Diagnostic performance of rapid antigen test for COVID-19 and the effect of viral load, sampling time, subject's clinical and laboratory parameters on test accuracy

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Abstract

The ongoing COVID-19 pandemic has highlighted the central role of diagnostic tests in pandemic control. Although reverse transcriptase quantitative real-time PCR (RT-qPCR) is the gold standard for the diagnosis of COVID-19, several rapid antigen tests (RAT) have been commercialized as rapid point-of-care diagnostics. To the best of our knowledge, there are limited data on the effect of patient's clinical and laboratory parameters on RAT performance and no studies exist that tested the importance of combining laboratory measurements in patient's blood in enhancing the performance of RAT. Here we tried to fill these gaps by evaluating the diagnostic performance of the RAT "Standard Q COVID-19 Ag" in participant's subgroups studying the influence of viral load, sampling time-post symptoms, clinical and laboratory features on test performance. Eighty-three nasopharyngeal and oropharyngeal swabs were tested for sever acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) by both RT-qPCR and RAT. Diagnostic accuracy of the RAT was evaluated for participant's subgroups that have various features. Support vector machine model was then used to investigate whether laboratory measurements in subject's blood would enhance the predictive accuracy of this RAT. The sensitivity, specificity and accuracy of the RAT were 78.2, 64.2 and 75.9%, respectively. Samples with high viral load and those that were collected within one week post-symptom showed the highest sensitivity and accuracy. Measuring Laboratory indices did not enhance the predictive accuracy of this RAT. It is concluded that "Standard Q COVID-19 Ag" should not be used alone for COVID-19 diagnosis due to its low diagnostic performance. This RAT is best used at early disease stage and in patients with high viral load.

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Running title: Performance of RAT in COVID-19 diagnosis

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Summary

The ongoing COVID-19 pandemic has highlighted the central role of diagnostic tests in pandemic control. Although reverse transcriptase quantitative real-time PCR (RT-qPCR) is the gold standard for the diagnosis of COVID-19, several rapid antigen tests (RAT) have been commercialized as rapid point-of-care diagnostics. To the best of our knowledge, there are limited data on the effect of patient's clinical and laboratory parameters on RAT performance and no studies exist that tested the importance of combining laboratory measurements in patient's blood in enhancing the performance of RAT. Here we tried to fill these gaps by evaluating the diagnostic performance of the RAT "Standard Q COVID-19 Ag" in participant's subgroups studying the influence of viral load, sampling time-post symptoms, clinical and laboratory features on test performance. Eighty-three nasopharyngeal and oropharyngeal swabs were tested for sever acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) by both RT-qPCR and RAT. Diagnostic accuracy of the RAT was evaluated for participant's subgroups that have various features. Support vector machine model was then used to investigate whether laboratory measurements in subject's blood would enhance the predictive accuracy of this RAT. The sensitivity, specificity and accuracy of the RAT were 78.2, 64.2 and 75.9%, respectively. Samples with high viral load and those that were collected within one week post-symptom showed the highest sensitivity and accuracy. Measuring Laboratory indices did not enhance the predictive accuracy of this RAT. It is concluded that "Standard Q COVID-19 Ag" should not be used alone for COVID-19 diagnosis due to its low diagnostic performance. This RAT is best used at early disease stage and in patients with high viral load.

Key words

Accuracy, COVID-19, Diagnosis, SARS-CoV-2, Rapid antigen test

Introduction

Infection with Coronavirus (CoV) diseases (COVID-19), which is caused by novel sever acute respiratory syndrome CoV-2 (SARS-CoV-2), was firstly reported in Wuhan, China in December 2019 (Akashi et al., 2019). On 11 March 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic of global concern (WHO, 11 March 2020.). After one year from the initial China outbreak, there have been tremendous increase in the number of confirmed cases as well as death records worldwide. As of 21 January 2021, ~ 95 million COVID-19 cases were confirmed worldwide and more than two million deaths were reported (WHO, 11 January 2020.). Egypt was among the first 10 countries in Africa that experienced COVID-19 cases (Nkengasong & Mankoula, 2020). By 3 January 2021, Egypt has reported 144.583 confirmed cases with 5.4% of them (7.918) died of the pandemic (WHO, 11 January 2020.). The sudden and unprecedented surge

in the number of reported cases is overwhelming the capacity of the national healthcare system, particularly in the developing countries (Leung et al., 2020). Central to the containment of the ongoing pandemic is the availability of rapid and accurate diagnostic tests that could pinpoint patients at early disease stages before further spread occur.

Reverse transcriptase quantitative real-time PCR (RT-qPCR) assay has been the gold standard for diagnosing COVID-19 in many health sectors and laboratories (Shen et al., 2020; Tang, Schmitz, Persing, & Stratton, 2020; van Kasteren et al., 2020). However, this assay often done in large centralized hospitals or laboratories away from the access of local inhabitants, it requires long time-to-results, skilled staff and specialized instruments and is of high cost. This is particularly the case in many developing countries, including Egypt (Anjum, Anam, & Rahman, 2020). In Egypt, the RT-qPCR diagnosis is mostly done in Cairo, the capital or in capitals of governorates leading to an overall turn-around-time of ˜ 24 hours at best between shipping the samples and obtaining the results (Sheridan, 2020). Indeed, suspected individuals often go first to the local clinics for emergency where RT-qPCR might not be available. The RT-qPCR may not be able to cope with the testing or screening needs in the low and middle-income countries due to limited infrastructure, low fund and limited human resources (Olalekan et al., 2020). To fill in this gap and to improve this situation, rapid antigen tests (RATs) are being developed and are in use as point-of-care diagnostic tools in local settings and emergency departments (Wee et al., 2020). They offer the advantage of being quick and can be done simply without need for special equipment (Lambert-Niclot et al., 2020; Nalumansi et al., 2020; Scohy et al., 2020). Determining the diagnostic performance of commercialized RAT is crucial because this gives indication about their reliability and clinical utility during the time of pandemic.

There have been many RAT available for diagnosis of COVID-19 (reviewed in (Olalekan et al., 2020)), yet their clinical applicability is questionable because their accuracy is low as compared to RT-qPCR, their diagnostic performance is highly variable, even when the same assay was applied in two different population with different ethnicity background (Chaimayo et al., 2020; Nalumansi et al., 2020) and their accuracy is host- and virus-dependent (Chaimayo et al., 2020; Tang et al., 2020). Similar to other countries, several RATs have been under development in Egypt, but the available studies lack detailed characterization of RAT performance, especially the impact of patients criteria, clinical features, sampling time and viral load on the test performance. Actually, only one RAT (BIOCREDIT COVID-19 antigen test) has been recently evaluated in Egypt (A. M. Abdelrazik, S. M. Elshafie, & H. M. Abdelaziz, 2020). In the current study, we aimed to add to the current knowledge by evaluating the clinical utility of a recently commercialized RAT, Standard Q COVID-19 Ag, in a number of Egyptian participants, who are suspected of having COVID-19. We also studied the influence of various factors on the assay performance and tested the hypothesis that measuring laboratory parameters could enhance RAT predictive accuracy when RT-qPCR is not available.

Material and methods

Study setting, Sample size and participant's data

This is a cross-sectional study conducted at the premises of Zagazig University Hospitals and the affiliated Scientific & Medical Research Centre, Faculty of Medicine, Zagazig University in the period from June 2020 through October 2020. Sample size was estimated using the online tool Openepi version 3.1 (Dean AG, 2013) considering hypothesized frequency of outcome in the population (p) equal to 50%±5, confidence limits as % of 100 (absolute ± %)(d): 5%, design effect (for cluster surveys-DEFF): 1 and sample size at confidence level 95%: 152. Eighty-three individuals were enrolled in the study after being referred to the COVID-19 isolation unit in the previously mentioned settings. Criterion for participants inclusion was that the participant should be suspected of having COVID-19 infection due to either he/she was in contact with COVID-19 positive individuals or was admitted to the hospital with symptoms suggestive of having COVID-19. Participant's metadata (age and gender), symptoms, radiological findings and fourteen laboratory parameters (Hemoglobin; HB, urea, platelet, white blood cells; WBC, creatinine, alanine aminotransferase; ALT, aspartate amino transferase; AST, lactate dehydrogenase; LDH, serum ferritin; S. ferritin, C. reactive protein; CRP, prothrombin time; PT, international normalized ratio; INR and polymorphonuclear leukocytes; PNL) were collected at the time of admission and during the course of illness. The laboratory measurements

were done at the clinical Pathology Department, Faculty of Medicine, Zagazig University.

Sample collection and preparation

Eighty-three nasopharyngeal (NP) and an oropharyngeal swabs (OP) were obtained by trained health staff at the isolation units. Based on recommendation from Center for Disease Control and Prevention (CDC) (CDC, 2020) and to maximize sensitivity and to limit the use of test resources, the two NP and OP swabs taken from one participant were admixed in a 3-ml tube containing viral transport medium (VTM, Ismailia free zone, Egypt. Ref: 1/V T01.001.0001) and stored at -80 °C until further analyses.

RNA extraction and detection of SARS-CoV-2 RNA by standard RT-qPCR

RNA extraction was done under BSL-2 on 410 µl of the VTM of both swabs using the QIAamp® Viral RNA mini kit (cat. no. 52906, Qiagen) according to the manufacturer's recommendation. During the extraction, RNase-free DNase set (cat no. 79254, Qiagen) was used to treat the RNA samples to eliminate the possibility of genomic DNA contamination. RNA quality and quantity were determined with the Nanodrop S1000 spectrophotometer (Thermo fisher Scientific). A one-step RT-qPCR was done on extracted RNA using real-time PCR kit (Primerdesign Ltd, Ref: Z-Path-COMD-19-CE, UK) in Stratagene Mx3000P qPCR System (Agilent). This assay targets RNA-dependent RNA polymerase (*RdRP*) gene within SARS-CoV-2. The 20 µl reaction mix formed of 10 µl 2X RT-qPCR Master Mix, 2 µl of COVID-19 Primer & Probe and 8 µl sample extract. A positive control template and negative amplification control with nuclease-free water were included in each run. In the one-step protocol, the reverse transcription (complementary DNA; cDNA; formation) was done by heating the mix at 55 °C for 10 min. and the cDNA was heated at 95 °C for 2 min. (initial denaturation) followed by 45 cycles, each consists of denaturation at 95 °C for 10 sec., annealing and extension at 60 °C for 1 min. The cycle threshold (Ct) values were recorded for each sample. The analyzed samples were considered negative if they have a Ct value [?]40 or no Ct values were reported. For positive samples, SARS-CoV-2 RNA content was categorized according to the Ct values into high ($Ct <$ 29), moderate (Ct = 29-36) and low (Ct = 37-39).

Detection of SARS-CoV-2 antigen by rapid antigen test (RAT)

The RT-qPCR-characterized samples were tested with the RAT Standard Q COVID-19 Ag test (SD Biosensor, Inc., Republic of Korea). The standard Q COVID-19 Ag test is an immunoassay that detects SARS-CoV-2 nucleocapsid protein by lateral flow technique. The test device consists of a membrane with control and test lines that are pre-coated with mouse monoclonal anti-SARS-CoV-2 antibody and anti-chicken IgG antibody, respectively. The mouse monoclonal anti-SARS-CoV-2 antibody conjugated with color particles are used as detectors for SARS-CoV-2 antigen. A colored test line would be visible, with various intensity, if SARS-CoV-2 nucleocapsid protein were present in the specimen. The test procedure was all done under BSL-2 following the manufacturer's instruction. Briefly, the VTM, containing the NP and OP fluids, was first vortexed for 20 seconds and only 100 µl thereof was placed into the sample port of the cassette and incubated at room temperature for 15-30 minutes until reading the results in a blinded approach (i.e. without knowing the RT-qPCR results of the samples).

Ethical statement

Written informed consent was obtained from each enrolled participant and the study was approved by the ethical committee at Faculty of Medicine, Zagazig University (IRB number: 6263, issued on 14.07.2020).

Data analyses and statistics

Descriptive statistics were used to describe the patient demographic, clinical and laboratory characteristics. Continuous variables (e.g. age) were expressed as median ± SD and were compared using Mann Whitney U test. Categorical variables were expressed as numbers and percentages and were compared using *?2* or Fisher's exact test. Correlation and agreement between RAT and RT-qPCR results were calculated using Pearson's correlation (r) and Cohn's kappa ($\varkappa$), respectively (Watson & Petrie, 2010). Measurements of diagnostic performance of RAT (sensitivity, specificity, positive predictive value, negative predictive value,

accuracy and likelihood ratio) for the whole subjects and subject's subgroups were calculated on contingency tables containing the numbers of each outcome. The confidence intervals (CI) were calculated using the Wilson-Brown method (Brown, Cai, & DasGupta, 2001). Participant's categories based on *Ct* values were defined following a previous report (Nalumansi et al., 2020). Receiver operating characteristic curve (ROC) was generated to provide another assessment for the diagnostic power of the RAT. These two analyses were done using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, (www.graphpad.com). To investigate whether combining measurements of blood parameters would by any means enhance the predictive accuracy of the RAT and thus raises its clinical utility, a support vector machine (SVM) model with Monte-Carlo cross validation was applied as described previously (de Araujo et al., 2019) and the performance of top ranked combination (best model) was evaluated for sensitivity, specificity and accuracy using class probability analyses. This analysis was done on data from 68 subjects (the other 15 subjects had no data on any of the laboratory feature). Random forest classification was utilized to reveal the demographic and clinical parameters that are most important in determining individuals with positive and negative results for both RAT and RT-qPCR. In both SVM and random forest models, singular value decomposition method was used to impute the missing values (Stacklies, Redestig, Scholz, Walther, & Selbig, 2007). These analyses were done using Metaboanalyst online server (Pang, Chong, Li, & Xia, 2020).

Results

Demographic, clinical and laboratory characteristics of enrolled subjects

The demographic and baseline clinical characteristics of the participants are summarized in **Tables 1 and S1.** The range of ages was 22-87 (Median = 55.5±18.5). More than half (59%, 49/83) of the subjects were male and 41% (34/83) were females. Data on the sampling time post-symptoms were available in only 70 participants. Samples were collected between 0-7 days post-symptoms in 54.2% (38/70), between 8-16 days post-symptoms in 38.5% (27/70) and >16 day post-symptoms in 5.7% (4/70) of the participants. In only one participant (1.2%), symptoms appeared 5 days after sampling. Clinical data of 47 (56.6%) subjects were only available, the majority of whom (89.3%, 42/47) were symptomatic as they showed at least one of the following symptoms [fever (n = 40), pharyngitis (n = 38), chest pain and dyspnea (n = 37, each), cough (n = 36) and diarrhea (n = 8)] and 5 subjects (10.6%) were asymptomatic. Radiological data were available in 44 participants, 84% (37/44) of whom showed radiological findings of various grades and 7 participants (15.9%) had no radiological findings.

Reverse transcriptase quantitative real-time PCR (RT-qPCR) detection of COVID-19 patients

The average *Ct* values of COVID-19 subjects was 31.1±7.4 (min = 15.7, max = 40.5). The majority of the subjects (83.1%, 69/83) were positive by RT-qPCR and 16.8% (13/83) were negative. As shown in**Table** 2, according to the *Ct* values, 29 (42%), 29(42%) and 11 (15.9%) subjects have strong, moderate and weak RT-qPCR positive results.

Diagnostic performance of StandardQ COVID-19 Ag

As shown in **Table 2** and **Figure 1** , out of the 69 RT-qPCR positive subjects, 54 were positive by the RAT (sensitivity = 78.2%). Of the 14 RT-qPCR negative subjects, nine were negative by the RAT (specificity = 64.2%). The RAT revealed 15 and 5 false negative and false positive subjects, respectively. The accuracy (overall concordance rate) of the RAT tests was 75.9%. Overall, the results of RT-qPCR and the RAT correlated weakly positive (r = 0.3, *P-value* = 0.005) and agreed fairly [Cohen's kappa ($\varkappa$) = 0.3 (CI = 0.16-0.59), SD = 0.1,*P-value* = 0.0006)] in a significant manner. The *Ct*values were significantly different (*P-value* < 0.0001) between RAT positive and negative subjects. The RAT showed the highest sensitivity (96.5%), specificity (44%) and accuracy (63.2%) in COVID-19 patients that have the highest viral nucleic acid content (*Ct* values < 29) compared to the groups with the lower viral load (*Ct* values [?] 29)**.** Other measures for accuracy for subject's subgroups based on *Ct* values are shown in**Table S2.** The ROC analysis yielded an AUC value of 0.7 ±0.08 (Ci = 0.0.5-0.8, *P* -value = 0.02) **(Figure 2 A)** .

Effect of subjects' demographic, clinical criteria and sampling time on the results of the RAT

Tables 3 and **S2** show the diagnostic performance of the RAT in various subject's subgroups. Although RAT results did not differ significantly between male and female subjects, it was slightly more sensitive and more accurate in female (sensitivity = 78.5% and accuracy = 79.4%) than in male (sensitivity = 76.1% and accuracy = 71.4%) participants and its specificity in female was almost twice (83.3%) that in male subjects (42.8%). High sensitivity and accuracy were evident when swabs were collected 0-7 days post-symptoms (n of subjects = 38) followed in order by the case when swabs were taken at 8-16 (n of subjects = 27) and >16 days post-symptoms (n of subjects = 4). There was no significant differences in the RAT results between symptomatic, radiology-positive subjects and asymptomatic, radiology-negative subjects, respectively. However, RAT was more sensitive and accurate in symptomatic subjects relative to the asymptomatic ones. RAT proved positive in 3 (60%) out of the 5 asymptomatic participants, one of these was considered asymptomatic COVID-19 carrier at the time of sampling as evidenced by the high RNA content (Ct = 17.6), showed strong positive RAT (i.e. strong line intensity) and showed symptoms 5-days after the sampling. RAT showed higher sensitivity and accuracy in subjects with no radiological findings than those with radiological findings. No radiological findings was evident in seven participants, 6 of whom were positive by both RAT and RT-qPCR.

Importance of combining laboratory parameters and RAT in diagnosing COVID-19 patients

Considering the intermediate diagnostic power (AUC = 0.7)**(Figure 2 A)** and low diagnostic performance of the RAT**(Tables 2** and **3)** , we investigated whether combining laboratory indices measured in blood and RAT would enhance the true identification of COVID-19 patients by the RAT. First, the SVM model revealed that HB, urea, RAT and S. ferritin were the top-4 parameters most frequently selected during the model building and cross validation followed by the other features **(Figure 2 B).** Various combination between these parameters (i.e. those listed in ascending order in **Figure 2 B**) yielded various accuracies in predicting true COVID-19 cases. The highest prediction accuracy (59.3%) was obtained when combining RAT with both HB and urea (top 2- features in**Figure 2 B).** Coupling RAT with HB, urea, S. ferritin and CRP (top 5- ranked features) yielded slightly lower prediction accuracy (58%) than the one produced by the 3-feature model. Combining all features together revealed low prediction accuracy of 48%. Subsequent evaluation of the "top 3-feature" model by predictive class probability analyses **(Figure 2 D)** revealed a sensitivity of 75.4%, where this model correctly identified 43 as positive subjects out of the 57 true positive ones (as determined by RT-qPCR). The model specificity was 81.8%, where the model correctly identified nine negative subjects out of the 11 true negative ones. The misclassified subjects are labelled in **figure 2 D** .

Important determinants for RT-qPCR and RAT results by random forest classification model

As shown in **Figure S1,** It was found that the sampling time post-symptom onset was the most significant parameter that determines the results in both assays. The order of importance of other parameters differed between the RT-qPCR and the RAT. Dyspnea and radiological findings were the top two parameters for the RT-qPCR results, whereas subject's age and chest pain were the most important features for the RAT.

Discussion

In this study, we aimed to evaluate the diagnostic performance of Standard Q COVID-19 Ag test, a recently commercialized RAT in Egypt and to investigate the factors that could influence test performance. With an overall accuracy of 75.9%, this RAT showed low performance as compared to the RT-qPCR. Combining laboratory parameters with RAT did not enhance RAT predictive accuracy. To the best of our knowledge, this study deems the first one in Egypt that provides detailed evaluation of the diagnostic performance of a RAT against RT-qPCR on Egyptian subjects.

Given that the ideal RAT should have a sensitivity > 95% and a specificity of 100% (Nalumansi et al., 2020), The Standard Q COVID-19 Ag studied here showed less than optimal performance. The observed 78.2% sensitivity means that this RAT test has falsely considered 21.7% (15/69) of the COVID-19 true positive cases as non-infected. Similarly, a specificity of 64.2% means that this RAT has falsely considered 35.7% (5/14) of the COVID-19 negative subjects as positive. The lack of sensitivity of the RAT could lead

to disease dissemination among population if the missed patients are infectious. Actually, an RT-qPCR-positive subject does not necessarily means that he/she is infectious. Our data indicated the majority of the 15 false negative patients by RAT had low viral load, although being symptomatic **(Table S1)** . Since we did not isolate live viruses from those patients, their infectiousness remains unknown and the presence of symptoms does not imply that the person is infectious as shown previously for COVID-19 patients with low viral load (Singanayagam et al., 2020). Symptoms in those groups could be attributed to virus-induced end-organ damage, which was obvious in their radiological findings, rather than presence of replicating virus. On the other side, the lack of specificity could lead to extra cost due to wrong decision of isolation or advising needless therapy. At the time of writing this paper, we are analyzing clinical data from big Egyptian cohort, which might solidify some of these conclusions. The current RAT showed higher sensitivity and lower specificity when it was applied in 262 Ugandan subjects (Nalumansi et al., 2020). This RAT had higher sensitivity (98.3%) and higher specificity (98.7%) than our results when applied on 454 subjects from Thailand (Chaimayo et al., 2020). This indicates that test results might be race/ethnicity- dependent. Our data added to the already known diversity in RATs result. The sensitivity of the current RAT was higher than that obtained by BIOCREDIT COVID-19 Ag test (43.1%) applied on nasal swabs in Egypt (A. M. Abdelrazik et al., 2020). In two independent studies, Ag Respi-Strip (Coris Bioconcept, Gembloux, Nelgium) exhibited specificity of 100% and sensitivity ranged from 30-50% (Lambert-Niclot et al., 2020; Scohy et al., 2020). Fluorescence RAT done on 239 participants in China showed low sensitivity of 68% and maximum specificity (100%) (Diao et al., 2020). The fluorescence immunochromatographic assay produced 93.9% sensitivity and 100% specificity when used on 127 subjects from Chile (Porte et al., 2020). The differences in test performance could be due to variabilities in the participant's clinical features, sample type and processing, PCR protocol and viral load in samples. When evaluating any RAT performance, it is worth noting that misdiagnosis of COVID-19 patients could be due to the difference between the virus strain contained in the sample and the one against which the antibodies coated in the RAT were raised. This is highlighted knowing that Standard Q COVID-19 Ag was designed to detect the original WUHAN-01 strain and that mutation rate is high in the antibody-target SARS-CoV-2 N protein (Rahman et al., 2020). It is therefore recommended to continuously evaluate and update the validity of this and other RAT when applied in different communities that might experience other SARS-CoV-2 strains especially with the beginning of second wave.

Our data showed that the Standard Q COVID-19 Ag was more sensitive and more accurate in patients with high viral load than those with low viral load. Similar results were shown for the same assay in Uganda (Nalumansi et al., 2020) and for other qualitative (Abeer Mohamed Abdelrazik, Shahira Morsy Elshafie, & Hossam M Abdelaziz, 2020; Lambert-Niclot et al., 2020; Porte et al., 2020) and quantitative (Akashi et al., 2019) RATs. In parallel, RAT showed the highest sensitivity and accuracy in the samples collected during the first week post-symptoms and sampling time was the top important feature that determines the results of both RT-qPCR and RAT as revealed by the our random forest classification **(Figure S1)** . These findings support previous reports that showed a 14% decrease in sensitivity of fluorescence immunochromatographic assay when performed on samples collected between 8-12 days post-symptoms relative to earlier samples (Porte et al., 2020). It is already known that SARS-CoV-2 load in upper respiratory tract samples often peak few days after symptom onset (Wolfel et al., 2020; Zou et al., 2020). This complement our results since 17 out of the 28 subjects with RAT positive and strong RT-qPCR were sampled between 0-7 days post-symptoms. Taken together, this suggests a triple relationship between high diagnostic performance of RAT, high viral load in the sample and the early time of sampling post-symptoms and highlights the clinical utility of this RAT in severely affected patients with high viral load and at early stages of COVID-19 infection.

Many studies are there that analyzed the performance of RAT, yet limited studies correlate patient's clinical and radiological features to the RAT performance. Our observation that Standard Q COVID-19 Ag test has higher sensitivity and accuracy in symptomatic than in asymptomatic subjects is in line with previous study done on 3410 Italian patients using the same assay, where the RAT's sensitivity declined from 89.9% in symptomatic subjects to 50% in the asymptomatic ones. As evidenced by one patient in our study,

our analysis suggests that Standard Q COVID-19 Ag test could detect, with very faint line, RT-qPCR negative subjects who are asymptomatic and had no radiological alteration. This highlights the importance of subjecting asymptomatic suspected individuals to the test and that this RAT might be sensitive enough to truly detect asymptomatic carriers, who likely account for significant portion of disease transmission events among humans (Cloutier et al., 2021). Our data indicate the low clinical value of radiological analyses in determining COVID-19 patients relative to RT-qPCR or even the RAT since all participants who had no radiological alteration proved positive by RT-qPCR (4 of them have high Ct value > 25) and five of them were also positive by RAT. Obviously, additional analyses are needed to generalize these observations.

From a diagnosis point of view, it might be useful to combine RAT results with laboratory measurements in patient's blood in pursuit of enhancing RAT performance, particularly when RAT is the only assay available. The machine learning approach employed here enabled us to test this hypothesis. The best-obtained and validated model (formed of RAT plus HB and urea) gave a predictive accuracy of 59.3% and other models with more features, that are COVID-19 related, gave even lower accuracy that this one. This analysis scheme suggests that using laboratory parameter might not afford the desired improvement in diagnostic performance of the RAT studied here, and possibly other RAT. Another point to consider for clinicians is the parameters that should be taken into consideration when performing the test given the differences in the results between RT-qPCR and RAT. The vast difference between determinants of both assays (as shown by random forest classification model) suggests that the differences between the results of both assay have reflected on the parameters to be considered as determinants for the assay.

We acknowledge that this analysis is limited by some factors that should be taken into account in upcoming studies: the small sample size and the unavailability of some participant's data were due to logistic hurdles during the pandemic time. Obviously, additional samples are required for evaluating this RAT. The limited fund at the time of the study and accelerated pressure for obtaining results precluded us from evaluating the influence of sample processing procedures on the RAT accuracy, such an important factor that might alter test results. We do believe that the strength of this study lies in its performance in real-life settings. We were able to link viral load, sampling time, clinical symptoms and laboratory parameters to the assay results and to test, by machine learning approach, the effect of measuring blood parameters on enhancing RAT performance.

Conclusion

Based on the real-world data described here, Standard Q COVID-19 Ag has the disadvantage of low diagnostic performance relative to the RT-qPCR; its sensitivity varies with sampling time and with the amount of viral nucleic acid contained in the sample. This test is best used for subjects with high viral load and when done early after COVID-19 symptom onset. Pending its application in large scale, our data recommend against using this test alone for COVID-19 diagnosis. This RAT, therefore, has no benefit in replacing or reducing the use of RT-qPCR assay for COVID-19 diagnosis at the time of pandemic.

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Conflict of interest

None

Data availability

The data contained in this article are available in the Supplementary materials

Reference

Abdelrazik, A. M., Elshafie, S. M., & Abdelaziz, H. M. (2020). Potential Use of Antigen-Based Rapid Test for SARS-CoV-2 in Respiratory Specimens in Low-Resource Settings in Egypt for Symptomatic Patients

and High-Risk Contacts. *Lab Med* . doi: 10.1093/labmed/lmaa104

Abdelrazik, A. M., Elshafie, S. M., & Abdelaziz, H. M. (2020). Potential Use of Antigen-Based Rapid Test for SARS-CoV-2 in Respiratory Specimens in Low-Resource Settings in Egypt for Symptomatic Patients and High-Risk Contacts. *Lab Med* . doi: 10.1093/labmed/lmaa104

Akashi, Y., Suzuki, H., Ueda, A., Hirose, Y., Hayashi, D., Imai, H., & Ishikawa, H. (2019). Analytical and clinical evaluation of a point-of-care molecular diagnostic system and its influenza A/B assay for rapid molecular detection of the influenza virus. *J Infect Chemother, 25* (8), 578-583. doi: 10.1016/j.jiac.2019.02.022

Anjum, F. R., Anam, S., & Rahman, S. U. (2020). Novel Coronavirus disease 2019 (COVID-19): new challenges and new responsibilities in developing countries. *Hum Vaccin Immunother, 16* (10), 2370-2372. doi: 10.1080/21645515.2020.1766939

Brown, L. D., Cai, T. T., & DasGupta, A. (2001). Interval Estimation for a Binomial Proportion.*Statistical Science, 16* (2), 101-117.

CDC. (2020). Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19.

Chaimayo, C., Kaewnaphan, B., Tanlieng, N., Athipanyasilp, N., Sirijatuphat, R., Chayakulkeeree, M., . . . Horthongkham, N. (2020). Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand. *Virol J, 17* (1), 177. doi: 10.1186/s12985-020-01452-5

Cloutier, L., Merindol, N., Pepin, G., Marcoux-Huard, C., Vasil, P.-A., Houle, C., . . . Danylo, A. (2021). Asymptomatic carriers of COVID-19 in a confined adult community population in Quebec: A cross-sectional study. *American Journal of Infection Control, 49* (1), 120-122. doi: https://doi.org/10.1016/j.ajic.2020.08.015

de Araujo, L. S., Ribeiro-Alves, M., Leal-Calvo, T., Leung, J., Duran, V., Samir, M., . . . Pessler, F. (2019). Reprogramming of Small Noncoding RNA Populations in Peripheral Blood Reveals Host Biomarkers for Latent and Active Mycobacterium tuberculosis Infection. *mBio, 10* (6), e01037-01019. doi: 10.1128/mBio.01037-19

Dean AG, S. K., Soe MM. (2013). Open Source Epidemiologic Statistics for Public Health. Retrieved 2020/11/29, 2020

Diao, B., Wen, K., Chen, J., Liu, Y.-P., Yuan, Z., Han, C., . . . Wu, Y. (2020). Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. *medRxiv* . doi: 10.1101/2020.03.07.20032524

Lambert-Niclot, S., Cuffel, A., Le Pape, S., Vauloup-Fellous, C., Morand-Joubert, L., Roque-Afonso, A. M., . . . Delaugerre, C. (2020). Evaluation of a Rapid Diagnostic Assay for Detection of SARS-CoV-2 Antigen in Nasopharyngeal Swabs. *J Clin Microbiol, 58* (8). doi: 10.1128/JCM.00977-20

Leung, T. Y. M., Chan, A. Y. L., Chan, E. W., Chan, V. K. Y., Chui, C. S. L., Cowling, B. J., . . . Lau, C. S. (2020). Short- and potential long-term adverse health outcomes of COVID-19: a rapid review. *Emerging Microbes & Infections, 9* (1), 2190-2199. doi: 10.1080/22221751.2020.1825914

Nalumansi, A., Lutalo, T., Kayiwa, J., Watera, C., Balinandi, S., Kiconco, J., . . . Kaleebu, P. (2020). Field Evaluation of the Performance of a SARS-CoV-2 Antigen Rapid Diagnostic Test in Uganda using Nasopharyngeal Samples. *Int J Infect Dis* . doi: 10.1016/j.ijid.2020.10.073

Nkengasong, J. N., & Mankoula, W. (2020). Looming threat of COVID-19 infection in Africa: act collectively, and fast. *Lancet, 395* (10227), 841-842. doi: 10.1016/S0140-6736(20)30464-5

Olalekan, A., Iwalokun, B., Akinloye, O. M., Popoola, O., Samuel, T. A., & Akinloye, O. (2020). COVID-19 rapid diagnostic test could contain transmission in low- and middle-income countries. *2020, 9* (1). doi: 10.4102/ajlm.v9i1.1255

Pang, Z., Chong, J., Li, S., & Xia, J. (2020). MetaboAnalystR 3.0: Toward an Optimized Workflow for Global Metabolomics. *Metabolites, 10* (5). doi: 10.3390/metabo10050186

Porte, L., Legarraga, P., Vollrath, V., Aguilera, X., Munita, J. M., Araos, R., . . . Weitzel, T. (2020). Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *Int J Infect Dis, 99* , 328-333. doi: 10.1016/j.ijid.2020.05.098

Rahman, M. S., Islam, M. R., Alam, A., Islam, I., Hoque, M. N., Akter, S., . . . Hossain, M. A. (2020). Evolutionary dynamics of SARS-CoV-2 nucleocapsid protein and its consequences. *J Med Virol* . doi: 10.1002/jmv.26626

Scohy, A., Anantharajah, A., Bodeus, M., Kabamba-Mukadi, B., Verroken, A., & Rodriguez-Villalobos, H. (2020). Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *J Clin Virol, 129* , 104455. doi: 10.1016/j.jcv.2020.104455

Shen, L., Cui, S., Zhang, D., Lin, C., Chen, L., & Wang, Q. (2020). Comparison of four commercial RT-PCR diagnostic kits for COVID-19 in China. *J Clin Lab Anal* , e23605. doi: 10.1002/jcla.23605

Sheridan, C. (2020). Fast, portable tests come online to curb coronavirus pandemic. *Nat Biotechnol, 38* (5), 515-518. doi: 10.1038/d41587-020-00010-2

Singanayagam, A., Patel, M., Charlett, A., Lopez Bernal, J., Saliba, V., Ellis, J., . . . Gopal, R. (2020). Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020.*Euro Surveill, 25* (32). doi: 10.2807/1560-7917.ES.2020.25.32.2001483

Stacklies, W., Redestig, H., Scholz, M., Walther, D., & Selbig, J. (2007). pcaMethods–a bioconductor package providing PCA methods for incomplete data. *Bioinformatics, 23* (9), 1164-1167. doi: 10.1093/bioinformatics/btm069

Tang, Y. W., Schmitz, J. E., Persing, D. H., & Stratton, C. W. (2020). Laboratory Diagnosis of COVID-19: Current Issues and Challenges. *J Clin Microbiol, 58* (6). doi: 10.1128/JCM.00512-20

van Kasteren, P. B., van der Veer, B., van den Brink, S., Wijsman, L., de Jonge, J., van den Brandt, A., . . . Meijer, A. (2020). Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. *J Clin Virol, 128* , 104412. doi: 10.1016/j.jcv.2020.104412

Watson, P. F., & Petrie, A. (2010). Method agreement analysis: A review of correct methodology.*Theriogenology, 73* (9), 1167-1179. doi: https://doi.org/10.1016/j.theriogenology.2010.01.003

Wee, L. E., Fua, T. P., Chua, Y. Y., Ho, A. F. W., Sim, X. Y. J., Conceicao, E. P., . . . Tan, B. H. (2020). Containing COVID-19 in the Emergency Department: The Role of Improved Case Detection and Segregation of Suspect Cases. *Acad Emerg Med, 27* (5), 379-387. doi: 10.1111/acem.13984

WHO. (11 March 2020.). Coronavirus Diseases (COVID-19) global situation Report-51.

WHO. (11 January 2020.). COVID-19 global situation report

Wolfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Muller, M. A., . . . Wendtner, C. (2020). Virological assessment of hospitalized patients with COVID-2019.*Nature, 581* (7809), 465-469. doi: 10.1038/s41586-020-2196-x

Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., . . . Wu, J. (2020). SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med, 382* (12), 1177-1179. doi: 10.1056/NEJMc2001737

Table 1. Frequency of demographic and clinical characteristics of study participants

Features	**Subgroup**	**No. (%)**	**P-value** [?]
Age		Median: 55.5 ±18.4 (SD^+)	0.8

Gender	Male	49 (59)	0.56
	Female	34 (41)	
Radiology	With findings	37 (44.5)	0.56
	No findings	7 (8.4)	
	Not reported	39 (46.9)	
Days post-symptom onset	0-7	38 (45.7)	0.42
	8-16	27 (32.5)	
	>16	4 (4.8)	
	Symptoms Appeared after sampling	1 (1.2)	
Symptomatology*			
	Symptomatic	42 (50.6)	0.14
	Asymptomatic	5 (6.2)	
	Not reported	36 (43.3)	
Fever**	Fever**	40 (95.2)	0.87
Pharyngitis**	Pharyngitis**	38 (90.4)	0.73
Chest pain**	Chest pain**	37 (88)	0.44
Dyspnea**	Dyspnea**	37 (88)	0.44
Cough**	Cough**	36 (85.7)	0.4
Diarrhea**	Diarrhea**	8 (19)	0.44

Table 2. Diagnostic performance of Standard Q COVID-19 Ag test against the RT-qPCR at various *Ct* categories

Features	Subgroup	**Standard Q COVID-19 Ag test**	**RT-qPCR test** Positive (n = 69)	**RT-qPCR test** Negative (n = 14)	Sensitivity % (CI)	Specificity % (CI)	Accuracy %	Likelihood ratio
All	*Ct* values	Positive (n = 59)	54	5	78.2 (0.67-0.86)	64.2 (0.38-0.83)	75.9	2.1
		Negative (n = 24)	15	9				
Subgroups of RT-qPCR positive patients*	Strongly positive (< 29)	Positive	28	28	96.5 (0.83-0.99)	44 (0.31-0.57)	63.2	1.7
		Negative	1	22				
	Moderately positive (29-36)	Positive	21	38	72.4 (0.54-0.85)	29.6 (0.19-0.42)	44.5	1
		Negative	8	16				
	Weakly positive (37-39)	Positive	5	54	45.4 (0.21-0.71)	23.9 (0.15-0.35)	26.8	0.59
		Negative	6	17				

Table 3. Diagnostic performance of Standard Q COVID-19 Ag test in different subgroups of participants

Features	Subgroup		**RT-qPCR test**	**RT-qPCR test**	Sensitivity% (CI*)	Specificity% (CI)	Accuracy%	Likelihood ratio
		Standard Q COVID-19 Ag test	Positive	Negative				
Gender	Male (n = 49)	Positive	32	4	76.1 (0.61-0.86)	42.8 (0.27-0.64)	71.4	1.33
		Negative	10	3				
	Female (n = 34)	Positive	22	1	78.5 (0.60-0.89)	83.3 (0.15-0.74)	79.4	4.71
		Negative	5	5				
Symptomatology (total n = 47)	Symptomatic (n = 42)	Positive	32	0	76.1 (0.63-0.88)	NA	76.1	NA
		Negative	10	0				
	Asymptomatic (n = 5)	Positive	2	1	66.6 (0.61-0.98)	50 (0-0.94)	60	0.66
		Negative	1	1				
Radiology (total n = 44)	With findings (n = 37)	Positive	28	1	75.6 (0.59-0.86)	0 (0-0.94)	73.6	0.75
		Negative	9	0				
	No findings (n = 7)	Positive	6	1	85.7 (0.48-0.95)	0 (0-1)	75	0.75
		Negative	2	0				
Days post symptom onset (total n = 69) *	0-7 (n = 38)	Positive	31	1	83.7 (0.68-0.92)	0 (0-0.94)	81.5	0.83
		Negative	6	0				
	8-16 (n = 27)	Positive	19	0	70 (0.51-0.84)	NA	70.3	NA
		Negative	8	0				
	>16 (n = 4)	Positive	2	1	66.6 (0.11-0.98)	0 (0-0.94)	50	0.66
		Negative	1	0				

** This number includes one participant that showed symptoms 5 days after the sampling.

Figure legend

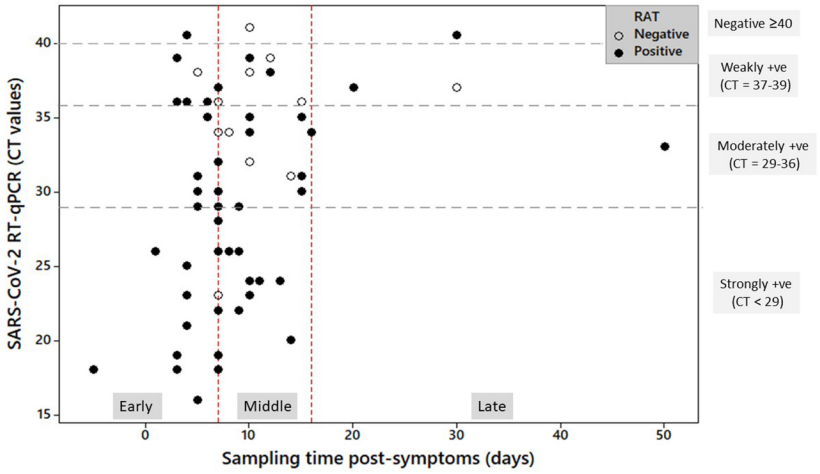
Figure 1. Distribution of participants with positive and negative results of RAT according to sampling time post-symptoms in days (x- axis) and Ct values as determined by RT-qPCR (y -axis). Sampling time post-symptom onset was classified into early (0-7 d), middle (8-16 d) and late (>16 d). RT-qPCR categories are indicated on the right side of the graph.

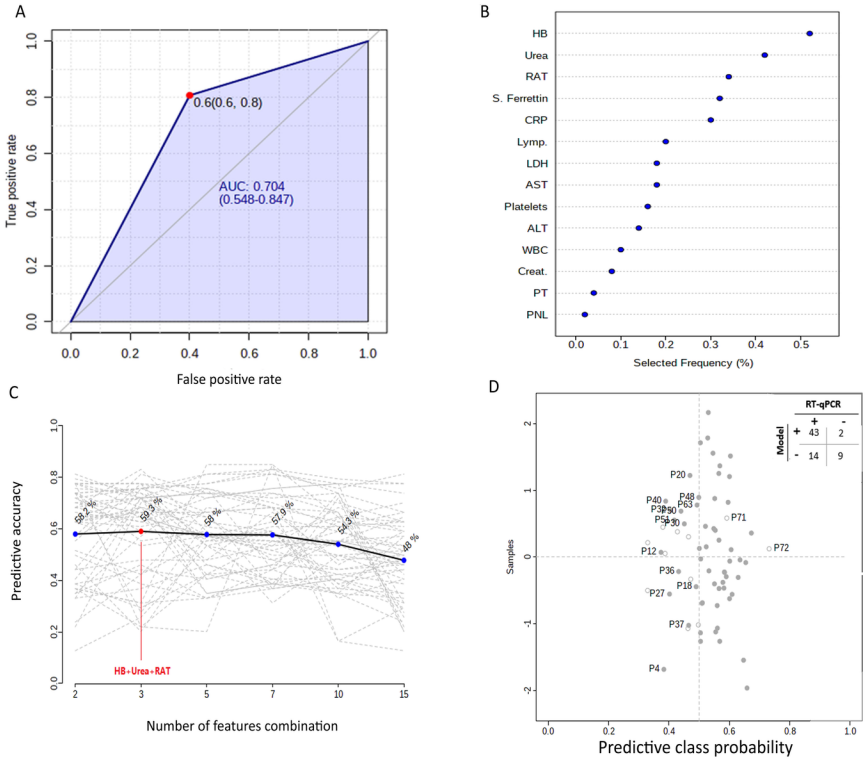
Figure 2. Diagnostic performance of RAT **A** . Receiver operating characteristic curve (ROC) analyses showing the diagnostic performance of the RAT with an area under the curve (AUC) of 0.7**B-C** . Support vector machine model. **B.** Top ranked features based on their frequency of being selected after the cross validation. **C.** Plot showing the predictive accuracy of feature combination in predicting the COVID-19 positive subjects as determined by RT-qPCR. The most accurate classifier gave an accuracy of 59.3% for the top 3-feature as revealed in **B** . **D.** Predicted class probability analyses to evaluate the performance of the 3- features model. Each dot refer to average prediction of one subject after cross-validation. Dark and light colored dots indicates positive and negative cases by RT-qPCR. The misclassified subjects by the 3-feature model are labeled. The classification boundary for COVID-19 positive subjects lies at the center of x -axis (x = 0.5, vertical dotted line). Values > 0.5 indicate probability of COVID-19 positive and closer to 1 indicate high probability. Confusion matrix shows the summary of the model performance.

Figure S1. Random forest classification model showing the ranked importance of subjects' demographic and clinical features in predicting the results of RAT and RT-qPCR assays. The features are ranked in an ascending order according to the mean decrease in accuracy (x -axis) when the respective feature was permuted.

Table S1. Demographic and clinical features of the study participants

Table S2. Diagnostic criteria for RAT for participant's subgroups





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