Identification of contagious SARS-CoV-2 infected individuals by Roche’s Rapid Antigen Test

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Abstract

Objectives: Rapid antigen tests (RAT) can provide valuable information on the presence or absence SARS-CoV-2 within 15 min without the need of a laboratory. The analytical and diagnostic characteristics of available RATs has led to the question whether they can safely distinguish between infectious and non-infectious patients in an acute care setting.

Methods: Three nasopharyngeal swabs for the analysis by RAT, reverse transcriptase real time polymerase chain reaction (RT-qPCR), and a cell culture based infection assay were collected from 67 patients that presented to the emergency department of the University Hospital of Graz (Austria). The first swab was used for on-site RAT testing in the emergency department using the Roche SARS-CoV-2 RAT. The second swab was sent to the central laboratory of the hospital for RT-qPCR with two independent methods (Cepheid Xpert® Xpress SARS-CoV-2 assay and Roche Cobas SARS-CoV-2 Test) and repeat RAT testing using the same commercial test. With the third swab a cell culture-based infection assay was performed.

Results: The RATs performed from independent samples showed substantial agreement (Cohen’s-kappa: 0.73, p<0.001). All patients with a positive RAT had positive RT-qPCR with cycle threshold (ct) values <25. Fifteen out of 55 RAT-negative samples were RT-qPCR positive with ct values between 25 and 40. The inoculation of cell cultures with RT-qPCR negative swabs and RT-qPCR positive swabs with ct values >25 did not induce cytopathic effects that were related to SARS-CoV-2. The infection assays from four RAT-negative patients showed cytopathic effects that were induced by other pathogens.

Conclusions: The SARS-CoV-2 RAT from Roche Diagnostics is a valuable tool for managing symptomatic patients. RAT-negative patients may be regarded as non-contagious.

Keywords: antibody assays; COVID-19; IgG; IgM; nucleocapside; SARS-CoV-2; spike.

Introduction

Since two years, coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), creates an enormous burden to healthcare systems worldwide [1, 2]. One of the multiple challenges that hospitals are facing is the rapid separation of SARS-CoV-2 positive patients from all other patients aiming to avoid infections of staff and propagation of the virus amongst SARS-CoV-2 negative patients that are hospitalized for conditions other than COVID-19. In the early phase of the pandemic, identification of the SARS-CoV-2 genome from swabs or respiratory tract fluid samples by reverse transcriptase real time polymerase chain reaction (RT-qPCR) was the only laboratory test available [3]. However, the utility of this test is limited by a rather long turn-around-time (TAT) that ranges between hours and days. In addition, laboratories where confronted with massive numbers of requests that overwhelmed their analytical capacity. Although they adapted their processes and instrumentation rapidly, TAT of traditional RT-qPCR tests cannot be reduced below 3–4 h because of their inherent complexity. Rapid point-of care RT-qPCR tests
have further reduced the time of analysis to a maximum of 60 min. However, the effective TAT can become significantly longer, when samples have to be transported to a laboratory rather than being analysed on-site. Further delays can occur when an excessive number of samples overwhelms the instrument capacity of the laboratory. As emergency departments and other acute care settings often have to take immediate decisions, a TAT of more than 1 h is still unacceptable [4–6].

The introduction of rapid SARS-CoV-2 antigen tests (RAT) has drastically changed the diagnostic landscape as they deliver a result within 15 min without the need for a clinical laboratory at a fraction of the cost of a RT-qPCR test. RATs are lateral flow immunoassays that detect the presence of SARS-CoV-2 spike protein (S) or nucleocapsid (NC) in nasopharyngeal swabs. Previous studies have shown that RATs are less sensitive than RT-qPCR tests, but offer good specificity [7, 8]. A recent meta-analysis performed a pooled analysis of 29 studies with a total of 17,171 COVID-19 suspects that underwent SARS-CoV-2 testing by RT-qPCR and RAT. The pooled specificity of RATs was 99.4% (95% CI: 99.1–99.8). However, the same meta-analysis also showed a sensitivity of only 68.4% (95% CI: 60.8–75.9). The limited sensitivity has raised concerns about the safe use of RATs in clinical practice. Especially hospitals, where the safety of staff and patients is a key priority, cannot afford missing contagious patient as they may cause a cluster that can threaten the entire operation of a hospital with severe consequences for acute patients that need urgent help.

Having to choose between a highly sensitive but slow RT-qPCR test or a less sensitive RAT, has created a diagnostic dilemma for clinicians that is yet to be resolved. Previous studies suggest that RATs have a better sensitivity in symptomatic patients [9]. Moreover, cycle threshold (ct) values are inversely related with sensitivity. These characteristics may open opportunities to identify strategies that allow a safe use of RATs in acute care settings, where rapid test results are pivotal. Considering that RT-qPCR is a highly sensitive method that detects viral RNA, but does not demonstrate the presence of intact virus particles, has led to speculations that weakly positive RT-qPCR results with high ct values are of limited clinical relevance [10]. Even if viable virus is present, high ct values indicate a low viral load limiting the contagiousness of such patients. In fact, several lines of indirect evidence suggest that RATs may align better with virus culture based test results than RT-qPCR [11]. Especially patients with ct values above 30 appear to have such a low viral load that may be insufficient to transmit the virus [12]. In addition, current SARS-CoV-2 RATs have been reported of having optimal performance profiles at time points that overlap with the expression profile of SARS-CoV-2 subgenomic RNAs, which are markers of replicating virus [13].

Existing knowledge of the analytical and diagnostic characteristics of available RATs has led to the question whether they can safely distinguish between infectious and non-infectious patients in an acute care setting [14–16]. To address this question, the present study performed parallel RAT and RT-qPCR testing in unselected patients that presented in the emergency department of a large tertiary teaching hospital. In addition, potential viral replication and accompanied contagiousness were assessed in cell culture-based infection assays.

Materials and methods

Study design

Sixty-seven patients that presented to the emergency department of the University Hospital of Graz (Austria) with symptoms suspicious of SARS-CoV-2 infection were asked to participate in this study. Patients were eligible for participation in this study if they were ≥18 years of age and had one or more self-reported symptoms compatible with COVID-19 leading to hospital admission. After consent to be included, patient signed the respective form. From each patient, three nasopharyngeal swabs were taken for RAT, RT-qPCR, and infection assays. The first swab was used for immediate on-site RAT testing. The second swab was sent to the Clinical Institute of Medical and Laboratory Diagnostics at the Medical (CIMCL) University of Graz (Austria) for RAT and rapid SARS-CoV-2 RT-qPCR testing. After completion of both tests, the remaining material sample was frozen at −80 °C and transferred in batches to the Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine at the Medical University of Graz (Austria) for confirmatory RT-qPCR testing with a different assay. The third swab was used for a cell culture-based infection assay. For this purpose, the respective samples were deep-frozen and transferred in batches to the BSL-3 laboratory of Institute of Pathology at the Medical University of Graz (Austria).

The study was approved by the Ethics Committee of the Medical University of Graz, Austria (33-082 ex 20/21), and performed in accordance with the guidelines of Good Clinical Practice and the Declaration of Helsinki.

Diagnostic SARS-CoV-2 testing

On-site RAT in the emergency department was performed by a trained healthcare professional using the SARS-CoV-2 Rapid Antigen Test (Roche Diagnostics, Rotkreuz, Switzerland). This lateral flow immunoassay detects the SARS-CoV-2 nucleocapsid (N) antigen in respiratory tract specimens. As described in the instructions of use, a sterile swab was introduced into the nasopharyngeal space of the patient and rotated gently 3–4 times. Then, the swab was dipped into plastic cups pre-filled with extraction buffer and rotated at least five times. After removal of the swab, the remaining liquid was transferred into the
sample application well on the lateral flow device. Results were read after 15 min of incubation. Finally, the test card was sent to the CIMCL for confirmatory reading within 30 min and documented photographically.

The second nasopharyngeal swab was collected as described above. Immediately after specimen collection, the swab was transferred into a Sigma Virocult 950S tube (Medicalwire, Corsham, Wiltshire, England) and transferred to the CIMCL. From this sample, the RAT was repeated by the CIMCL. In addition, rapid RT-qPCR testing was performed with the Xpert® Xpress SARS-CoV-2 assay on a GeneXpert instrument (Cepheid, Sunnyvale, CA). This cartridge-based, fully automated RT-qPCR test performs 45 qPCR cycles and detects two target genes, N2 and E. The result is usually available within 45 min. The laboratory performs daily quality control measurements and participates successfully in an external quality assurance program (QCMD, Glasgow, Scotland; https://www.qcmd.org/).

Tubes with left-over transport medium were frozen at –80 °C and transferred to the Molecular Diagnostics Laboratory at the Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine for confirmatory RT-qPCR testing with the cobas SARS-CoV-2 Test on a cobas 6800 system (Roche Molecular Diagnostics, Pleasanton, CA). Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1α/b nonstructural region that is unique to SARS-CoV-2. In addition, a conserved region in the structural protein envelope E gene is chosen for pan-Sarbecovirus detection. Furthermore, an RNA internal control is introduced into each specimen during sample processing to monitor the entire sample preparation and qPCR amplification process. The laboratory performs daily quality control measurements and participates successfully in an external quality assurance program (QCMD, Glasgow, Scotland; https://www.qcmd.org/).

Infection assay

African green monkey kidney epithelial cells (VeroE6), purchased from Biomedica (VC-FT6), were grown in MEM medium containing Earle’s Salts and L-Glutamine (Thermo Fisher Scientific, Waltham, USA), supplemented with 5% fetal calf serum (FCS) (Thermo Fisher Scientific, Waltham, USA) and 1% Penicillin-Streptomycin (PenStrep) (Thermo Fisher Scientific, Waltham, USA) at 37 °C and 5% CO₂. 24 h prior to infection VeroE6 cells were seeded in 48-well cell culture plates (30,000 cells per well) in MEM supplemented with 2% FCS and 1% PenStrep to reach 80% confluence on the day of infection.

Nasopharyngeal swabs were thawed and filtrated through a 0.2 µm syringe filter (Thermo Fisher Scientific, Waltham, USA). Subsequently, 200 µL eluate were incubated with cells for 1 h at 37 °C, 5% CO₂. Afterwards, cells were washed once with medium and incubated in fresh MEM supplemented with 2% FCS and 1% PenStrep for 72 h. Cytopathic effects (CPE) were documented after 72 h post infection via bright field microscopy and documented by taking digital images indicating viral replication.

In order to ascertain the presence or absence of virus genome, the swabs that induced CPE in VeroE6 cells after 72 h were RT-qPCR tested at the Institute of Pathology. Additionally, viral RNA was isolated from 140 µL of cell culture supernatant from wells showing CPE, to confirm SARS-CoV-2 replication by RT-qPCR. Viral RNA was isolated from the supernatant with QiaAmp Viral RNA Mini kit (QIAGEN GmbH Hilden, Germany) according to the manufacturer’s protocol. RNA was eluted in 60 µL water and stored at –80 °C until usage. SARS-CoV-2 viral RNA was analyzed by RT-qPCR.

Statistical analysis

The concordance of the results obtained by the on-site RAT and the laboratory RAT was examined using Cohen’s kappa coefficient. For the correlation of the SARS-CoV-2 specific ct values obtained by the Xpert® Xpress SARS-CoV-2 assay (E gene) and the cobas SARS-CoV-2 Test (ORF1α/b gene), Spearman’s rank correlation was used. Furthermore, infection assay data were analyzed with standard descriptive statistics. For computerized statistical analysis, SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) was employed.

Results

Patient characteristics

Thirty-three females and 34 males participated in this study. Mean age (standard deviation) was 56 [19] years. Based on the results from the Xpert® Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, CA), 27 patients were RT-qPCR positive whereas the remaining 40 patients were negative. In all patients, results of the target genes E and the N2 were concordant. Ct values ranged from 12 to 38 for the E target and from 14 to 40 for the N2 target.

RAT and RT-qPCR testing

RATs performed from independent swab samples at the emergency department and the CIMCL revealed 16 and 12 positive results, respectively (Table 1). In total, 61 out of 67 patients (91%) had concordant RAT results. Six results were found to be discrepant, five of them showed a positive result with the on-site RAT but a negative result with

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<th>Negative</th>
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Table 1: Comparison of RAT results from 67 replicate swab samples of the same patients performed in the emergency department (on-site) and in the central laboratory. Both RATs were performed from independent samples that were collected from an experienced operator at the same occasion.
laboratory RAT and one of them vice versa (Table 1). Cohen’s kappa was 0.73 (p<0.001), indicating substantial agreement.

All patients with a positive laboratory RAT performed at the CIMCL had positive RT-qPCR results with the Xpert® Xpress SARS-CoV-2 assay and the cobas SARS-CoV-2 test. All three tests were performed from the same sample. RAT positive samples had consistently ct values <25 for all targets (Figure 1). Fifteen out of 55 RAT negative samples were RT-qPCR positive with ct values between 25 and 40. When comparing the qualitative results from the two RT-qPCR assays, all patients were classified identically. In addition, the ct values from both assays showed excellent correlation with a Spearman correlation coefficients of 0.996 (<0.001) (Figure 2).

Figure 1: Illustration of the infection assay.
(A) Representative image of cytopathic effects (CPE) on VeroE6 cells caused by SARS-CoV-2 replication after 72 h. (B) Representative image of not infected VeroE6 cells.

Figure 2: Comparison of results gained by different RT-qPCR assays with the used rapid antigen test (RAT) and with the the infection assay. (A) Distribution of RAT positive and negative results in relation to the ct values of SARS-CoV-2 specific target genes obtained by independent RT-qPCR methods. For each patient, all three test were performed from the same nasopharyngeal swab sample. (B) Distribution of positive and negative infection assay results in relation to the ct values of two SARS-CoV-2 specific target genes obtained by independent RT-qPCR methods from nasopharyngeal swabs. Ct values for the E gene target were obtained with the Xpert® Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, CA), ct values for the ORF1a/b gene target with the cobas SARS-CoV-2 Test (Roche Molecular Diagnostics).
Infection assays

SARS-CoV-2 RNA was detected in 19 swabs, out of which seven had a ct value below 25. Seventy-two hours after the inoculation of cell cultures, cell morphology was assessed microscopically for CPE. Successful replication of virus manifests in detachment and rounding of cells. In contrast, not infected cells form an intact monolayer (Figure 1).

In 12 cases, cells infected with those samples showed CPE after 72 h (Figure 2). To ensure that CPE resulted from SARS-CoV-2 replication, viral RNA from the supernatants was measured by RT-qPCR. One swab had a ct value of 26.4, but showed no CPE after 72 h although viral replication was detected in the supernatant. Furthermore, four swabs negative for SARS-CoV-2 showed CPE, but no SARS-CoV-2 RNA was detected in the cell culture supernatant (Figure 2, grey dots). These individuals also had a negative RAT, supporting an infection with a pathogen other than SARS-CoV-2. All other SARS-CoV-2 RNA negative and positive swabs with ct value above 25 did not show CPE in cell culture indicating absence of infection and viral replication.

Discussion

The present results demonstrate that the SARS-CoV-2 RAT from Roche Diagnostics is a valuable tool for managing symptomatic patients in an acute care setting where rapid decision making is critical. Although a negative RAT does not rule out a SARS-CoV-2 infection, such patients may be regarded as non-contagious. Nevertheless, RATs cannot replace RT-qPCR testing as they are less sensitive. Another key finding is the limited concordance of RATs from independent swabs that have been collected at the same time.

Our results support previous studies showing that RATs are less sensitive than RT-qPCR tests, but offer good specificity [7, 8, 17, 18]. Amongst the 15 RAT negative swab samples that were tested RT-qPCR positive, ct values ranged between 25 and 40. However, none of the RAT-negative swabs induced a CPE suggesting a low viral load or the presence of non-viable viral material in these samples. In contrast, RAT positive swabs consistently had ct values <25 suggesting that RATs perform better on specimens with higher viral loads. However, this study did not aim to establish a ct-threshold that is associated to RAT positivity as this would require a considerably larger sample size. In addition, the sensitivity of different RATs and qPCR tests varies, so that any threshold would be test-specific. An increased sensitivity of RATs in specimens with lower ct value is consistent with findings from other RAT studies [19, 20]. Most RAT positive samples in the present study were also able to infect cultured cells as shown by CPE and positive RT-qPCR results from the supernatant. Furthermore, swab samples with lower ct values were more likely to produce a positive result in the infection assay. However, this inverse correlation between ct values and the likelihood of infecting cultured cells is imperfect, which is primarily due to the qualitative nature of SARS-CoV-2 RT-qPCR testing. Using ct values from such a qualitative test as a surrogate of the patient’s viral load is critical. Ct values are strongly influenced by pre-analytical factors, such as swab collection and sample storage conditions. In 10% of the patients, consecutive swab collected at the same time also delivered discordant RAT results. Besides pre-analytical aspects, also analytical factors, such as assay variability and RNA extraction efficiency, contribute to variable ct values [21–23].

Although infection assays are less standardized than most other diagnostic tests, the robustness of our results is ensured by RT-qPCR of the original sample, microscopy of the cell cultures and additional RT-qPCR tests from the supernatant after 72 h. The present data strongly supports the hypothesis that weak-positive RT-qPCR results with high CT values are of limited clinical relevance as they indicate a low viral load so that such patients are not contagious [10]. In line with a previous study from Pickering et al. we were able to show that Roche’s RATs aligns better with cell culture based infection assays than RT-qPCR [11]. La Scola and colleagues suggested that the viral load of patients with ct values above 30 might be too low for successful virus transmission [12]. In addition, Wölfel et al. have shown that infected patients may continue to shed viral RNA although symptoms have resolved for several weeks. Despite persistently positive RT-qPCR results, SARS-CoV-2 could not be cultured from swabs of such patients suggesting that there is little or no viable virus [12, 24]. In contrast, Strömer et al. [25] have shown that RAT positive swabs are associated with ct values <30 and positive cell culture infection assays. In the present study, none of the RT-qPCR positive swab samples with a ct value above 25 induced CPE caused by viral replication in VeroE6 cells. Therefore, a ct value >25 obtained from a properly collected nasopharyngeal swab sample seems to rule out a relevant risk of virus transmission. However, this cut-off is only valid for the RT-qPCR tests used in this study. Furthermore, the commutability of our infection assay results is limited by the cells used for culture. While VeroE6 cells are the preferred cell type for SARS-CoV-2 in-vitro culture, they differ from epithelial cells of the human respiratory tract, which may result in a
somewhat different susceptibility to SARS-CoV-2 infection. Also, our infection assay is characterized by rather static incubation conditions, whereas the human nasopharyngeal mucosa is permanently drained and ventilated. Some additional uncertainty regarding the precise cut-off is due to the relatively small sample size. Nevertheless, public authorities have recognized the limited risk of virus transmission in individuals with a low viral load by introducing a ct threshold of ≥30 for lifting quarantine.

The present results also demonstrate that the collection of swab samples is critical and represents a major source of variability. In 10% of our patients, the RAT result could not be reproduced when the tests were performed from independent samples collected immediately one after the other by the same operator. It should be considered that the setting of our study was optimized in order to reduce variability. Therefore, in clinical practice, the impact of sample collection and other preanalytical factors may be much greater. In fact, previous studies that have explored the reproducibility of SARS-CoV-2 RT-qPCR results from independent swab samples have found that the probability of a qPCR positive result depends on the day of sampling, the site of sampling, the quality of sample taking, the transport medium and the time from sample collection to analysis [26–32]. Although such a limited reproducibility is unsatisfactory, it is not surprising, as the salivary sample matrix of a nasopharyngeal swab is much less homogenous than a blood or urine sample. Dense mucus particles with a high viral load may be found in some areas, but not in others. Moreover, previous food consumption, tooth cleaning or drinking may alter salivary composition and impact RAT and RT-qPCR results. These points to a frequent problem in daily practice, where discrepant results from independent swab samples motivate patients and healthcare professionals to question the accuracy of RAT and RT-qPCR tests. Here we were also able to demonstrate that the RT-qPCR results from two laboratories that work in accordance with ISO 15189 standards and use validated methods with CE-IVD label can be expected to provide comparable results across the entire analytical range when the same sample is used.

It is important to recognize that the results presented here apply exclusively to the RAT from Roche Diagnostics. Other SARS-CoV-2 lateral flow antigen tests may offer different diagnostic performances [33]. For example, a Korean study reported a sensitivity of 98.3% and a specificity of 98.7% for the Standard Q COVID-19 Ag test (SD Biosensor®, Chuncheonbuk-do, Republic of Korea) [34] whereas the COVID-19 Ag Respi-Stripa (Coris BioConcept, Gembloux, Belgium) offers a sensitivity of only 57.6% and a specificity of 100% [35]. In addition to qualitative lateral flow devices, different quantitative immunoassay formats have also been developed. A comparison of four automated quantitative SARS-CoV-2 antigen assays has shown specificities ≥97% and sensitivity between 17 and 52% [36]. However, all these tests require proper laboratory equipment and have a longer TAT, which makes them less suitable for the acute care setting. Moreover, sensitivities and specificities have exclusively been calculated on the basis of RT-qPCR reference tests. None of these studies performed infection assays to determine the presence of viable virus and contagiousness.

Our study has several limitations. The number of samples is relatively small and participants were exclusively symptomatic patients that presented to the emergency department. However, the study design was rather involved and infection assays cannot be performed on large sample sets. Even if the sample size is rather small, the results clearly show that the RAT has a very high negative predictive value for contagiousness on the basis of a gold standard infection assay. Furthermore, RT-qPCR assays are intended for the qualitative detection of SARS-CoV-2 RNA and linearity across multiple virus concentrations has not been established. Furthermore, infection assays were performed from a different swab sample than the RAT and RT-qPCR tests. Although all nasopharyngeal swabs were collected by experienced operators at the same occasion, viral load may differ between these samples. To address this issue, swab samples that induced CPE in the infection assay were re-tested by RT-qPCR at the Institute of Pathology. Finally, during the study period, the alpha variant was the predominant variant in the study population.

In conclusion, the RAT used in this study is a valuable tool for the management of symptomatic patients in a critical care setting. Despite lower sensitivity than RT-qPCR, the RAT offered similar specificity and identified all culture positive specimens. In the present set of samples, a negative RAT results excluded a clinically relevant risk of virus transmission and was reliably associated with ct values above 25. Therefore, Roche’s SARS-CoV-2 RAT can safely be used for the triage of symptomatic patients and guide immediate clinical decisions until the RT-qPCR result is available. In addition, this test appears to be a safe and cost-effective tool for the screening of healthcare workers independent of vaccination or recovery state. As RATs may react differently with individual virus variants, such as Delta or Omicron, the diagnostic performance of needs to be checked, when new variants arise. Finally, it has to be emphasized that SARS-CoV-2 RAT and RT-qPCR tests may deliver discordant
results when performed from different swab samples, which highlights the critical importance of correct sample collection.

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Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors’ Institutional Review Board (33-082 ex 20/21).

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