to symptoms that these patients were presenting in the ED, there was no difference between the groups of positive and negative antigen tests in those presenting with or without general symptoms (lack of appetite, headache, weakness, or muscle pain), with or without upper airway symptoms (rhinorrhea or a sore throat) and with or without lower respiratory symptoms (cough, chest pain, excess sputum, or shortness of breath) as seen in Table 1. A statistical difference was noted in patients who presented without gastrointestinal symptoms (abdominal pain, diarrhea, nausea, or vomiting) between the negative antigen group and the positive antigen group (51, 32.3% and 107, 67.7% respectively with an OR of 0.48, a CI 0.25-0.92 and P value of 0.027).

Cycle Threshold (CT) refers to the number of PCR cycles required to identify a positive signal, with fewer cycles equating to higher viral RNA load. The mean CT in the subjects with the negative antigen test was 29.1 with a SD of 5.2 while it was 20.9 with a SD of 4.7 in the subjects with a positive antigen test (OR of 0.71 CI of 0.65-0.78 and a P-value was <0.001). This shows that patients with a positive antigen test had higher viral loads compared to patients with negative antigen tests.

As noted previously (in the text above and in Table 1), the time from symptoms onset to the time of testing was significantly different between the positive antigen group and negative antigen. Every day added from the beginning of the symptoms to the date of testing lowered the odds for a positive test by 0.926 which translates to a 7.4% reduction. This accumulates to a lower OR of 0.58, or a 42% reduction in the chance of a positive antigen test when testing after 7 days since beginning of the symptoms (Figure 1).

We also evaluated the relation between the different parameters we collected and the CT of positive PCR tests, using ANOVA modeling. Our linear regression models revealed that for every extra day from onset of symptoms there was a significant increase in the CT by 0.31 (p=0.001). As for the incidence of gastrointestinal symptoms, there was a significant increase of the CT by 2.54 (0.013). This is shown in Table 2, and it is noticeable that these are the two variables

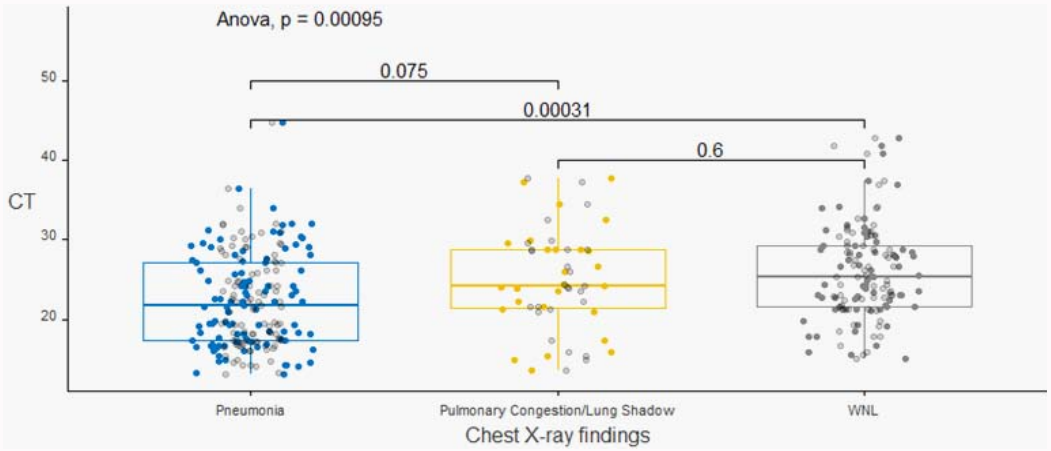

Figure 2: Clinical findings and RT-PCR results-according to cyclic time.

Table 2: Variables analysis.

Variables	B (SE)	95% CI	p
Age (years)	0.03 (0.02)	-0.01-0.07	0.97
ER discharge	0.29 (0.88)	-1.44-2.02	0.74
Exposure to verified COVID-19 patient	-1.61 (1.45)	-4.47-1.26	0.27
Time from onset of symptoms	0.31 (0.09)	0.13-0.50	0.001
Fever (≥ 38.0°C)	-1.11 (0.88)	-2.84-0.61	0.204
Pulse	-0.02 (0.02)	-0.06-0.02	0.236
Oxygen Saturation	-0.10 (0.08)	-0.26-0.07	0.254
General	-1.36 (0.89)	-3.11-0.40	0.129
Upper respiratory symptoms	-1.72 (1.34)	-4.36-0.92	0.201
Lower respiratory symptoms	0.45 (0.92)	-1.36-2.26	0.624
Gastrointestinal symptoms	2.54 (1.01)	0.55-4.53	0.013

that were found to have a correlation that was significant statistically with a positive antigen test.

Another finding our ANOVA model revealed, as shown in Figure 2, was a significant association between chest X-ray findings and CT values ($p<0.001$). Multiple comparison exhibited significant difference between pneumonia findings and WNL, as the CT values were higher within patients with a normal chest X-ray.

Discussion

Our Data supports the data collected in previous studies, demonstrating that the Ag test is highly specific with variable sensitivity, depending on the patient's group characteristics [8-10]. Notably, this low sensitivity (in contrast to the very high septicity) falls lower than the 80% sensitivity required by the WHO for these tests [5] and the sensitivity of 89% reported by the manufacturer of this kit. In trying to understand what factors affect the Ag test results, we revealed that longer time from symptoms, absence of GI symptoms, and normal chest X-ray can predict false negative Ag test results.

According to our data, none of the patients had a positive antigen test at ≥ 2 weeks since the symptoms start, thus we recommend using the test up to 2 weeks. Beyond 2 weeks one should rely solely on the PCR results. We also concluded that each extra day from the onset of symptoms increases, significantly, the PCR CT by 0.31 ($p=0.001$) thus lowering the sensitivity.

Furthermore, there was a correlation between the Ag test results and the PCR CT, reflecting the sample's viral load. This correlation is not surprising and is in accordance with previous reports [8-12].

In this regard, we noticed that no positive Ag test was detected when the CT was beyond 30 and that all the patients with CT lower than 17 had a positive antigen test.

Importantly, in the daily reality of the ED, CT results are generally obtained retrospectively and are not available upon admission, thus not being helpful for the medical staff at the triage of these suspected patients. We therefore ran another logistic regression analysis aimed to identify patient characteristics which can predict the CT results (which demonstrated a significant correlation with the Antigen test result).

Our study expands and supports previous studies that showed the limitations of various Ag based tests, mainly, the sensitivity that was lower than desired, the high specificity, the link between a positive antigen test and a lower PCR CT, and the time frame within which these tests are optimal- within the first 7 days since symptoms began [8-10]. However, our study further revealed the relation between various symptoms and signs and the possibility of a positive Ag test. To the best of our knowledge, this was not described in previous studies. In this respect, we showed a significant correlation between the absence of gastrointestinal symptoms and a normal chest X-ray with a positive antigen test. This can be helpful for a clinician evaluating a suspected COVID-19 patient that has GI symptoms or a normal CXR and should not be admitted to a non-COVID department if the Ag test is negative due to the higher rate of false negative tests in these subgroups. In these patients we recommend waiting for a PCR test to be negative before admitting to a non-COVID department.

According to this data, we have changed the policy of admitting patients to COVID-19 wards in our hospital. In light of the high demonstrated specify, we started admitting patient to COVID-19 wards (if clinically appropriate) on the basis of a positive antigen test without waiting for a positive PCR test. This policy shortened the stay in the emergency department for these patients and increased the turnover of available beds - a highly needed resource during this period.

Limitations

Our study has some limitations: it is not certain that these results

would be similar across all different variants of the SARS-CoV-2, as this study was performed during the alpha variant circulation in Israel. Additionally, the study examined only one brand of antigen test- the Bionote. Now check COVID-19 Ag test- and the applicability of the findings to other antigen tests should be further examined. The data was collected from symptomatic patients and might not accurately reflect the test parameters in asymptomatic infected individuals. Due to the fact that during this time span the prevalence in Israel and in Jerusalem was changing according to the pandemic waves we used averaged prevalence when calculating the PPV and NPV.

Conclusion

In conclusion, we found that positive COVID-Ag tests correlate with earlier presentation, absence of gastrointestinal symptoms and lower cycle time which correlates with a higher viral load in the individuals. Thus, we recommend using the COVID-Ag test in the ED setting up to 2 weeks. Furthermore; in facing new endemic disease, when no enough data is available, studies should be conducted analyzing the data as this could potentially lead to use practical steps affecting and even changing the local policy providing better insights for COVID-19 patient's management.

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