



Evaluation of a Rapid Antigen Test To Detect SARS-CoV-2 Infection and Identify Potentially Infectious Individuals

Michael Korenkov,^a Nareshkumar Poopalasingam,^a Matthias Madler,^a Kanika Vanshylla,^a Ralf Eggeling,^b Maïke Wirtz,^a Irina Fish,^a Felix Dewald,^a Lutz Gieselmann,^{a,c} Clara Lehmann,^{c,d,e} Gerd Fätkenheuer,^{c,d} Henning Gruell,^a Nico Pfeifer,^{b,f,g} Eva Heger,^a Florian Klein^{a,c,e}

^aInstitute of Virology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

^bMethods in Medical Informatics, Department of Computer Science, University of Tübingen, Tübingen, Germany

^cGerman Center for Infection Research, Partner Site Bonn-Cologne, Cologne, Germany

^dDepartment I of Internal Medicine, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

^eCenter for Molecular Medicine Cologne, University of Cologne, Cologne, Germany

^fFaculty of Medicine, University of Tübingen, Tübingen, Germany

^gGerman Center for Infection Research, Partner Site Tübingen, Tübingen, Germany

Michael Korenkov and Nareshkumar Poopalasingam contributed equally to this article. Author order was determined alphabetically.

ABSTRACT The identification and isolation of highly infectious SARS-CoV-2-infected individuals is an important public health strategy. Rapid antigen detection tests (RADT) are promising tools for large-scale screenings due to timely results and feasibility for on-site testing. Nonetheless, the diagnostic performance of RADT in detecting infectious individuals is not yet fully determined. In this study, RT-qPCR and virus culture of RT-qPCR-positive samples were used to evaluate and compare the performance of the Standard Q COVID-19 Ag test in detecting SARS-CoV-2-infected and possibly infectious individuals. To this end, two combined oro- and nasopharyngeal swabs were collected at a routine SARS-CoV-2 diagnostic center. A total of 2,028 samples were tested, and 118 virus cultures were inoculated. SARS-CoV-2 infection was detected in 210 samples by RT-qPCR, representing a positive rate of 10.36%. The Standard Q COVID-19 Ag test yielded a positive result in 92 (4.54%) samples resulting in an overall sensitivity and specificity of 42.86 and 99.89%, respectively. For adjusted C_T values of <20 ($n=14$), <25 ($n=57$), and <30 ($n=88$), the RADT reached sensitivities of 100, 98.25, and 88.64%, respectively. All 29 culture-positive samples were detected by the RADT. Although the overall sensitivity was low, the Standard Q COVID-19 Ag test reliably detected patients with high RNA loads. In addition, negative RADT results fully corresponded with the lack of viral cultivability in Vero E6 cells. These results indicate that RADT can be a valuable tool for the detection of individuals with high RNA loads that are likely to transmit SARS-CoV-2.

KEYWORDS SARS-CoV-2, antigen test, RT-qPCR, virus culture, infectiousness

Timely diagnosis of SARS-CoV-2 infection with subsequent contact tracing and rapid isolation is a critical public health strategy to contain the COVID-19 pandemic (1–3). The current gold standard of SARS-CoV-2 testing is based on real-time reverse transcription-PCR (RT-qPCR) (4). However, due to higher costs, longer turnaround times, and higher sensitivities, RT-qPCR is less suited for rapid point-of-care identification of infectious individuals, since it is also able to detect nonreplicating viruses (5–7). Therefore, there is a need for an inexpensive alternative testing method to directly detect infectious individuals that can be deployed widely without the use of specialized equipment (8, 9).

One promising approach is the use of lateral flow immunochromatographic assays, commonly referred to as rapid antigen detection tests (RADT), designed to detect viral

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Address correspondence to Florian Klein, florian.klein@uk-koeln.de.

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antigens. RADT are of particular use for community-based screenings due to low turnaround times and feasibility for on-site testing (10, 11). Numerous tests have already been approved for clinical use; however, performance studies under real-life conditions evaluating the quality of RADT using not only RT-qPCR but also virus cultures as a reference for infectivity are limited (12, 13). In published studies, reported test characteristics, such as sensitivity, varied greatly depending on cohort composition (24.3 to 89%) (14–26).

As high RNA loads are typically associated with a higher probability of infectiousness, the diagnostic performance of RADT in the context of infectivity models is yet to be determined (27–29). Therefore, there is a need for large-scale field studies with a focus on virus cultivability to be able to appropriately interpret RADT results. Here, we examined the performance of RADT in detecting both infected and infectious individuals in a routine diagnostic center using RT-qPCR and virus culturing as reference methods.

MATERIALS AND METHODS

Study design. Between 26 October 2020 and 11 January 2021, all individuals were tested for SARS-CoV-2 infection by RT-qPCR at the University Hospital Cologne, Department I of Internal Medicine, Cologne, Germany. SARS-CoV-2 testing was available for individuals from the general population with COVID-19 symptoms, suspected disease, or SARS-CoV-2 exposure as part of routine diagnostics, as well as for hospital staff members as part of screening measures. For quality control, an RADT was simultaneously performed after verbal consent. Two swab specimens, each collected from oro- and nasopharynges, were obtained by the same trained personnel. The first swab was transferred into virus transport and preservation medium (Biocomma, Shenzhen, China) or BD ESwab (Becton Dickinson, Franklin Lakes, NJ, USA) for RT-qPCR testing, and the second swab was used for on-site RADT testing. All samples for RT-qPCR were routinely processed within 12 h after collection. Upon approval by the Institutional Review Board of the University of Cologne, results were retrospectively analyzed, including clinical data retrieved from a symptoms diary webtool that all individuals registering for a SARS-CoV-2 test are asked to complete. Due to several implausible self-reported entries, only webtool entries not older than a week from the time of testing were included into symptom analysis. Patients were categorized as symptomatic if the reported symptom duration at the time of testing was ≤ 14 days and one of the following symptoms was found: fever, cough, rhinorrhea, nausea, diarrhea, shortness of breath, and/or a new olfactory or taste disorder.

Rapid antigen detection test. The Standard Q COVID-19 Ag test (SD Biosensor, Inc., Suwon-si, Republic of Korea/Hoffmann La Roche AG, Basel, Switzerland) is a rapid chromatographic immunoassay for the qualitative detection of SARS-CoV-2 nucleocapsid protein. The test was performed according to the manufacturer's instructions using the enclosed dry swab for sample collection with one modification. Instead of a nasopharyngeal swab only, a combined oro- and nasopharyngeal swab was performed to ensure comparability with RT-qPCR sample collection. The operating instructions, in brief, were as follows. The collected swab was mixed in the provided tube of collection medium, and three drops were applied through a nozzle cap onto the test strip. Results were read out visually after 15 to 20 min by medically trained and instructed personnel. In accordance with the manufacturer's reference guide, faint lines were considered positive if the control line was also present.

Real-time reverse transcription-PCR. RT-qPCR was performed using different SARS-CoV-2 RNA detection protocols that were normalized according to the same standard. The following SARS-CoV-2 detection protocols were utilized. (i) Nucleic acid extraction was done for 935 (46.10%) samples using the MagNA Pure 96 system DNA and viral NA large volume kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. After RNA purification from 500 μ l of viral transport medium and elution into 100 μ l of elution buffer, multiplex RT-qPCR was performed using an in-house N-gene primer set on a LightCycler 480 II system (Roche Diagnostics). The LightMix SarbecoV E-gene plus equine arteritis virus (EAV) control kit (TIB Molbiol, Berlin, Germany) was included in every RT-qPCR run. (ii) A cobas SARS-CoV-2 test kit running on the cobas 6800 (Roche Diagnostics) was used for 407 (20.07%) samples targeting the viral E-gene and ORF1a/b regions according to the manufacturer's instructions. (iii) A SARS-CoV-2 AMP kit running on an Alinity m (Abbott, Chicago, IL, USA) was used for 63 (3.11%) specimens targeting the viral N and RdRp genes according to the manufacturer's instructions. (iv) A multiplex RT-qPCR with a LightMix SarbecoV E-gene (TIB Microbiol), an in-house N-gene primer/probe set, and a human β -globin primer set as an internal control, running on the Panther Fusion (Hologic, Wiesbaden, Germany), was used for 36 (1.78%) samples. (v) For 579 (28.55%) specimens, samples from up to 10 asymptomatic employees were pooled and tested for SARS-CoV-2 using the methods described in steps i, ii, and iii. Positive pools were resolved, and samples were tested separately as described previously in steps i to iv. (vi) An Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA) test kit was used for 8 (0.39%) samples according to the manufacturer's instructions.

To enable comparison of cycle threshold (C_T) values obtained by the different RT-qPCR methods, C_T values were translated into copies/ml and then converted to a cobas 6800 adjusted C_T value. For this purpose, seven serial dilutions from a high titer SARS-CoV-2 sample were tested by all five RT-qPCR methods described above. Standard curves for each amplification method were generated by a regression model. For the following conversion of device-specific C_T values into copies/ml, two SARS-CoV-2 samples with a quantified RNA load from INSTAND (Society for the Promotion of Quality Assurance in Medical Laboratories, e.V., Düsseldorf, Germany; in cooperation with the Robert Koch-Institute and the

Institute of Virology, Charité, Berlin) were tested on every device and subsequently used for C_T -based absolute RNA quantification.

SARS-CoV-2 culture. Vero E6 cells (ATCC CRL-1586) were cultured in complete medium (CM) consisting of Dulbecco modified Eagle medium (Thermo Fisher Scientific/Gibco, Waltham, MA, USA) supplemented with 10% fetal calf serum (FCS; GE Healthcare, Chicago, IL, USA), 200 U/ml penicillin, 200 μ g/ml streptomycin, 0.25 μ g/ml amphotericin B, 2 mM L-glutamine, and 1 mM sodium pyruvate (all from Thermo Fisher Scientific/Gibco) at 37°C in an incubator with 5% CO₂. One day prior to infection, 3×10^5 cells were seeded onto T25 flasks in 5 ml of CM. Retained swab samples were stored for up to 8 h at 4°C in transport medium until RT-qPCR results became available. For virus cultures, 250 μ l of SARS-CoV-2 RT-qPCR-positive samples were diluted 1:5 in infection medium (IM) consisting of complete medium with FCS reduced to 2%. After removal of the cell culture supernatant, the diluted samples were added to Vero E6 cells and incubated for 1 h at 37°C and 5% CO₂. After washing with 5 ml of phosphate-buffered saline (Thermo Fisher Scientific/Gibco), 5 ml of IM was added, and the cells were cultured for 7 days at 37°C and 5% CO₂. The cells were checked for the presence of cytopathic effects (CPE) on days 4 and 7. On both days, 1 ml of culture supernatant was harvested and stored at -80°C for conformation of positive cultures through RT-qPCR as described above. An increase in RNA load and unchanged RNA loads of $>9 \log_{10}$ copies/ml between day 4 and day 7 were considered successful virus cultivation. All virus isolation experiments were performed under BSL-3 conditions.

Dilution series. To validate our SARS-CoV-2 culturing approach, four dilution series with sample CT values ranging from 15.5 to 17.6 were analyzed for virus recovery and RADT result. Two hundred fifty microliters of retained samples was thawed and serially diluted in IM (1:5). Virus cultivation and RT-qPCR were performed as described above. RADT were performed with 250 μ l of original samples and each dilution as described above. Virus cultivation and RADT testing showed that all SARS-CoV-2-positive cultures were previously detected by RADT (see Fig. S1 in the supplemental material).

Statistical analysis. Sensitivity (positive percent agreement) and specificity (negative percent agreement), as well as positive and negative predictive values, were calculated using RT-qPCR as a reference. Culture and RADT results were evaluated by a contingency table, and a *P* value was calculated with Fisher exact test. Confidence intervals (CI) were calculated using the Wilson/Brown method. Mann-Whitney U-test (MWU) was used to compare differences between medians. *P* values of <0.05 were considered significant. Probit regression was carried out using a generalized linear model (R-function glm) with the probit link function. To correct for repeated measurements from the same individual on different days, the basic analyses were modified as follows. (i) Confusion matrices for the calculation of all performance measures were calculated in a weighted manner so that the contribution of a single test is inversely proportional to the number of tests taken from the corresponding individual. (ii) To compare two populations of data points, a generalized estimating equation (R-function gee::gee) was fitted using each patient as its own cluster and an exchangeable correlation structure. (iii) Probit regression was carried out by fitting a generalized linear mixed model with probit link function and random intercepts for each individual (R-function GLMMadaptive::mixed_model). Marginal means and confidence bands were calculated with R-function ggeffects::ggpredict. Data analysis was performed using Microsoft Excel 16.44 (Microsoft), Prism 9 (GraphPad Software, Inc.), Python 3.8.3, and R 3.6.3.

Ethics. The Institutional Review Board of the University of Cologne acknowledged and approved the study under application 21-1039.

Data availability. All data used in the analysis will be made available upon request.

RESULTS

RT-qPCR and RADT testing in a large cohort under real-life conditions. To validate RADT performance, two swabs, each obtained from oro- and nasopharynges, were collected and tested using both RT-qPCR and RADT. RT-qPCR-positive samples were additionally cultivated in Vero E6 cells to determine the ability of the RADT to detect replication-competent virus in individual specimens. A total of 2,032 samples were tested, 4 of which were excluded due to 3 RADT results not recorded and 1 incorrect execution of the RADT leaving 2,028 (99.80%) samples from 1,849 individuals eligible for analysis. SARS-CoV-2 was detected in 210 samples by RT-qPCR representing a study prevalence of 10.36% (Fig. 1A). Data on symptoms were obtained and analyzed for 1,676 (82.64%) of 2,028 samples. At the time of sampling 866 (42.70%) swabs were taken from symptomatic individuals, while 810 (39.94%) specimens were collected from asymptomatic individuals. For 352 (17.35%) samples, the symptom status of the respective patients was unknown at the time of analysis. For 599 specimens (69.17%), individuals reported up to three symptoms, for 247 (28.52%) more than three symptoms, and for 20 samples (2.31%) the number of symptoms was not reported by the respective subject. In our cohort 320 (15.78%) samples were obtained from 141 (7.62%) individuals who were tested twice or more (Fig. 1B). A total of 1,239 (61.09%) samples were taken from female and 789 (38.91%) from male individuals. Participants had a median age of 32.25 years (interquartile range [IQR] = 26.15 to 43.12) (Fig. 1C). The

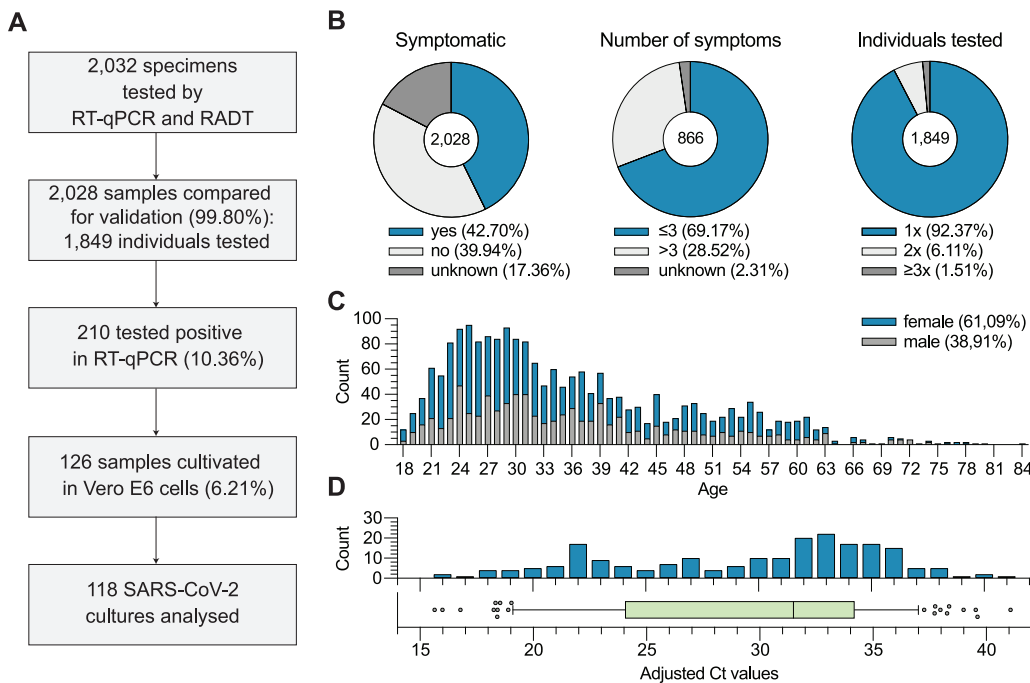


FIG 1 Study procedure and cohort description. (A) Flowchart including cohort sizes selected for RT-qPCR, RADT, and cell culture assays. Percentages in boxes 3 and 4 refer to all analyzed samples ($n = 2,028$). (B) Distribution and number of symptoms, as well as test frequencies, among individuals. (C) Age and gender distribution of the cohort. (D) Distribution of cycle threshold (C_T) values (adjusted to cobas 6800).

median adjusted C_T value determined by RT-qPCR was 31.49 (IQR = 24.19 to 34.16) (Fig. 1D). Of 210 RT-qPCR-positive and 2 RT-qPCR-inconclusive samples, 126 (60%) were cultivated in Vero E6 cells on the same day of sample collection (Fig. 1A). Of these, 8 (4.80%) were excluded due to culture contaminations ($n = 6$) or negative RT-qPCR results upon retesting of inconclusive samples ($n = 2$) (Fig. 1B). A detailed cohort description can be found in Table S1 in the supplemental material.

Reliable detection of high RNA load samples by RADT. The Standard Q COVID-19 Ag test yielded a positive result in 92 (4.54%) and a negative result in 1,936 (95.46%) samples (Fig. 2A). Using the results of the RT-qPCR as a reference, RADT classified 90 samples (4.44%) as true positive, 1,816 (89.54%) as true negative, 120 (5.92%) as false negative, and 2 (0.10%) as false positive, resulting in an overall sensitivity and specificity of 42.86% (95% CI = 36.35 to 49.62) and 99.89% (95% CI = 99.60 to 99.98), respectively (Table 1). For positive RADT results, the median C_T was 23.32 (IQR = 21.48 to 26.69) with a median copy number/ml of 6.69 \log_{10} (IQR = 5.57 \log_{10} to 7.3 \log_{10}) compared to 33.46 (IQR = 32.04 to 35.38) and 3.3 \log_{10} (IQR = 2.66 \log_{10} to 3.79 \log_{10}) for negative RADT results ($P < 0.0001$; Fig. 2B). Stratified by adjusted C_T values the RADT had sensitivities of 100% (14/14), 98.25% (56/57), 88.64% (78/88), and 50.57% (89/176) for adjusted C_T values of <20 , <25 , <30 , and <35 , respectively (Table 1). Diagnostic sensitivities of 93.33, 55.55, and 22.22% are reached for adjusted C_T values between 25 and 26, 27 and 28, and 29 and 30. Sensitivities of 86.36, 29.17, and 9.68% are reached for 6 \log_{10} , 5 \log_{10} , and 4 \log_{10} copies/ml, respectively (Fig. 2C). We conclude that observed RADT sensitivity declines at adjusted C_T values above 27 or below 6 \log_{10} copies/ml. However, the RADT reliably detects samples with higher RNA loads.

Decreased RADT sensitivity over the course of symptom duration. We determined that 130 (15.01%) and 73 (8.43%) of 866 samples from symptomatic subjects tested positive in RT-qPCR and RADT, respectively. Symptom duration at the time of sampling was reported for 860 (99.31%) samples with a median duration of 2 days since symptom onset for both RT-qPCR-positive (IQR = 1 to 6) and -negative (IQR = 1 to 4) samples (Fig. 3A). Of samples tested positive by RT-qPCR, RADT detected 56 (68.29%) samples within 4 days since symptom onset. When reported, median symptom duration for RT-qPCR-positive samples with either a RADT-positive ($n = 69$) or -negative ($n = 55$) result was 2 days (IQR = 1 to 3;

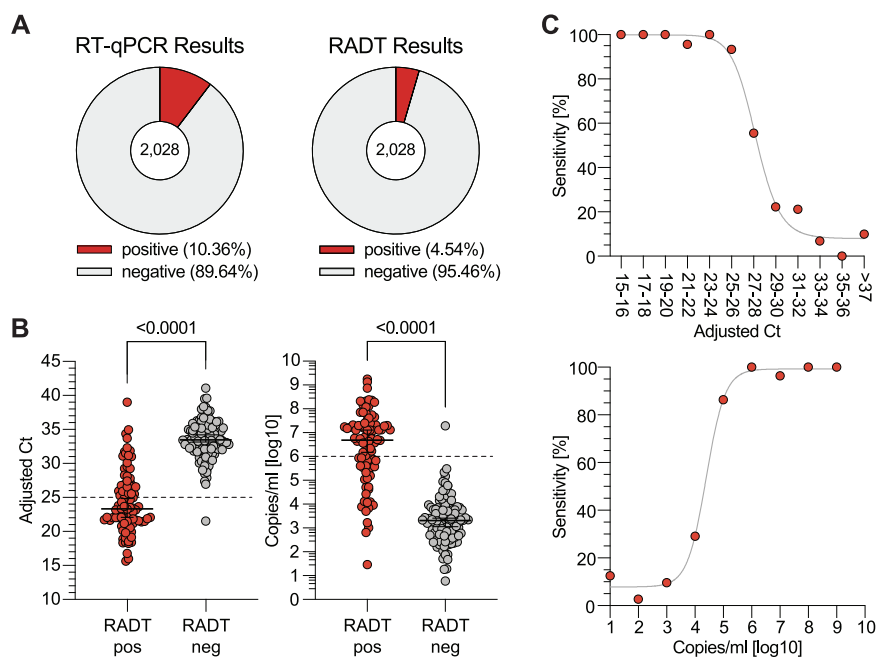


FIG 2 Head-to-head comparison of SARS-CoV-2 detection by RT-qPCR and RADT. (A) RT-qPCR and RADT results of all 2,028 specimens. (B) The 210 RT-qPCR-positive samples are plotted by adjusted C_T values and SARS-CoV-2 RNA load, respectively, and stratified by their RADT result ($P < 0.0001$, MWU). (C) The sensitivity of the RADT is stratified by adjusted C_T values and RNA load in log copies/ml. The top graph shows data points for two C_T units each. Due to low sample sizes, C_T values of >37 were combined.

Fig. 3B). For samples from symptomatic patients, the median adjusted C_T and median copies/ml were 28.90 (IQR = 23.17 to 32.80) and 4.83 log₁₀ (IQR = 3.53 log₁₀ to 6.74 log₁₀), respectively. In individuals not reporting any symptoms, detected RNA load was significantly lower (median adjusted C_T = 33.45 [IQR = 31.26 to 35.31]; median copies/ml = 3.31 log₁₀ [IQR = 2.69 log₁₀ to 4.05 log₁₀; $P < 0.0001$]) (Fig. 3C). Samples with RNA concentrations of >6 log₁₀ were only observed up to 6 days after symptom onset. Thus, we conclude that the sensitivity of the RADT decreased with symptom duration as RNA loads declined (Fig. 3D).

Negative RADT result corresponds with lack of viral cultivability. For 118 inoculated cultures, CPE was observed in 29 (24.58%). To confirm virus replication, RT-qPCR of culture supernatant taken on days 4 and 7 was performed as described previously. The observed CPE and positive RT-qPCR results matched in 116 (98.30%) cases. Since CPE is operator dependent, RT-qPCR results were used for further analysis (Table 2). Initial C_T values and copies/ml of positive cultures ranged from 15.64 to 24.97 and 6.14 log₁₀ to 9.25 log₁₀, whereas negative cultures ranged from 21.5 to 38.27 or 1.71 log₁₀ to 7.30 log₁₀, respectively

TABLE 1 Performance data of the Standard Q COVID-19 Ag test^a

Subgroup	Total no.	Sensitivity [% (95% CI)]
Overall ^b	2,028	42.86 (36.35–49.62)
$C_T < 20$ ($\hat{=}$ >7.80 log ₁₀ copies/ml)	14	100.00 (72.25–100.00)
$C_T < 25$ ($\hat{=}$ >6.13 log ₁₀ copies/ml)	57	98.25 (90.71–99.91)
$C_T < 30$ ($\hat{=}$ >4.46 log ₁₀ copies/ml)	88	88.64 (80.33–93.71)
$C_T < 35$ ($\hat{=}$ >2.80 log ₁₀ copies/ml)	176	50.57 (43.25–57.86)
Symptomatic	866	55.39 (46.81–63.65)
Asymptomatic	810	22.50 (14.73–32.79)

^aThe RADT sensitivity, specificity, and positive and negative predictive values (PPV and NPV) were calculated using RT-qPCR as a reference. RADT sensitivity was stratified by cycle threshold (C_T) values and symptom status.

^bPPV, 97.83% (95% CI = 92.42 to 99.61); NPV, 93.80% (95% CI = 92.64 to 94.79); specificity, 99.89 (95% CI = 99.60 to 99.98).

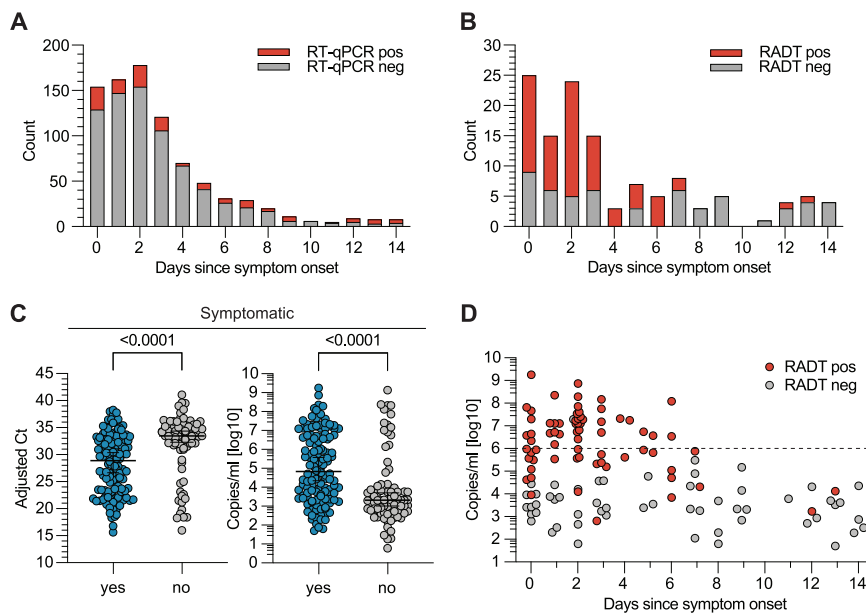


FIG 3 RT-qPCR and RADT results in symptomatic and asymptomatic individuals. (A) The number of RT-qPCR-positive and -negative specimens from symptomatic individuals is plotted by the days since symptom onset. (B) The number of RADT-positive and -negative specimens within the RT-qPCR-positive samples of symptomatic participants ($n=130$) is stratified by the days since symptom onset. (C) Symptomatic and asymptomatic RT-qPCR positives are plotted by adjusted C_T value and RNA load ($P < 0.0001$, MWU). (D) RT-qPCR-positive samples of symptomatic individuals are plotted by RNA load and stratified by the number of days since symptom onset. The RADT result is indicated in corresponding colors.

(Fig. 4A). All (29/29) positive SARS-CoV-2 cultures had been previously identified as positive by RADT. Of 89 negative cultures, 64 (71.91%) had been previously classified as RADT negative and 25 (28.09%) as RADT positive, resulting in a sensitivity of 100.00% (95% CI = 88.30 to 100.00) and a specificity of 71.91% (95% CI = 61.82 to 80.19) for detecting viral cultivability by RADT ($P < 0.0001$; Table 2). Therefore, the calculated PPV of RADT for viral cultivability in Vero E6 cells was 53.70% (95% CI = 40.61 to 66.31) and the NPV was 100% (95% CI = 94.34 to 100.00; Table 2). For RADT- and culture-positive results, the median adjusted C_T was 20.8 and the median RNA load in copies/ml was 7.53 \log_{10} compared to 33.39 and 3.34 \log_{10} for RADT- and culture-negative results. The median adjusted C_T and copies/ml for RADT-positive but culture-negative results were 25.43 (IQR = 23.39 to 27.72) and 5.99 \log_{10} (IQR = 5.23 \log_{10} to 6.67 \log_{10}), respectively (Fig. 4B). With viral RNA declining over the course of disease, the ability to isolate virus decreased, with no positive culture after 6 days since symptom onset (Fig. 4C). Probit regression of RADT and cell culture analyses show greater probabilities of positive result (PPR) for low adjusted C_T values and high RNA loads, respectively. The virus culture assay shows 90 and 50% PPR for an adjusted C_T value around 21.45 or 7.31 \log_{10} copies/ml and 23 or 6.8 \log_{10} copies/ml, respectively. RADT show a PPR of 90 and 50% at an adjusted C_T value of 24.7 or 6.24 \log_{10} copies/ml and 29.0 or 4.78 \log_{10} copies/ml, respectively (Fig. 4D). In summary, these data show that a negative RADT result can reliably predict noninfectiousness in Vero E6 cells.

Adjustments for repeated testing reveal no statistically significant difference.

To evaluate whether repeated testing of several individuals affects our conclusions, additional statistical analyses were carried out (see Materials and Methods). Correction for repeated RADT measurements was based on weighted counts (see Fig. S2A) rounded to whole numbers, yielding very similar results compared to the unweighted variant (see Fig. S2). In addition, the performance of the RADT was analyzed for the first measurement of each subject only (simplified analysis; Fig. S2A). Calculated sensitivities showed almost identical values as overall sensitivities described above (see Fig. S2B to D). For all studies that applied an MWU test (Fig. 2B, 3C, and 4A and B), the P values remain $P < 0.0001$ according a generalized estimating equation (GEE).

TABLE 2 Comparison of RADT and culture results^a

Parameter	Culture positive	Culture negative	Total
No.			
RADT positive	29	25	54
RADT negative	0	64	64
Total	29	89	118
%			
Sensitivity	100.00 (88.30–100.00)	PPV	53.70 (40.61–66.31)
Specificity	71.91 (61.82–80.20)	NPV	100.00 (94.34–100.00)

^aAnalysis of RADT performance in the context of culture infectivity ($P < 0.0001$, Fisher exact test; weighted analysis $P < 0.0001$; 95% CI values are indicated in parentheses).

DISCUSSION

RADT are inexpensive and fast diagnostic tools that can be immediately performed at the point of care. Here, we present comprehensive data on the use of Standard Q COVID-19 RADT for high-throughput testing of a large cohort tested under real-life conditions in a SARS-CoV-2 outpatient diagnostic center.

Implementation and execution of the RADT testing was completed without any difficulties. Upon following the manufacturer’s instructions, there were only two cases in which the result could not be read out clearly. As a consequence, we were able to conduct 2,028 paired RT-qPCR and RADT tests directly on site and cultivate virus on the same day without prior sample freezing. While the sensitivities of other RADT vary between 24 and 93% in different studies (33–35), the reported Standard Q RADT sensitivities are mostly in the range from 68 to 90% (14–24). The overall diagnostic sensitivity observed in our study was 42.86%. However, the investigated cohort of nonhospitalized patients was to a large extent comprised of individuals

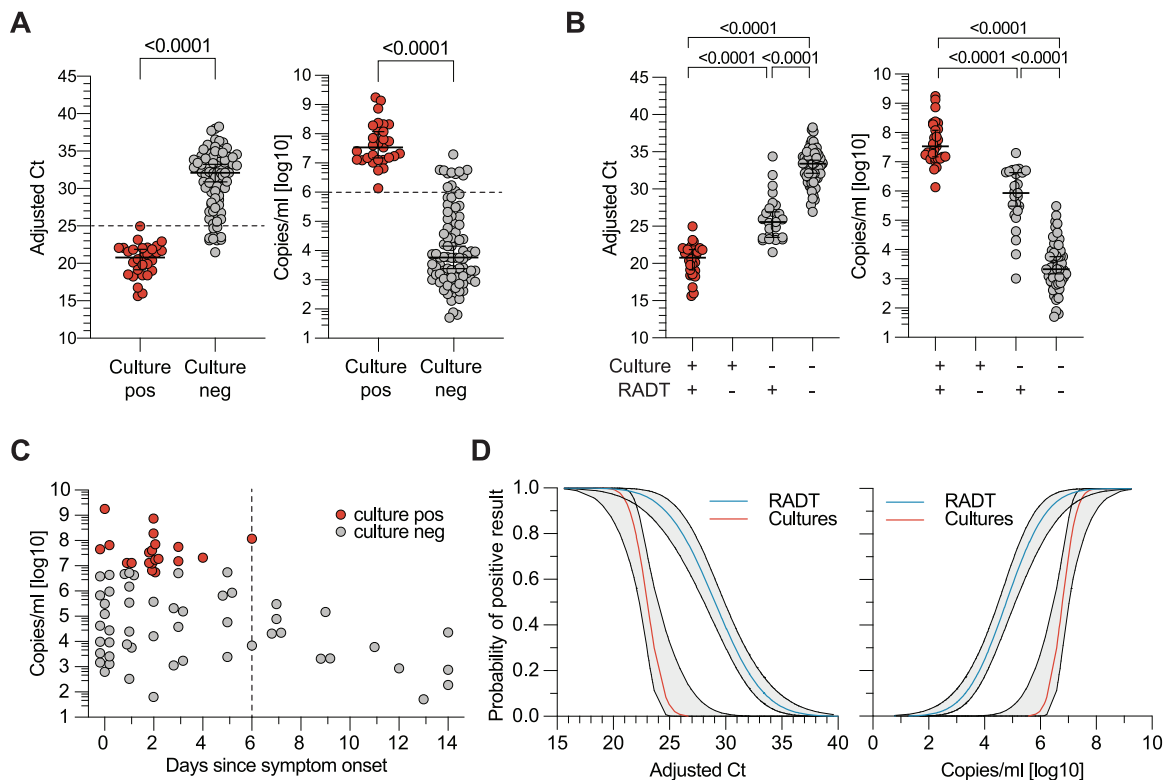


FIG 4 Virus culture analysis and RADT performance in the detection of replication-competent SARS-CoV-2. (A) Positive and negative viral cultures plotted by adjusted C_T and RNA load of the original swab medium ($P < 0.0001$, MWU). (B) Adjusted C_T values and RNA loads of cultured samples are stratified by culture and RADT result ($P < 0.0001$, MWU). (C) Culture-positive and -negative samples are plotted by RNA load of original swab medium and stratified by the days since symptom onset. (D) Probability of positive result for RADT and viral cultures in the context of adjusted C_T values and RNA load (Probit-Model, R-function GLM).

with adjusted C_T values over 30 (122/210). Stratification by RNA load revealed that the Standard Q RADT performed reliably for patients with RNA loads over $5.4 \log_{10}$ or below C_T 27, which is in accordance to recent studies (14, 23, 25, 36). Since an RNA concentration of $6 \log_{10}$ copies/ml is commonly suspected to be the threshold for contagiousness, we aimed to investigate the correlation between RADT result and SARS-CoV-2 *in vitro* infectivity (28, 29).

In contrast to highly sensitive nucleic acid-based detection methods that do not specifically test for intact viral particles required for transmission, viral culture is a commonly used, albeit laborious, method outside of routine diagnostics to determine the presence of infectious virus in samples (7, 30–32, 37). Although the detection of viable virus in cell culture models is strong evidence of infectiousness, a negative result does not eliminate the possibility of human transmission (11, 38). Moreover, the validity of viral culture as a surrogate for infectivity may depend on the susceptibility of the cell line used (31, 32, 37). However, loss of infectious titer in classical Vero E6 cells has been associated with a lack of transmission despite detection of viral RNA in preclinical models (39).

To investigate whether the Standard Q RADT might be able to reliably detect culture-positive samples in Vero E6 cells, we attempted virus isolation from samples positive for SARS-CoV-2 RNA. All samples from which virus could be recovered had previously tested positive in RADT. Moreover, none of the samples tested negative by RADT contained infectious virus determined by cell culture. Furthermore, when taking symptom duration into account, we detected no positive culture 6 days after symptom onset, indicating a decreased probability of recovering viable virus as symptom duration increases (29, 30, 40–42); at the same time, the RADT identified positive samples for up to 9 days. While some groups have described virus isolation from samples above C_T 30 (31, 43), our study only observed positive cultures from samples with higher RNA loads, which is in accordance with previous observations (10, 36, 41, 44). Taking the time of suspected exposure and duration of symptoms into account (31, 38), our results suggest that RADT testing is of potential use for estimating infectivity at the time of sampling.

This study, however, is subject to some limitations. Although the examined single-center study population was large, our cohort might not be considered representative of the general population due to young age and disproportionate gender distribution. The data on symptoms and their duration are only reliable to a limited extent, since they were retrospectively analyzed from mostly self-reported symptoms entered into a web tool. Furthermore, instead of a nasal swab, we used a combined oro- and nasopharyngeal swab to investigate RADT performance, which impedes the feasibility for the general public. Lastly, although we have corrected for using different SARS-CoV-2 detection assays by reporting data in RNA loads, the diverse set of amplification methods remains a limitation.

In combating overdispersed SARS-CoV-2 transmission, rapid detection and isolation of highly infectious individuals is a primary goal (8, 9, 45–49). In our investigation the Standard Q RADT was able to reliably detect high RNA loads, as well as all culture-positive samples. Therefore, this test could be used as a fast surrogate marker for viral cultivation to identify and prevent SARS-CoV-2 transmissions by highly infectious individuals. Although less sensitive than RT-qPCR and therefore less suited for an inpatient setting, RADT could compensate for this disadvantage through easy and feasible mass screenings in a community setting (8, 50). Furthermore, one might suspect that RT-qPCR positive, but RADT-negative individuals do not pose a high risk of transmissions, since all samples remained culture negative in our experimental setup. However, individual results must be interpreted with caution, as SARS-CoV-2 infection could remain undetected in early stages. In summary, our results suggest that SARS-CoV-2 transmission could be reduced by systematic RADT, despite the fact that some infected individuals will not be detected by the test (51). Simple to perform

and applicable anywhere, RADT enable mass testing as a complementary method to RT-qPCR to more effectively combat the SARS-CoV-2 pandemic.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.7 MB.

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