Comparison of Abbott ID NOW COVID-19 Rapid Molecular Assay to Allplex 2019-nCoV and VIASURE SARS-CoV-2 Detection in Nasal Swabs

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Abstract

BACKGROUND: Readily available, accurate, and rapid diagnostic technologies are of high priority to contain emerging and re-emerging pandemics and to properly allocate personal protective equipment usage and preventing nosocomial spread with subsequent community transmission. Detection of positive 2019-nCoV nucleic acids by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR)-based assays remains the gold standard for COVID-19 diagnostics. However, these assays take an average over 3–5 h to generate results and the PCR tests require certified laboratories, expensive equipment, and trained technicians to operate. Therefore, there is an urgent need for rapid point of care molecular tests that can be readily used in a healthcare setting that generates reliable results within few hours. Those tests should provide reliable results in the setting to facilitate the diagnosis and rapid decision-making.

AIM: The present study aimed to evaluate the diagnostic performance of Abbott ID NOW SARS-CoV-2 compared to two gold standard assays (Allplex 2019-nCoV and VIASURE SARS-CoV-2) and to detect the relation between viral load and the sensitivity of ID NOW SARS-CoV-2 assay.

METHODS: A total of 86 and 42 nasopharyngeal swabs collected from patients attending the Reference Laboratory of Egyptian University Hospitals during the period from January 2022 to May 2022, were tested by our reference methods of RT-PCR for COVID-19 detection; VIASURE kit and Allplex kits, respectively. Corresponding dry nasal swabs were collected from the same patients for ID NOW SARS-CoV-2 ribonucleic acid (RNA) detection assay.

RESULTS: As regards the results of the 86 nasopharyngeal swabs tested by both VIASURE kit and ID NOW, there was a good agreement between both methods (95%) (kappa = 0.924), where the ID NOW method was not able to detect three COVID-19-positive samples (3/86, 5.1%). ID NOW exhibited specificity and sensitivity of 100% and 94.9%, respectively. As for comparing results of ID NOW with Allplex kit, the results of the 42 nasopharyngeal swabs tested by both tests revealed good agreement between both methods (kappa = 0.908). In comparison with Allplex kit, ID NOW exhibited specificity and sensitivity of 90% and 100%, respectively. Regarding the relation between the viral load detected by VIASURE kit and results of the ID NOW test, we found that ID NOW showed a sensitivity of 82.35% in samples with low viral load (CT > 30), while for samples with intermediate (CT: 20–30) and high (CT < 20) viral loads, sensitivity was 100%.

CONCLUSION: ID NOW assay in our study exhibited a high diagnostic performance when evaluated with the gold standard RT-PCR methods. Our study further substantiates the high sensitivity of ID NOW in the presence of intermediate and high viral loads detected by molecular RT-PCR SARS-CoV-2 testing. Its analytical performances, combined with the very short 13 min reactional time and the friendly device-guided handling procedure, constitute an additional advantage of ID NOW COVID-19 for setting up a rapid diagnosis within the clinical laboratories and for timely identification of outbreaks allowing for aggressive contact tracing and containment.

Background

In December 2019, a novel viral pneumonia case due to an unknown cause was reported in Wuhan, China, with evidence of person-to-person transmission [1]. By January 2020, a novel coronavirus was confirmed by the World Health Organization (WHO) to be the cause of pneumonia that affected groups of people in Wuhan and proposed to name the virus as “2019 novel coronavirus (2019-nCoV)” [2]. On February 2020, the Coronavirus Study Group (CSG) of the International Committee for Taxonomy of Viruses recommended 2019-nCoV to be classified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3]. Since the first outbreak of the coronavirus disease 2019 (COVID-19) in China, it has spread
rapidly across the world and has reached the status of global pandemic as announced by the WHO on March 2020 [4]. By December 1, 2020, over 61 million cases and over 1.4 million deaths have been reported throughout the world [5].

This pandemic was confirmed to have reached Egypt by February 2020. Egypt's Health Ministry announced the first case in the country at Cairo International Airport involving a Chinese national on February 14. The first confirmed death was on March 8, 2020 [6]. By January 1, 2021, there have been over 141,000 confirmed cases, 113,000 recoveries, and 7741 deaths, indicating rapid rise in cases reflective of community transmission as well as the scaling up of SARS-CoV-2 viral detection tests in Egypt [7].

Coronavirus disease 2019 (COVID-19) has an extensive spectrum of manifestations ranging from asymptomatic infections and mild disease of upper respiratory tract system to severe viral pneumonia with acute respiratory distress syndrome and eventually death. Asymptomatic and subclinical infections are a major challenge of COVID-19 public health concerns as they can spread the infection and remained undiscovered in the community [8].

2019-nCoV is a single-stranded RNA, enveloped beta-coronavirus with a diameter of 60–140 nm [9]. It has genomic characteristics that are different from the Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV [10]. In addition, 2019-nCoV has 14 open reading frames (Orfs) and encodes for 27 proteins. The 5’ end of the genome has the Orf1ab and orf1a genes and encodes pp1ab and pp1a proteins, respectively. However, the 3’ end of the genome contains four structural proteins, which are spike glycoprotein (S), small envelope protein (E), nucleocapsid protein (N), and membrane glycoprotein (M) [11].

A well-established and comprehensive strategy, including good surveillance system, accurate diagnostics, research, clinical treatment, and development of effective vaccines, is urgently needed to win the battle against COVID-19 [12]. Thereafter, readily available, accurate, reliable, and rapid diagnostic tests and technologies remain a top priority to contain the emerging and re-emerging pandemics and to properly allocate the usage of personal protective equipment (PPE) and prevent rapid nosocomial with subsequent community transmission. Hence, various diagnostic tests for COVID-19 detection are available [13]. These tests are based on different methods and techniques including: (i) Detection of the presence of 2019-nCoV nucleic acids by RT-PCR; (ii) identification of positive 2019-nCoV-specific IgM and IgG antibodies in serum using chemiluminescence immunoassays (CLIA), enzyme immune assays (EIA-methods), and a number of point-of-care rapid lateral flow immunoassay (LFIA) test assay kits [14], and (iii) viral gene sequencing to detect known 2019-nCoV sequences [15].

Detection of 2019-nCoV nucleic acids by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR)-based assays performed on both upper and lower respiratory samples remains the gold standard for COVID-19 diagnosis. However, these real-time PCR test assays have some limitations [14]. They generally take long time with an average of 3–5 h to give positive or negative results and the PCR tests require well-established certified laboratories, expensive automated techniques, and equipment in addition to well-trained technicians to operate [16].

Molecular diagnostic tests using real-time RT-PCR technique target different SARS-CoV-2 genes regions, including the ORF1b or ORF8 regions, the spike (S) protein, the nucleocapsid (N) protein, the envelope (E), and the RNA-dependent RNA polymerase (RdRP) genes [17].

Several commercially available assays for SARS-CoV-2 RT-PCR are available but few of them are assessed. Thereby, more studies that evaluate their performance are essential [18].

There is an urgent need for rapid point-of-care molecular tests that can be readily available to be used in a health-care setting that can generate reliable results within few hours. Those tests should also provide accurate results in the setting to facilitate the diagnosis and rapid decision-making [19].

These tests include; (i) ID NOW COVID-19 assay performed on the Abbott instrument platform (Abbott Diagnostics Scarborough, Inc., Scarborough, ME) which qualitatively detects SARS-CoV-2 viral nucleic acids from upper and lower respiratory tract samples including; nasal, nasopharyngeal, and throat swabs. It utilizes the isothermal nucleic acid amplification technology to amplify a unique region of the RdRp genome, giving positive results within 5–13 min and negative results within 13 min [20]. (ii) VIASURE SARS-CoV-2 RT-qPCR for the detection of ORF1ab and N genes with high sensitivity and specificity but this test lacks RNA quality control probe [21]. (iii) Aliplex™ 2019-nCoV Assay (Seegene) which detects the E, N, and RdRP genes rapidly within 1 h and 50 min after extraction with high sensitivity and specificity [22].

**Objectives**

This study aimed to compare Abbott ID-NOW SARS-CoV-2 and two commercial kits including Aliplex 2019-nCoV and VIASURE SARS-CoV-2 and to determine the relation between viral load as detected by VIASURE SARS-CoV-2 and the sensitivity of ID NOW SARS-CoV-2 assay.

**Study design**

This was a cross-sectional study.
Materials and Methods

Sample collection

A total of 86 and 42 nasopharyngeal swabs collected from patients attending RLEUH (with symptoms suspected of COVID-19 infection) including adults >18 years and non-pregnant women during the period from January 2022 till April 2022, were tested by our reference methods of RT-PCR for COVID-19 detection; VIASURE kit and Allplex kits, respectively. Corresponding dry nasal swabs were collected from the same patients for ID NOW SARS-CoV-2 ribonucleic acid (RNA) detection assay.

Sample processing

SARS-CoV-2 ribonucleic acid (RNA) detection, using ID NOW RT-PCR (Abbott Diagnostics Scarborough Inc., Scarborough, ME)

ID NOW COVID-19 assay performed on the ID NOW instrument is a rapid molecular in vitro diagnostic test utilizing an isothermal nucleic acid amplification technology intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in direct anterior nasal (nasal), nasopharyngeal, or throat swabs from individuals who are suspected of COVID-19 by their healthcare provider within the first 7 days of the onset of symptoms.

The assay amplifies a unique region of the RdRp genome with a manufacture’s claimed LOD of 125 genome equivalents/ml. Positive results are available within 5 to 13 min and negative results within 13 min.

This test is used only under the Food and Drug Administration’s Emergency Use Authorization (EUA) [20].

The swabs provided in the test kit were used for collecting nasal specimen and were transported immediately to the laboratory in a dry sterile screw capped tube at room temperature. The components of the kit were stored at 4–8°C and they were brought to room temperature before use. Processing was performed as per instructions from manufacturer within an hour of sample collection.

The ID NOW COVID-19 kit contains all components required to carry out an assay for SARS-CoV-2 on the ID NOW instrument. It is comprised of a sample receiver, containing elution/lysis buffer, a test base, comprising two sealed reaction tubes, each containing a lyophilized pellet, a transfer cartridge for transfer of the eluted sample to the test base, and the ID NOW instrument.

The test base contains the reagents required for amplification of SARS-CoV-2, as well as an internal control. The templates (similar to primers) designed to target SARS-CoV-2 RNA amplify a region of the RdRp gene. Fluorescently labeled molecular beacons are used to specifically identify each of the amplified RNA targets. The internal control is designed to control for sample inhibition and assay reagent function. The positive and negative control swabs provided with the kit are to be used with a new lot.

The reference test was done on nasopharyngeal collected in Viral Transport Medium (VTM) and transported at 4–8°C for RT-PCR testing.

Real-time reverse-transcription polymerase chain reaction using VIASURE SARS-CoV-2 detection kit

Total nucleic acid was extracted from the samples, using Chemagic Viral NA/gDNA Kit Special. PerkinElmer:960 preparations from 200UI sample. Detection was done by a commercial rRT-PCR kit (VIASURE Real-Time PCR detection kits by Certest Biotec, Spain), following manufactures manual on CFX96 Real-Time System, BioRad.

Real-time reverse-transcription polymerase chain reaction using Allplex 2019-nCoV (Seegene, Seoul, Korea) detection kit

Total nucleic acid was extracted using STARMag 96 × 4, Seoul, Korea, all extracts were subjected to detection using Allplex TM SARS-CoV-2 Master Assay (Seegene, Seoul, Korea), according to manufacture manual on CFX96 Real-Time System, BioRad.

Statistical analysis

Data are analyzed using the statistical package Statistical Package for the Social Sciences software version 25. Frequency (count) and relative frequency (percentage) are used for the categorical data. The sensitivity, specificity, positive predictive value, and negative predictive value are calculated, along with the 95% confidence interval. The measurement agreements between tests are evaluated with Cohen’s kappa (κ) statistics. The comparison between the categorical data is done using the Chi-square (χ²) test. Fisher’s exact test is used instead when the expected frequency is <5. p ≤ 0.05 is considered statistically significant.

Results

In the present study, a total of 86 and 42 nasopharyngeal swabs obtained from attendees to RLEUH were tested by our reference methods for
COVID-19: VIASURE kit and Allplex kits, respectively. Then, all samples were analyzed using the ID NOW test.

**Results of VIASURE kit versus ID NOW**

As regards the results of the 86 nasopharyngeal swabs tested by both VIASURE kit and ID NOW, Table 1 shows that there was a good agreement between both methods (95%) (kappa = 0.924), where the ID NOW method was not able to detect three COVID-19-positive samples (3/86, 5.1%). ID NOW exhibited specificity and sensitivity of 100% and 94.9%, respectively. The diagnostic performance of ID NOW versus VIASURE detection kit is summarized in Table 2.

**Relation between the viral load detected by VIASURE kit and results of the ID NOW test**

We divided the positive samples into three groups according to the viral load obtained by the VIASURE. The first group represented samples with low viral load (CT > 30), the second group included samples with intermediate viral load (CT: 20–30), and the third group contained those with high viral load (CT < 20). We calculated the sensitivities exhibited by ID NOW among each group. We found that ID NOW showed a sensitivity of 82.35% in the first group, while the second and third group, sensitivity was 100%. The rest of the results are summarized in Table 3.

**Results of Allplex kit versus ID NOW**

As for comparing results of ID NOW with Allplex kit, the results of the 42 nasopharyngeal swabs tested by both tests are shown in Table 4. The results revealed good agreement between both methods (kappa = 0.908). In comparison with Allplex kit, ID NOW exhibited specificity and sensitivity of 90% and 100%, respectively. The diagnostic performance of ID NOW versus Allplex detection kit is summarized in Table 5.

**Discussion**

There is an urgent need for rapid point-of-care molecular tests that can be readily available to be used in a health-care setting that can generate reliable results within few hours. Those tests should also provide accurate results in the setting to facilitate the diagnosis and rapid decision-making [19].

These tests include Abbott ID NOW COVID-19 assay which qualitatively detects SARS-CoV-2 viral nucleic acids from nasal, nasopharyngeal, and throat swabs, giving positive results within 5–13 min and negative results within 13 min [20].

Detection of positive 2019-nCoV nucleic acids by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR)-based assays performed on upper and lower respiratory specimens remains the gold standard for COVID-19 diagnostics [14], [23]. However, these real-time PCR test assays have some limitations, they generally take an average over 3–5 h to generate results and the PCR tests require well-established laboratories, expensive techniques and equipment, as well as trained technicians to operate the assay [16].

Thereby, VIASURE SARS-CoV-2 and Allplex 2019-nCoV real-time reverse transcriptase-polymerase chain reaction (rRT-PCR)-based assays are considered reference methods, their analytical performance has been successfully evaluated in many studies including this done by Freire et al. (2021) and Bogiel et al. (2021) [24], [25]. Freire et al. (2021) reported that the VIASURE SARS-CoV-2 detection assay sensitivity was 91.9% and the specificity was 100% as compared to FDA EUA 2019-nCoV CDC kit (IDT, USA) as a gold standard [24]. Bogiel et al. (2021) who confirmed the high sensitivity (98.7%) and specificity (100%) of VIASURE SARS-CoV-2 when two-gene tests are used [25].

Therefore, in the present study, we aimed at the comparison between Abbott ID NOW SARS-CoV-2 RT-PCR (Abbott Diagnostics Scarborough Inc., Scarborough, ME) for ribonucleic acid (RNA) detection...
and real-time reverse-transcription polymerase chain reaction using Allplex 2019-nCoV (Seegene, Seoul, Korea) and VIASURE SARS-CoV-2 detection kits as the gold standards.

**Table 5: Diagnostic performance of ID NOW versus Allplex kit**

<table>
<thead>
<tr>
<th>ID NOW versus Allplex kit</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Overall agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID NOW</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td></td>
</tr>
<tr>
<td>Positive, No (%)</td>
<td>90</td>
<td>97.87</td>
<td>100</td>
<td>99.24</td>
<td>(0.8456–1.00)</td>
</tr>
<tr>
<td>Negative, No (%)</td>
<td>(0.6830–0.9877)</td>
<td>(0.7300–0.9937)</td>
<td>(0.8384–0.9942)</td>
<td></td>
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A total of 86 and 42 paired nasopharyngeal swabs collected from patients presented with signs and symptoms of COVID-19 infection at the Reference laboratory of Egyptian University Hospitals including adults >18 years and non-pregnant women during the period of January 2022 till April 2022.

As regards the results of our study, of the 86 nasopharyngeal swabs tested by both VIASURE kit and ID NOW, there was a good overall agreement (96.5%) between both methods (kappa = 0.924), where the ID NOW method was not able to detect three COVID-19-positive samples (3/86, 5.1%). ID NOW also exhibited specificity and sensitivity of 100% and 94.9%, respectively. Moreover, positive and negative predictive value (PPV and NPV) of ID NOW were calculated compared to VIASURE showing results of 100% and 90%, respectively.

Moreover, as for comparing results of ID-NOW with Allplex kit, the results of the 42 nasopharyngeal swabs revealed good agreement (95.24%) between both methods (kappa = 0.908). In comparison with Allplex kit, ID NOW exhibited specificity and sensitivity of 90% and 100%, respectively. Moreover, positive and negative predictive values (PPV and NPV) of ID NOW were calculated compared to Allplex showing results of 91.67% and 100%, respectively.

Similar to our results, other studies reported good agreement and high specificity of Abbott ID NOW COVID-19 RNA detection assay as compared to other gold standard real-time reverse-transcription polymerase chain reaction assays. Mitchell and George (2020) [26] determined the analytical performance of the ID NOW assay for detecting SARS-CoV-2 in comparison to CDC and New York EUA assays, which served as reference methods. They found that ID NOW assay specificity like our study was 100%, while its sensitivity was less (71.7%), they attributed this decrease in sensitivity to the fact that ID NOW performs well for strong and moderately positive samples but has reduced sensitivity for weakly positive samples [26].

Moreover, Sepulveda et al. (2021) [27] found that the overall agreement between ID NOW and Xpert was 96.3% (Cohen’s kappa = 0.786), the percent positive agreement was 70.0% (95% CI = 60.5–78.4%) and the overall percent negative agreement was 99.5% (95% CI = 98.8–99.9%) [27]. However, unlike our study, this report demonstrates that the overall sensitivity of the ID NOW assay compared to RT-PCR was only 70%, but like our study, they also found that ID NOW has very high sensitivity for the detection of patients with high levels of SARS-CoV-2 RNA load (100% for estimated viral loads ≥ 945 GE/mL) [27].

In a study from New York City during the first surge, the clinical sensitivity of a SARS-CoV-2 RT-PCR assay was estimated to be as low as 58% in repeat tested patients, possibly due to sampling too early [28], whereas in another study, clinical sensitivity was 82–97% [29].

Our present study showed two false-positive results (10%) out of 42 samples detected by ID NOW when compared to Allplex kit, reasons for those positive results might be attributed to laboratory errors including clerical error, cross-contamination from another positive sample, or problems with the reagents used (such as chemicals, enzymes, and dyes). The numbers of samples are too small to verdict these false positive results. Hence, before issuing our statement of judgment, larger number of samples is recommended to be used.

Low diagnostic sensitivity of SARS-CoV-2 assays may be attributed to impaired timing of the sampling relative to the course of the disease, inappropriate sampling technique, type of swabs used, transportation media, and other pre-analytical factors contribute to loss of sensitivity including the dilutional effect of VTM versus dry swabs for the ID NOW [20].

Abbott ID NOW provides fast results but has been criticized for its low sensitivity in samples with low viral loads. Many studies have suggested that the SARS-CoV-2 viral load might predict the potentiality of disease severity and transmission. In the 2020 SARS-CoV epidemic, a higher viral load was related to increase emergency care requirements, intensive care, and overall poor prognosis [30, 31].

Therefore, in the present study, we studied the relation between the viral load detected by VIASURE kit and results of the ID NOW test by dividing the positive samples into three groups according to the viral load obtained by the VIASURE. The first group represented samples with low viral load (CT > 30), the second group included samples with intermediate viral load (CT = 20–30), and the third group contained those with high viral load
(CT < 20). We found that ID NOW showed a sensitivity of 82.35% in the first group with low viral load, while for the second and third group with intermediate and high viral loads, the sensitivity was 100%.

Similarly in a study by Ramachandran et al. (2021) [32], stratified analysis by low and high Ct values demonstrated reduction in sensitivity in patients with low viral loads: 91.7% (81.6% to 97.2%) in low Ct value patients versus 58.3% (27.7% to 84.8%) in high Ct value patients [32]. In addition, several studies have failed to detect SARS-CoV-2 from samples with low levels of viral RNA load [33], [34], [35].

Accordingly, cycle threshold analysis suggests a relationship between viral load and ID NOW sensitivity. Based on our findings, the ID NOW has a good utility as a rapid rule-in test for COVID-19 in samples with high viral load; however, Ct values are affected by pre-analytic, analytic, and post-analytical variables including specimen type, sampling time, collection technique, viral transport and storage conditions, nucleic acid extraction, viral RNA load, primer designing, real-time PCR efficiency, and Ct value determination method [36], therefore, we advise caution with its use as a single rule-out test, especially in samples with lower viral loads.

It should be of notice that there were some limitations in the present study. Although the main limitation is the sample size (a total of 128 nasopharyngeal swabs), our results support that SARS-CoV-2 ribonucleic acid (RNA) detection, using ID NOW RT-PCR (Abbott Diagnostics Scarborough Inc., Scarborough, ME) assay has a good diagnostic performance with high over all agreement and high sensitivity and specificity results when evaluated with the gold standard real-time reverse transcriptase-polymerase chain reaction (rRT-PCR)-based assays.

Another limitation was due to the current pandemic situation that led to an interrupted availability of the tests, so it was not possible to evaluate the same number of samples for all assays. Moreover, another limitation was that there were no clinical data available so we could not verify the clinical performance of ID NOW RT-PCR. In addition, data from our study did not provide information on the potential utility of ID-NOW in testing an asymptomatic patient population, since our study did not focus on that group of patients.

However, our study also had points of strength. First, we managed to evaluate viral load as detected by the Ct values of VIASURE assay kit and the results of ID NOW RT-PCR were low, intermediate and high viral loads results could be obtained. Furthermore, we evaluated the performance of ID NOW RT-PCR in comparison with two well-established real-time reverse transcriptase-polymerase chain reactions (rRT-PCR)-based assays.

Conclusion

Our study demonstrates that the ID NOW assay has a diagnostic performance when evaluated with the gold standard RT-PCR methods. Our study further substantiates the high sensitivity of ID NOW in the presence of intermediate and high viral loads detected by molecular RT-PCR SARS-CoV-2 testing. ID NOW COVID-19 assay presents molecular grade performance characteristics as compared to the more complex and time-consuming RT-PCR assays. Its analytical performances, combined with the very short 13 min detection time and the easy friendly device-guided handling procedure, constitute an additional advantage of ID NOW COVID-19 for establishing rapid diagnosis within the clinical laboratories. Moreover, using rapid and sensitive assays such as the ID NOW to detect individuals with high viral loads may assist in the timely identification of outbreaks allowing for effective and aggressive contact detection, tracing, and containment with proper isolation and prevention of disease spread.

Accordingly, POC testing has substantial advantages over laboratory-based testing when a patient presents with symptoms characteristic of COVID-19. Patients who are SARS-CoV-2 positive can be asked to isolate immediately, and patients who test negative can be reassured or retested using a more sensitive test for low viral loads, depending on the clinical point of view and accurate judgment. Thus, ID NOW provides a speedy and effective alternative to laboratory-based RT-PCR methods under many clinical circumstances.

In light of the obtained results, we recommend larger-scale studies involving patients with a wider range of symptoms to include mild, moderate, and severe COVID-19 infections and asymptomatic cases also and to verify the clinical performance of ID NOW RT-PCR and to study wither the sensitivity and specificity of ID NOW will change in case of asymptomatic patient's samples. Furthermore, we encourage the study of the relationship between viral load and infectivity of the disease as this topic has not been addressed in our study as it might be of great use in determining isolation of the patients and their contacts for better containment of the virus and rapid recovery of patients.

References

PMid:31978945
PMid:32015508


PMid:32007143

PMid:32327757

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