Clinical and Experimental Health Sciences

Comparison of the Rapid Antigen Test to RT-qPCR in Diagnosis of SARS-CoV-2: A University Experience in Northern Cyprus

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ABSTRACT

Objective: As an alternative to RT-qPCR assays used in the diagnosis SARS-CoV-2, antigen-detecting rapid diagnostic tests (Ag-RDTs) are available for the qualitative detection of SARS-CoV-2 in nasopharyngeal swab samples. The aim of this study was to assess the accuracy and reliability of Ag-RDTs as a diagnostic method of detecting SARS-CoV-2 positive cases within a given population.

Methods: In first phase of this investigation, 357 nasopharyngeal swab samples were screened for SARS-CoV-2 using Ag-RDTs. For the purposes of this study RT-qPCR was then applied to the same 357 nasopharyngeal swab samples in order to compare the reliability of the two detection methods. In the second phase of this investigation, Ag-RDTs were applied to an additional 75 nasopharyngeal swab samples that were already known to be RT-qPCR positive.

Results: In the first phase of this investigation, of the 357 samples screened using Ag-RDTs 14 samples were positive for SARS-CoV-2, in contrast, when RT-qPCR analysis was applied to the same 357 samples no SARS-CoV-2 samples were detected. Therefore, the false antigen positivity was determined to be at 3.9%. In the second phase of this investigation 75 RT-qPCR positive samples were re-evaluated with a rapid antigen test. Twenty-four of the 75 RT-qPCR positive sample were undetected.

Conclusion: Solely relying on rapid antigen tests to detect SARS-CoV-2 infections in the community could consequently result in infectious individuals remaining in the population. The impact of false negative rapid test results can be reduced by implementing confirmatory RT-qPCR analysis particularly in symptomatic patients.

Keywords: SARS-CoV-2, RT-qPCR, Antigen, Test, Northern Cyprus

1. INTRODUCTION

The COVID-19 (Novel Coronavirus Disease 19) pandemic, caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), led to an unprecedented public health crisis (1). While the pandemic continues worldwide, rapid and reliable tests are urgently needed for the diagnosis of COVID-19 (2). Nucleic acid amplification tests (NAATs), such as real time reverse transcription polymerase chain reaction (RT-qPCR) assays, are referred to as the gold standard test used in the diagnosis of SARS-CoV-2 acute infection. Although standard RT-qPCR protocols have high sensitivity and specificity, they can be time-consuming and expensive (3,4).

As an alternative to PCR assays, antigen detecting rapid diagnostic tests (Ag-RDTs) are available for qualitative determination of SARS-CoV-2 associated antigen (5). Antigen based tests are inexpensive and can return results within 15-30 minutes. These tests are designed to directly detect SARS-CoV-2 proteins (4,6,7). The accuracy of Ag-RDTs depends on several factors, such as the time elapsed from the onset of infection and symptoms, the viral load in the specimen, the processing of the specimen, and the quality of the reagents in the test kits (7). Sensitivity of Ag-RDTs in the detection of SARS-CoV-2 compared to NAATs SARS-CoV-2 detection sensitivity in nasal/nasopharyngeal swab samples were found to be highly variable. Their sensitivity ranging from 0-94%, while specificity is >97% (4). According to data by the World Health Organization (WHO), Ag-RDTs may generate false negative results in COVID-19 patients with low viral load. Consequently the need for further development of these tests is essential and are therefore not recommended for use in clinical diagnosis (5,8).

The aim of this study was to determine the accuracy of the SARS-CoV-2 Ag-RDT that is currently used in several countries as a diagnostic tool for the detection of SARS-CoV-2 found in nasopharyngeal/oropharyngeal swab samples.

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2. METHODS

Ethics committee approval was obtained for our study with the project number NEU/2021/95-1411 at the meeting held by the Near East University (NEU) Scientific Research Ethics Committee on 30.09.2021.

2.1. Study Groups and Study Design

The study involved two types of analyses. Initially, in the first phase of the study, Ag-RDTs and RT-qPCR were performed on 357 specimens that were collected from Near East College students and teachers between 1st and 30th April 2021. In the second phase of our study, Ag-RDTs were applied to 75 SARS-CoV-2 positive patients' samples, which were verified by direct swab specimen and RT-qPCR at the DESAM Research Institute, COVID-19 Diagnostic Laboratory between 28th May and 30th June 2021. For the second phase of the study, following the identification of 75 SARS-CoV-2 positive samples using RT-qPCR, Ag-RDTs were also applied immediately to the same swab samples collected from the 75 SARS-CoV-2 positive patients. All samples were collected as part of the routine COVID-19 screening protocol as set out by the Ministry of Health of Northern Cyprus.

2.2. Ag-RDT and RT-qPCR Analyses

The SARS-CoV-2 (COVID-19) Antigen Test (Softec, in-vitro diagnostic, Istanbul/Turkey) kit represented the selected Ag-RDT used in this investigation. This test is an immunological test that detects the nucleocapsid antigen (N) of the SARS-CoV-2, based on the principle of the double antibody sandwich technique. The sensitivity and specificity of the rapid antigen test kit according to the manufacturer was 96.38% and 99.17% respectively.

The Ag-RDT was performed in accordance with the recommendations of the manufacturer using nasopharyngeal/oropharyngeal swab samples collected from individuals. Briefly, nasopharyngeal swabs which were collected from individuals were transferred to a viral transport media immediately and 50 µl of the specimen was taken directly from the transport tube and applied onto the antigen test cassette, the results were evaluated after 15 minutes.

All RT-qPCR tests were performed using the Bio-speedy SARS-CoV-2 Double Gene RT-qPCR (Bioeksen, Ar-Ge Tekn. A.Ş., Istanbul/Turkey) kit. In accordance with the manufacturer's instructions, the kit was designed to detect *ORF1ab* and the *N* gene of SARS-CoV-2. In accordance with the FDA, a cycle threshold (CT) value less than 38.00 in a given RT-qPCR run of a sample was considered negative to the presence of SARS-CoV-2 (9). Each RT-qPCR study included a negative and positive control for quality control purposes.

The 75 RT-qPCR positive specimens which were also re-evaluated with Ag-RDTs were initially categorized into groups based on the samples Ct value. The groups were as follows: Group A: Ct of 10-19.99; Group B: Ct of 20-29.99; Group C: Ct of 30-38.

2.3 Statistical and Simulation Analysis

Statistical analysis of the data obtained was conducted using SPSS (Statistical Package for the Social Sciences) Demo Version 22.0 (SPSS Inc., Chicago, IL, USA) program. Pearson Chi Square and One Way ANOVA were used to determine the statistical significance and $p<0.05$ values were considered statistically significant.

The simulation analysis was used to simulate the collective results of SARS-CoV-2 positive cases which were detected in the DESAM Research Institute, COVID-19 Diagnostic Laboratory using RT-qPCR and Ag-RDT. A total of 865 SARS-CoV-2 RNA positive cases which were diagnosed using RT-qPCR between 01 July 2020 and 25 June 2021 were included in the simulation analysis. The SARS-CoV-2 RNA positive cases were separated into 3 groups (Group D: 10.00-19.99, Group E: 20.00-29.99, Group F: 30.00-38.00) based on their Ct values. A Ct value above 38.00 were not included into the groups. One Way ANOVA test were used to determine similarities among the groups (D to F).

3. RESULTS

In the first phase of this study, a total of 357 Ag-RDTs samples were re-evaluated for SARS-CoV-2 using RT-qPCR. Initially a total of 14 Ag-RDTs positive samples were obtained, however the RT-qPCR results of these 14 antigen positive samples together with the remaining 343 samples all tested negative for SARS-CoV-2 using the extracted RNA (as the template) from the specimens. Thus, the false positive rate of the antigen test (Ag-RDTs) used was 3.9%.

To further assess the reliability of rapid antigen tests in the detection of SARS-CoV-2, 75 RT-qPCR positive samples were re-evaluated with a rapid antigen test. While 51 (68%) of the 75 RT-qPCR positive samples included in the study were antigen positive, 24 (32%) were negative. These 75 SARS-CoV-2 RT-qPCR positive samples were assessed and categorized according to the Ct values (Table 1). The mean Ct values in antigen positive and negative samples were 20.46±3.95 (between 12.05-31.40), 26.03±3.28 (between 18.84-31.60) respectively. The difference between the mean Ct values of antigen positive and negative patients was statistically significant ($p=0.001$) (Figure 1). Accordingly, it was determined that the antigen test may not detect patients with a low viral load (Ct: 30.00-38.00) and in turn giving a false negative result. Table 2 displays the sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) of the antigen kit tested.

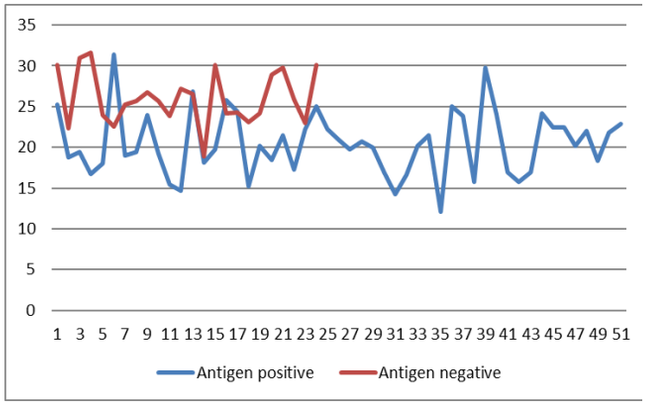

Figure 1. *Distribution of Ct values in antigen positive and negative samples*

These RT-qPCR positive samples were further separated into three groups based on the Ct values; in such group A: Ct of 10-19.99, group B: Ct of 20-29.99, group C: Ct of 30-38, respectively. None of the Ct values of the 75 samples were higher or equal to a Ct of 38. Group A was composed of 25 samples, 24 of which tested both positive for SARS-CoV-2 by RT-qPCR and antigen testing. On the other hand, Group C contained six samples, five of which are RT-qPCR RNA positive and antigen test negative (false negative). The false negative rate of Group A, B and C were determined and shown in the Table 1. The false negativity rate of the antigen test in patients with low viral load was 83.3%. According to Table 1, as the Ct values increase, the false negative rate of the antigen test also increases significantly (p=0.001).

Table 1. *Comparison of Ct values of SARS-CoV-2 RT-qPCR positive samples with antigen test results, n (%)*

Group	RT-qPCR RNA Positive / Antigen Test Positive	RT-qPCR RNA Positive / Antigen Test Negative	Total	False Negative Rate
Group A (Ct: 10.00-19.99)	24	1	25	4.0%
Group B (Ct: 20.00-29.99)	26	18	44	40.9%
Group C (Ct: 30.00-38.00)	1	5	6	83.3%

RT-PCR: Real time polymerase chain reaction; RNA: Ribonucleic acid; Ct: Cycle threshold

Table 2. *Performance evaluation of the tested antigen kit*

	RT-qPCR positive	RT-qPCR negative	Total
Antigen positive	51	14	65
Antigen negative	24	343	367
Total	75	357	432
Sensitivity	68.0%		
Specificity	96.1%		
Positive predictive value (PPV)	78.5%		
Negative predictive value (NPV)	93.5%		

RT-qPCR: Real time reverse transcription polymerase chain reaction

Based on the results of this study, a simulation was conducted to predict the reliability of the rapid antigen test in a larger sample size. A total of 865 RT-qPCR positive samples were grouped based on the Ct values of the samples and separated into groups D, E, F (Table 3). The Ct values of Group A and Group D, Group B and Group E, Group C and Group F were separately compared between against each other and there were no statistical differences among the compared groups based on the Ct values (p=0.994, p=0.195, p=0.260 respectively).

In the simulation context, Groups A, B and C false negative antigen test rates can be used to simulate Group D, E and F accordingly. The patients were categorized into A, B and C based on Ct values, this is an indication of the viral load. Thus, if all 865 RT-qPCR positive samples were re-tested with a rapid antigen test, it is predicted that, 402 positive cases (46.53%) would not to be detected as a positive case. In summary, the false negative rate of rapid antigen tests based on real data would be 46.53%.

Table 3. *Simulation analysis results*

Ct Values Range	Antigen Test False Negative Rate (%)	SARS-CoV-2 RNA Positive Cases (n)	If only antigen test was used amount of false negative cases (n)
10.00-19.99 Ct	4.00 (Group D)	120	4.80
20.00-29.99 Ct	40.91 (Group E)	526	215.19
30.00-38.00 Ct	83.33 (Group F)	219	182.49
Total	-	865	402.48

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; RNA: Ribonucleic acid; Ct: Cycle threshold

4. DISCUSSION

COVID-19 pandemic has brought about the need for rapid and sensitive diagnostic tests to detect the SARS-CoV-2 as quickly as possible in order to avoid the spread of the virus and manage patients infected with SARS-CoV-2 appropriately. Rapid antigen tests are cost-effective, easy to use, and can be manufactured in large quantities (10). Ag-RDTs as their name suggests are quick in providing a result, but as this study has confirmed, the performance is dependent on the viral load, the quality of the specimen and the processing phase (11). In this study, the performance of the antigen test used in the routine screening of SARS-CoV-2 was determined by re-confirming the antigen test results with RT-qPCR and vice versa. Although the sample size in each Ct group is not evenly distributed, the preliminary data at hand suggests that the sensitivity of the antigen test may be dependent on viral load.

The sensitivity and specificity of the Softec SARS-CoV-2 Antigen Test (Turkey) kit as advertised by the manufacturer is 96.38% and 99.17%, respectively. The first phase of this study comprised 357 asymptomatic individuals who were part of a routine screening process of college students and teachers for SARS-CoV-2, as part of the Northern Cyprus COVID-19 public screening program. To further assess the

sensitivity and specificity of the antigen test kit, 75 SARS-CoV-2 RT-qPCR positive samples which were initially detected with RT-qPCR as part of routine public screening, were also tested with the antigen test kit. Collectively, based on these results, the sensitivity and specificity of the Softec SARS-CoV-2 Antigen Test kit was 68.0% and 96.1%, respectively. Our study suggests that the antigen test kit sensitivity was relative to the patient's viral load, that is, the patients with relatively higher Ct values (Ct>26) were more likely to generate false negative rapid antigen test results. These findings suggest that rapid antigen tests are less effective amongst asymptomatic individuals, when compared with RT-qPCR results. In addition, the PPV of antigen testing amongst asymptomatic individuals was calculated to be 78.5%. The correlation between lower viral loads and false-negative results by rapid antigen tests has also been noted by others (12,13). Previous studies have also demonstrated that PPV and sensitivity being particularly low in asymptomatic individuals. In the study of Barrera-Alvalos et al., 55 patient samples with positive RT-qPCR results at different Ct values were investigated. Ag-RDTs was performed on all of these samples, and none of the samples above >30 ct values were found to be positive. The sensitivity of the Ag-RDT was 90% in samples with RT-qPCR Ct value between 20≤Ct<25, 10% between 25≤Ct<30 and 0% in samples with >30 Ct value (14). In accordance with these collective findings, the WHO has announced that the sensitivity of antigen tests varies and negative diagnostic testing results should be assessed depending on the circumstances of the population and should be considered as presumptive results (15).

Phase 2 of this investigation is summarized in Table 3. The Ct values in Group A (10.00-19.99), B (20.00-29.99) and C (30.00-38.00) is representative of 865 SARS-CoV-2 RT-qPCR positive patients and is an indication of the reality of viral load in the North Cyprus population. According to statistical simulation the false negative rate following rapid antigen testing of real data is predicted to be 46.53%. To further validate the results from the current study a larger sample number of SARS-CoV-2 PCR positive samples with Ct values between 30-38 is required.

5. CONCLUSION

In this study, it has been demonstrated that antigen test kit sensitivity appears to be dependent to the patient's viral load, higher Ct values (Ct>26) were more likely to generate false negative rapid antigen test results. As indicated by the second phase of the investigation, relying solely on rapid antigen tests to detect SARS-CoV-2 infections could consequently result in infectious individuals remaining in the population. In agreement with the recommendation by the CDC (2021), the impact of false negative rapid test results can be reduced by implementing confirmatory RT-qPCR particularly in symptomatic patients.

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Design of the study: TŞ, KS, EG, MÇE, PT
Acquisition of data for the study: EG, FT, EŞ, MÇE, PT, BD, CSÖ, GT
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