



OPEN

Complementary methods for SARS-CoV-2 diagnosis in times of material shortage

Thaisa Lucas Sandri^{1,2,3}, Juliana Inoue¹, Johanna Geiger², Johanna-Marie Griesbaum¹, Constanze Heinzl¹, Michael Burnet², Rolf Fendel^{1,4,5}, Peter G. Kremsner^{1,4,5}, Jana Held^{1,4,5,6} & Andrea Kreidenweiss^{1,4,5,6}✉

The pandemic caused by SARS-CoV-2 resulted in increasing demands for diagnostic tests, leading to a shortage of recommended testing materials and reagents. This study reports on the performance of self-sampled alternative swabbing material (ordinary Q-tips tested against flocked swab and rayon swab), of reagents for classical RNA extraction (phenol/guanidine-based protocol against a commercial kit), and of intercalating dye-based one-step quantitative reverse transcription real-time PCRs (RT-qPCR) compared against the gold standard hydrolysis probe-based assays for SARS-CoV-2 detection. The study found sampling with Q-tips, RNA extraction with classical protocol and intercalating dye-based RT-qPCR as a reliable and comparably sensitive strategy for detection of SARS-CoV-2—particularly valuable in the current period with a resurgent and dramatic increase in SARS-CoV-2 infections and growing shortage of diagnostic materials especially for regions limited in resources.

At the end of 2019, the new coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) was discovered in Wuhan, China, causing the respiratory disease COVID-19. The following outbreak, classified by the World Health Organisation (WHO) as a pandemic situation on 11th March 2020, resulted in an increasing demand for fast and reliable diagnostic tests to identify infected individuals to prevent the spread of the virus^{1,2}. Many health institutions reported shortages of required materials for pharyngeal specimen collection, sample extraction, and PCR materials. To ensure reliable diagnosis of SARS-CoV-2 infections in the event of a resurgence in infection rates, such as those observed in Europe and North America in autumn/winter 2020/2021, suitable alternative materials need to be urgently identified^{1,2}.

Although rapid tests for SARS-CoV-2 antigen detection are now widely available, reverse transcription quantitative real-time PCR (RT-qPCR) will remain the gold standard for diagnosing SARS-CoV-2 infections due to its excellent sensitivity. Until very recently, there were only few approved materials, reagents, and procedures for SARS-CoV-2 molecular diagnostics^{3–5}. Guidelines recommended the preferred use of flocked swabs stored in transport medium over dry swabs collected by specialists⁶ and listed only kit-based RNA extraction methodologies followed by a hydrolysis probe-based RT-qPCR assay. These materials and methods became the gold standard procedure for detection of SARS-CoV-2.

With the increasing demand of SARS-CoV-2 diagnostic tests for people suspected of having COVID-19 and population screenings, there is a continuing shortage of recommended swabs, RNA extraction kits, and reagents for hydrolysis probe-based RT-qPCR. In addition, the difficulty of providing rapid access and the high costs of recommended consumables pose a barrier for wider application of this diagnostic method for low-resource countries. Therefore, adaptation of diagnostic procedures to available and alternative reagents is necessary to ensure continued reliable diagnosis. For this purpose, we tested self-sampled, ordinary cotton swabs “Q-tips” as an alternative to medical swabs for oropharyngeal sampling, the classical RNA extraction protocol, and a cost-saving intercalating dye-based RT-qPCR assay for the detection of SARS-CoV-2.

¹Institute of Tropical Medicine, Eberhard Karls University Tübingen, Wilhelmstrasse 27, 72074 Tübingen, Germany. ²Synovo GmbH, Paul-Ehrlich-Strasse 15, 72076 Tübingen, Germany. ³Laboratory of Molecular Immunopathology, Department of Clinical Pathology, Federal University of Paraná, Curitiba, Brazil. ⁴German Center for Infection Research (DZIF), Partner Site Tübingen, Wilhelmstrasse 27, 72074 Tübingen, Germany. ⁵Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon. ⁶These authors contributed equally: Jana Held and Andrea Kreidenweiss. ✉email: andrea.kreidenweiss@uni-tuebingen.de

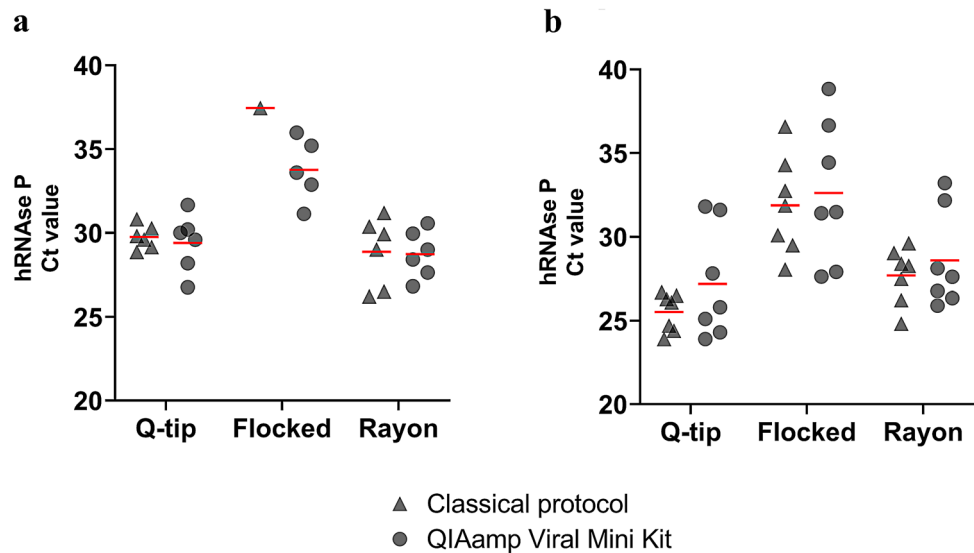


Figure 1. Comparison of swab types and RNA extraction methodologies for RNA extraction efficiency. Q-tips, flocked swabs, and rayon swabs were used, and RNA was extracted either by a classical protocol or the commercial kit. Ct values for hRNase P amplification of oropharyngeal samples from (a) non-infected participants ($n=6$); and (b) SARS-CoV-2-infected participants ($n=7$). Means are represented by red lines.

Results

To provide alternatives for molecular detection of SARS-CoV-2 infections during material shortages, we compared the following: ordinary cotton swab “Q-tips” available in supermarkets against medical sampling approaches (here: flocked swabs stored in transport medium, and rayon swabs stored dry), a classical (phenol/guanidine-based) protocol for RNA extraction against the commercially available kit for viral RNA extraction, and a cost-saving intercalating dye-based one-step, reverse transcription quantitative real-time PCR (SYBR RT-qPCR) protocol against the gold standard hydrolysis probe-based one-step, reverse transcription quantitative real-time PCR (probe RT-qPCR) for detection of SARS-CoV-2. These alternative options could also be beneficial for SARS-CoV-2 diagnosis in laboratories in low-resource settings. Samples were collected by self-swabbing⁷.

Comparison of sampling approaches and RNA extraction methods. Participants self-sampled themselves using two sets of three different swab types (2× Q-tips, 2× flocked swabs, 2× rayon swabs). Swabs were stored according to recommended procedures, i.e. flocked swabs were stored in saline and served as the recommended gold standard. Q-tips and rayon swabs were kept dry, and the latter served as direct comparator for Q-tips under investigation. RNA was extracted from each swab type either by using the classical protocol or the commercial QIAamp Viral Mini kit. To ensure best quality and high concentrated RNA extracts, we strictly followed recommended procedures and accordingly applied respective volumes of starting material given per sampling approaches. This means for flocked swabs kept in 2 ml saline, 140 µl entered into RNA extraction by both methodologies. Rayon swabs and Q tips were stored dry and RNA extraction started by either immersing the swabs in 1 ml QIAzol for the classical protocol or in 560 µl AVL buffer for the commercial kit and the complete volumes entered into downstream RNA extraction procedures. RNA was eluted with 60 µl volume, either DEPC water in the classical protocol or buffer (AVE) in the commercial kit. To evaluate the different swabs as options for self-sampling⁷, the housekeeping gene human RNase P (hRNase P) was quantified to serve as endogenous control by RT-qPCR. Of non-infected participants, Q-tips swabs plus classical RNA extraction protocol was as efficient for RNA recovery as the ones extracted by the commercial QIAamp Viral Mini kit, with a Ct mean of 29 ± 0.7 and 29 ± 1.7 , respectively (Fig. 1a). Of SARS-CoV-2 infected participants, RNA recovery with Q-tips plus classical extraction was more efficient than the kit-based RNA extraction (Ct: 25 ± 1.4 vs 27 ± 3.3) (Fig. 1b). Overall, sampling with ordinary Q-tips was as good as with medical swabs with rather slightly lower Ct values (indicating more RNA recovery in the extraction). Likewise, the classical RNA extraction protocol was at least as good as the commercial kit, when the control gene hRNase P was amplified. As expected, flocked swab samples resulted in higher Ct values reflecting less recovery of RNA material compared to Q-tips and rayon swab, most likely due to the recommended sample dilution. Flocked swabs are immersed in saline solution after sampling and only a fraction (140 µl of 2000 µl) is used for RNA extraction according to the standard protocol. This finding was consistent for sampling of uninfected as well as for SARS-CoV-2 positive individuals (Fig. 1a,b).

Establishment of an intercalating dye-based RT-qPCR for SARS-COV-2 detection. Next, we simplified the available SARS-CoV-2 probe RT-qPCR protocol towards an intercalating dye-based RT-qPCR (SYBR RT-qPCR) plus melting curve analysis as an alternative to the costly probe RT-qPCR assay. This was done first to detect the control gene hRNase P and then for the SARS-CoV-2 genes E and RdRp. Performance of the E

Gene	Primer concentration	58 °C ^a		60 °C ^a	
		T _m (°C)	LOD (copies)	T _m (°C)	LOD (copies)
E	Fwd, Rv: 200 nM	–	–	81.02 (± 0.07)	50
	Fwd, Rv: 400 nM	81.06 (± 0.03)	50	81.07 (± 0.02)	5
RdRp	Fwd: 600 nM Rv: 800 nM	80.6 (± 0.03)	1000	80.6 (± 0.06)	100

Table 1. Performance of primers of the intercalating dye-based RT-qPCR protocol for SARS-CoV-2 detection. Fwd: forward primer; Rev: reverse primer; T_m: melting temperature; LOD: limit of detection. ^aPrimer annealing temperature.

gene assay was more stable and more sensitive at a primer concentration of 400 nM and the annealing temperature set to 60 °C than primers at 200 nM and/or at 58 °C temperature as in the original protocol (Table 1). The RdRp gene assay also showed a better performance at 60 °C (Table 1). In silico melting curve analysis predicted a melting temperature (T_m) of 85 ± 0.43 °C for hRNase P, 81 ± 0.43 °C for E gene, and 80.5 ± 0.43 °C for RdRp. The hRNase P, E, and RdRp genes assays presented a specific melting peak at 84.5 °C, 80.6 °C, and 81.1 °C, respectively. A few negative samples showed a unique unspecific melting peak at 75.9 °C in the E gene assay that could be differentiated from the specific one. For all melting curves, see Supplementary Figure S1.

When comparing the SYBR RT-qPCR to probe RT-qPCR, hRNase P (LOD: 10 copies for both) and E gene (LOD: 5 copies for both) assays showed similar performance, while the RdRp gene assay using SYBR RT-qPCR was less sensitive compared to the probe RT-qPCR (LOD 100 vs. 10 copies) (Fig. 2 and Supplementary Figure S2).

Hydrolysis probe-based RT-qPCR assay. All target genes were consistently detected from swabs of all types (Fig. 3). Considering the type of swab and the number of target gene copies, RNA recovered from Q-tips was similarly high as from rayon swabs (Q-tips: hRNase P: 4.6 ± 0.8 log copies, E: 5.2 ± 1.2, and RdRp: 2.7 ± 0.5 versus rayon swab: hRNase P: 4.2 ± 0.5 log copies, E: 5.5 ± 0.7, and RdRp: 2.9 ± 0.5). In contrast, flocked swab (hRNase P: 3 ± 0.8 log copies, E: 4.8 ± 0.6, and RdRp: 2.5 ± 0.5) was less suitable for cellular sampling and RNA yield (Fig. 3).

SYBR RT-qPCR assay for SARS-CoV-2 detection as an alternative to probe RT-qPCR. Paired samples tested with both intercalating dye-based and probe-based assays showed a mean variance in the Ct values of 0.1 cycle for hRNase P, 0.4 cycle for E gene, and 2.2 cycles for RdRp. The RdRp gene assay using intercalating dye gave earlier Ct values when compared to the probe-based. The intercalating dye-based assay failed in detecting one sample for both E gene (probe-based Ct value: 32.8) and RdRp gene (probe-based Ct value: 31.6) assays. Interestingly, both samples were extracted using the classical method. Despite those failures, both intercalating assays could detect even later Ct values of other samples (the latest Ct value detected in field samples was 35.2 for E gene and 35 for the RdRp gene). Regarding the log of gene copies compared to hydrolysis probe-based assays, the hRNase P intercalating dye-based assay resulted in lower values when using rayon swab and Q-tips (R: 3.0 ± 0.4 vs. 4.2 ± 0.5 and Qt: 3.3 ± 0.6 vs. 4.6 ± 0.8) and equivalent results when using flocked swab and saline (2 ± 0.8 vs. 3 ± 0.8); E gene intercalating dye-based assay gave lower values when using rayon swab and Q-tips (R: 4.4 ± 1.2 vs. 5.5 ± 0.7 and Qt: 4.2 ± 1.4 vs. 5.2 ± 1.2) and equivalent results when using flocked swab and saline (4.2 ± 0.9 vs. 4.8 ± 0.6); and RdRp gene assays gave consistent results among the different techniques utilized (R: 2.5 ± 0.6 vs. 2.9 ± 0.5, Qt: 2.8 ± 0.6 vs. 2.8 ± 0.5, and FS: 2.2 ± 0.5 vs. 2.5 ± 0.5) (Fig. 3). Raw data as RNA yield and Ct values for all assays is displayed in the Supplementary Tables S3 and S4.

Discussion

The current pandemic due to the SARS-CoV-2 rapid spread demands more testing and there is an urgent need for alternative but reliable materials for diagnosis. With a continuous global public health emergency state, it is predicted that several million people will become infected in the upcoming months of 2021^{8,9}. Thus, reliable and accessible materials, reagents, and methods for diagnosis of SARS-CoV-2 infections are of paramount importance. Here, we compared the performance of ordinary Q-tips commonly available at supermarkets against medical flocked swabs and rayon swabs, a classical protocol for RNA extraction against a commercial kit and established a cost-efficient intercalating dye-based RT-qPCR for detection of SARS-CoV-2. This approach is not only urgently needed, it is also helpful to regions which suffer from limited financial resources. Overall, the performance of self-sampled oropharyngeal Q-tips, the phenol/guanidine-based (classical) protocol for RNA extraction, and the intercalating dye-based RT-qPCR for detection of SARS-CoV-2 genetic material was—individually as well as in combination—as reliable and sensitive as their respective comparators recommended by the authorities.

Here we also report the convenience and feasibility of swab self-sampling. SARS-CoV-2 infected participants received kits with swabs, sampled themselves, and called the study team to collect them, thereby reducing SARS-CoV-2 spread and the study team exposure. In addition to this, in a pandemic scenario, self-sampling can also improve the mass screening participation, reduce the medical staff workload, and keep the clinics, hospitals, and even emergency rooms dedicated to the ones really in need of medical care. Also, in places with limited access to medical care, self-sampling with Q-tips swabs (available at the supermarket) may be an interesting option for accelerated diagnosis.

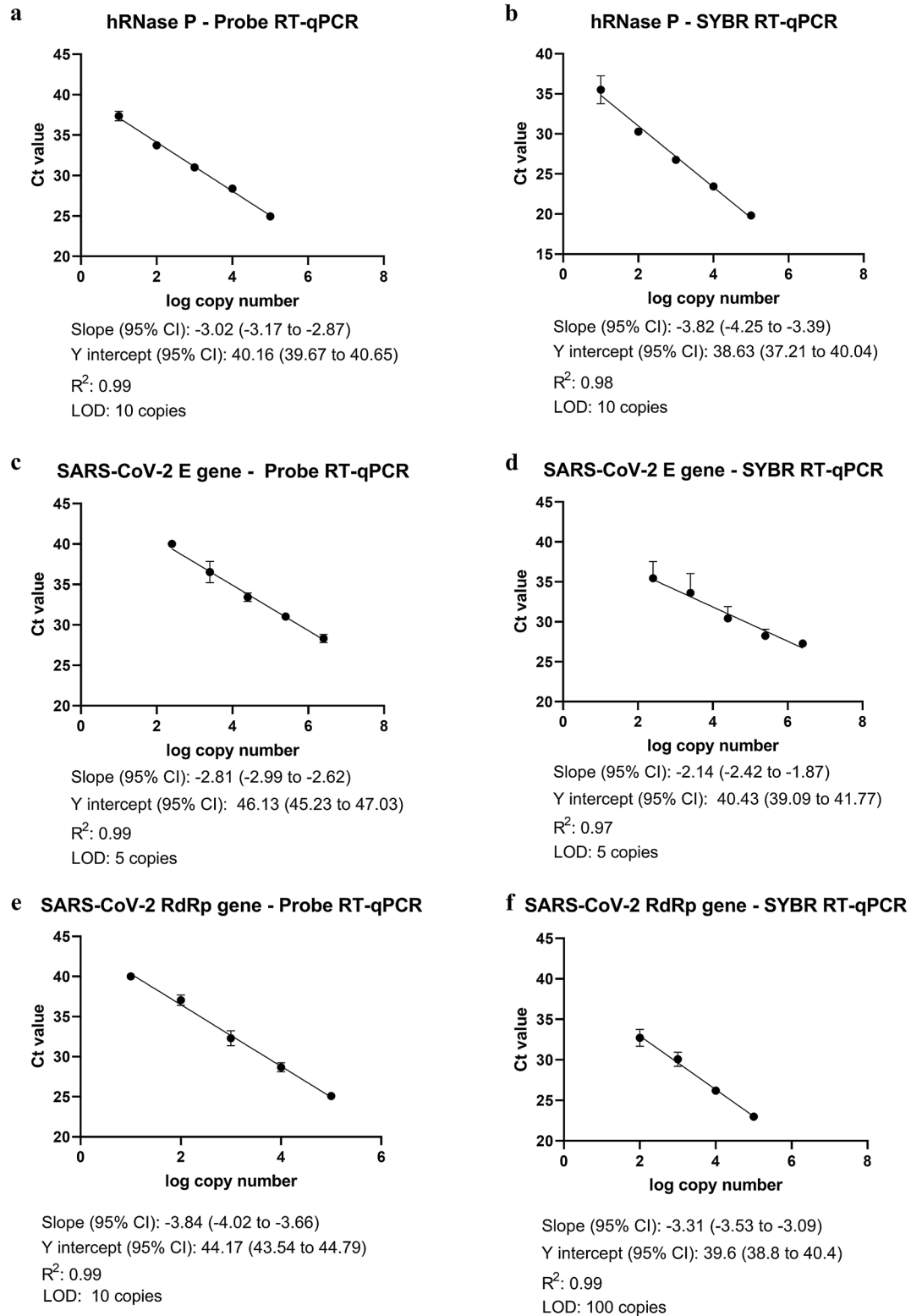


Figure 2. Standard curves of the targeted genes generated with SYBR green-based and hydrolysis probed-based RT-qPCR assays.

In all RT-qPCR assays either detecting hRNase P, which served as an endogenous control for sample integrity and proper RNA extraction, Ct values obtained for samples taken by Q-tips were similar to those obtained by rayon swab¹⁰, while samples taken by flocked swab showed fewer copies. As recommended, flocked swabs are

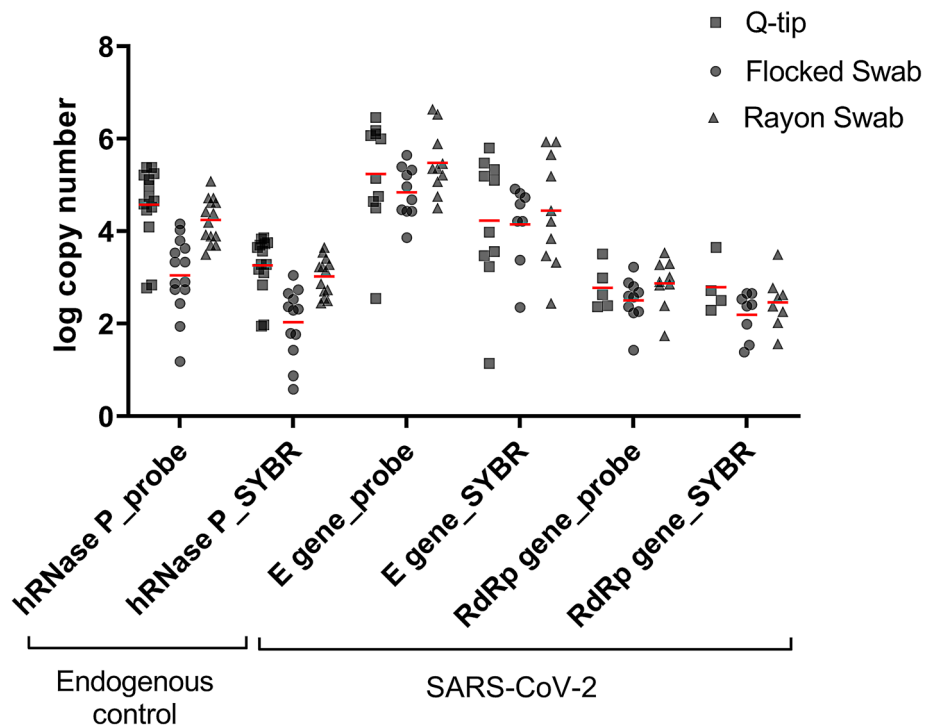


Figure 3. Comparison of the performance of the SYBR RT-qPCR and hydrolysis-probe based RT-qPCR methods in detecting the target genes in different swab types of SARS-CoV-2 infected participants ($n=7$). RNA was extracted with the classical protocol or the commercial kit. Means are represented by red lines.

stored in 2 ml of saline after sampling and only 1/14 of the volume is used in the subsequent RT-qPCR assay. Thus, reduced sensitivity is most likely the result of the small sample fraction tested.

The classical procedure for RNA extraction, done before the introduction of commercial kits became widely available, requires multiple handling steps including cell lyses using phenol/guanidine (or a commercially available solution such as QIAzol used here), phase separation with chloroform and RNA precipitation with isopropanol. The classical procedure resulted into yields and qualities of extracted RNA that did not differ in Ct outputs of RT-qPCR amplification compared to RNA extracted with the commercial kit with simplified and standardized pipetting steps. Interestingly, despite the fact that the used commercial kit is designed to isolate mainly viral RNA (QIAamp Viral RNA Mini Handbook), this did not result into lower Ct values of SARS-CoV-2 RT-qPCR and thus more starting copies compared to the classical protocol which extracts human and viral RNAs. The reduced sample throughput is a limit to the classical RNA extraction protocol.

In contrast to hydrolysis probe-based RT-qPCR, intercalating dye-based assays do not depend on modified oligonucleotides, where special manufacturing and supply can be critical in the current times. Intercalating dye-based RT-qPCR protocols have occasionally been used for SARS-CoV-2 diagnosis. Here, we expanded the available Charité protocol¹¹ towards a SYBR one-step RT-qPCR protocol for SARS-CoV-2 detection. In silico analysis of the primers and melting temperature peaks showed specificity and feasibility. The assay performance was sensitive and robust after increasing the annealing temperature from 58 to 60 °C compared to the probe-based protocol. The intercalating dye-based assays for hRNase P and E genes were as sensitive as the hydrolysis probe-based assays, but the RdRp gene showed ten times less sensitivity. Dorlass and collaborators¹² also reported good performance of E gene primers published by Corman et al.¹¹ using intercalating dye-based assays in nasopharyngeal swabs collected in hospitals when compared to probe-based assay. Only one SARS-CoV-2 RT-qPCR positive individual who was positive for RNase P by SYBR RT-qPCR was not positive for the E and RdRp genes when material from Q-tips plus classical RNA extraction was tested. In addition, one sample was not positive for the RdRp SYBR RT-qPCR assay despite the other two assays were successful. Thus, the intercalating dye-based RT-qPCR is a promising alternative for SARS-CoV-2 molecular diagnosis.

In pandemic scenarios, a shortage of recommended materials for molecular diagnostics can limit the identification of infected individuals which may have a negative impact on the efficiency of isolation measures and contributes to the spread of the virus. Moreover, the costs of the recommended materials often cannot be afforded by low-resource settings. Here, we showed that Q-tips available at very low costs in local supermarkets can be used for oropharyngeal self-sampling, RNA can be extracted with a classical (phenol-guanidine-based) protocol, and intercalating dye-based RT-qPCR is a valuable and sensitive diagnostic test reliably detecting SARS-CoV-2 infected individuals.

	Assay	Reaction mix	Template volume (µl)	Total reaction volume (µl)	Reverse transcription	Initial melting	Cycle melting	Cycle annealing	# Cycles	Melting curve
Hydrolysis probe assays ^{a11}	RNase P	0.4 mM each primer 0.1 mM probe	3	15	50 °C, 15 min	95 °C, 2 min	95 °C, 15 s	58 °C, 45 s	×45	–
	SARS-CoV-2 E gene	0.4 mM each primer 0.2 mM probe	3	15	50 °C, 15 min	95 °C, 2 min	95 °C, 15 s	58 °C, 45 s	×45	–
	SARS-CoV-2 RdRp gene	0.6 mM forward primer 0.8 mM reverse primer 0.2 mM probe	3	15	50 °C, 15 min	95 °C, 2 min	95 °C, 15 s	58 °C, 45 s	×45	–
Intercalating dye assays ^b	RNase P	0.4 mM each primer	2	10	45 °C, 10 min	95 °C, 2 min	95 °C, 5 s	60 °C, 30 s	×45	60–95 °C (5 acquisitions/°C)
	SARS-CoV-2 E gene	0.4 mM each primer	2	10	45 °C, 10 min	95 °C, 2 min	95 °C, 5 s	60 °C, 30 s	×45	60–95 °C (5 acquisitions/°C)
	SARS-CoV-2 RdRp gene	0.6 mM forward primer 0.8 mM reverse primer	2	10	45 °C, 10 min	95 °C, 2 min	95 °C, 5 s	60 °C, 30 s	×45	60–95 °C (5 acquisitions/°C)

Table 2. Nucleic acid amplification assays conditions. ^aSuperScript™ III Platinum™ One-Step qRT-PCR Kit (ThermoFisher). ^b SensiFAST One-Step SYBR Green No-Rox Master Mix (Bioline GmbH, Luckenwalde, Germany).

Methods

Study participants. A total of 13 participants were enrolled in the study, seven individuals with acute SARS-CoV-2 infection and six uninfected control individuals. Study participants were requested to sample themselves and the procedure of oropharyngeal swabbing was explained to each participant. Each participant was provided with two kits containing three different swab types each that were collected by the study team within two hours after sampling. All individuals gave written informed consent. The study was approved by the Ethics Committee of the Universitätsklinikum Tübingen (Ref. number 20/231/B01).

Sample collection. Three different swab types were compared for their applicability to oropharyngeal sampling and detection of SARS-CoV-2 infections: (1) Q-tips, these are ordinary cotton swabs with a paper stick usually used for ear cleaning and available in local supermarkets, (2) flocked swab embedded in 2 ml of 0.9% NaCl solution after sampling (COPAN FLOQswab 501CS01, Copan, Italy), and (3) dry rayon swab (COPAN 155C Rayon, Copan, Italy). Swabs were kept at 4 °C until RNA extraction but no longer than 4 h.

RNA extraction. Two different RNA extraction methodologies were compared for their efficiency in RNA yield: a classical protocol using phenol/guanidine thiocyanate (QIAzol Lysis Reagent, QIAGEN) for cell lysis and RNase inhibition, chloroform and isopropanol for RNA purification and precipitation, respectively, following manufacturer's protocol¹³, and the commercial kit QIAamp Viral RNA Mini kit (QIAGEN). To start the RNA extraction, the complete cotton material of Q-tips and rayon swabs was cut and either immersed in 1 ml QIAzol or 560 µl AVL buffer containing carrier RNA when utilizing QIAamp Viral RNA Mini kit and kept for 10 min at room temperature before continuing with the respective protocols. Flocked swabs were stored in 2 ml saline after sampling, 140 µl of this solution was used for RNA extraction for each one of the two extraction methodologies. Total RNA was eluted with 60 µl nuclease-free water for the classical extraction or elution buffer provided by the kit. RNA concentration and quality per extraction were measured using NanoDrop 1000 (NanoDrop Technologies).

Endogenous human control. Detection of human RNase P (hRNase P) RNA in swab material can serve as an endogenous control, to ensure sample integrity and proper RNA extraction as suggested by the CDC⁵. Here, we used hRNase P one-step reverse transcription quantitative real-time PCR (RT-qPCR) (Table 2) to ensure proper sampling and RNA extraction when using different swab types and to evaluate RNA extraction protocols using samples from SARS-CoV-2 non-infected participants. Synthetic hRNase P RNA was used for the standard curves and as positive control.

SARS-CoV-2 assays. For SARS-CoV-2 detection, the protocols for hydrolysis probe-based, one-step, reverse transcription quantitative real-time PCR (probe RT-qPCR) from the Institute of Virology, Charité, Berlin, Germany, targeting the envelope (E) and the RNA dependent RNA polymerase (RdRp) genes of SARS-CoV-2 were utilized¹¹. These protocols were further modified to establish intercalating dye-based, one-step, reverse transcription quantitative real-time PCR (SYBR RT-qPCR). Assays are detailed in Table 2. The prediction of the melting temperature (T_m) was performed in silico using the software uMELT Quartz (<https://dna.utah.org/umelt/quartz/>)¹⁴. To establish the SYBR RT-qPCR, different primer concentrations (for E gene) and annealing temperatures (58 °C as described at the original protocol and 60 °C for all genes) were tested (Table 1).

Synthetic RNA fragments from SARS-CoV-2 target genes were used as positive controls and for the generation of the standard curves for all assays.

Data analysis. LightCycler 480 II software was used and threshold cycles (Ct) were calculated applying the second derivate maximum method (mathematical approach to calculate the cycle where the second derivative of the real-time fluorescence intensity curve reaches the maximum). All samples, including the standard curves, were measured in triplicates (technical replicates). Synthetic positive controls for each gene (used as serial dilution) were measured to allow: (i) Linear regression analysis (plus goodness-of-fit (R^2)) using mean Ct values and respective virus concentration (log copy number), (ii) Limit of detection (LOD) was interpolated from a parametric logistic regression, representing the lowest number of copies detected. All analysis was performed with GraphPad Prism Software (version 9.0.2). All data is displayed in the text as mean and \pm standard deviation, in figures the individual data points and means are indicated.

All methods were carried out in accordance with relevant guidelines and regulations.

Received: 7 December 2020; Accepted: 21 May 2021

Published online: 07 June 2021

References

- Patel, R. *et al.* Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: Value of diagnostic testing for SARS-CoV-2/COVID-19. *mBio*. <https://doi.org/10.1128/mBio.00722-20> (2020).
- Cheng, M. P. *et al.* Diagnostic testing for severe acute respiratory syndrome-related coronavirus-2. *Ann. Intern. Med.* <https://doi.org/10.7326/M20-1301> (2020).
- WHO. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance. World Health Organization. <https://apps.who.int/iris/handle/10665/331329> (2020).
- ECDC. Laboratory support for COVID-19 in the EU/EEA. *European Centre for Disease Prevention and Control*. <https://www.ecdc.europa.eu/en/novel-coronavirus/laboratory-support> (2020).
- CDC. Interim guidelines for clinical specimens for COVID-19|CDC. *Centers Dis. Control Prev.* **2019**, 1–5 (2020).
- RKI—Coronavirus SARS-CoV-2—Hinweise zur Testung von Patienten auf Infektion mit dem neuartigen Coronavirus SARS-CoV-2. *Robert Koch Institut*. https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Vorl_Testung_nCoV.html (2020).
- McCulloch, D. J. *et al.* Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection. *JAMA Netw. Open* **3**, e2016382 (2020).
- Nesteruk, I. Long-term predictions for COVID-19 pandemic dynamics in Ukraine, Austria and Italy. *medRxiv* <https://doi.org/10.1101/2020.04.08.20058123> (2020).
- Ciufolini, I. & Paolozzi, A. Prediction of the time evolution of the Covid-19 Pandemic in Italy by a Gauss Error Function and Monte Carlo simulations. *medRxiv* <https://doi.org/10.1101/2020.03.27.20045104> (2020).
- Parikh, B. A., Wallace, M. A., McCune, B. T., Burnham, C.-A.D. & Anderson, N. W. The effects of “Dry Swab” incubation on SARS-CoV-2 molecular testing. *J. Appl. Lab. Med.* <https://doi.org/10.1093/jalm/jfab010> (2021).
- Corman, V. M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* **25**, 2000045 (2020).
- Dorlass, E. G. *et al.* Lower cost alternatives for molecular diagnosis of COVID-19: Conventional RT-PCR and SYBR Green-based RT-qPCR. *Braz. J. Microbiol.* **51**, 1117–1123 (2020).
- QIAGEN. QIAzol Lysis Reagent Quick-Start Protocol. *QIAGEN*. <https://www.qiagen.com/de/resources/resourcedetail?id=6c452080-142a-44a7-a902-9177dea57d7clang=en> (2011).
- Dwight, Z., Palais, R. & Wittwer, C. T. uMELT: Prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application. *Bioinformatics* **27**, 1019–1020 (2011).

Acknowledgements

All authors are grateful to the participants. This publication was supported by the European Virus Archive Global (EVA-GLOBAL) project that has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No 871029.

Author contributions

Conceptualization: T.L.S., J.H., A.K.; methodology: T.L.S., J.I., J.M.G., J.G., C.H., R.F., J.H., A.K.; data curation: T.L.S., J.I., J.M.G., J.G.; formal analysis: T.L.S.; validation: T.L.S., A.K.; investigation: T.L.S., J.I., J.M.G., J.G.; writing the original draft: T.L.S.; resources: M.B., P.K., A.K.; review and editing: J.H., A.K. All authors reviewed the manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-021-91457-z>.

Correspondence and requests for materials should be addressed to A.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021