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Short Communication

Fast SARS-CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples

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ABSTRACT

Objectives: The gold-standard COVID-19 diagnosis relies on detecting SARS-CoV-2 using RNA purification and one-step retrotranscription and quantitative PCR (RT-qPCR). Based on the urgent need for high-throughput screening, we tested the performance of three alternative, simple and affordable protocols to rapidly detect SARS-CoV-2, bypassing the long and tedious RNA extraction step and reducing the time to viral detection.
Methods: We evaluated three methods based on direct nasopharyngeal swab viral transmission medium (VTM) heating before the RT-qPCR: a) direct without additives; b) in a formamide-EDTA (FAE) buffer, c) in a RNAsnap™ buffer.
Results: Although with a delay in cycle threshold compared to the gold-standard, we found consistent results in nasopharyngeal swab samples that were subject to a direct 70°C incubation for 10 min.
Conclusions: Our findings provide valuable options to overcome any supply chain issue and help to increase the throughput of diagnostic tests, thereby complementing standard diagnosis.

Introduction

The ongoing coronavirus disease 2019 (COVID-19) pandemic due to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) worldwide infection (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports) has imposed an unexpected high burden on the health care systems worldwide leading to an increasing demand for daily diagnostic screening. The current standard assay for diagnosis is based on the extraction of RNA from respiratory samples, especially from nasopharyngeal swab viral transport media (VTM), and subsequent one-step reverse transcription and real-time quantitative PCR (RT-qPCR) targeting one or several sequences from SARS-CoV-2 (Corman et al. 2020). However, this standard procedure usually takes 3.5-4.0 h considering the manual interventions, and there is a risk of reagent shortage in major kit suppliers, particularly for the RNA extraction step. Alternatives to accelerate this procedure have been proposed in consequence, the most efficient relying on Loop-mediated Isothermal Amplification (LAMP) (Esbin et al. 2020).

Here we aimed to simplify the current diagnostic standard for COVID-19 by skipping the RNA extraction step. We tested three

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simple approaches based on direct nasopharyngeal swab VTM heating before the RT-qPCR: a) directly without additives (Direct); b) in a formamide-EDTA (FAE) buffer (Shedlovskiy et al. 2017); and c) in a RNAsnap™ buffer (Stead et al. 2012).

Materials and Methods

The study was conducted at the University Hospital Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain) during March 2020. For the exploratory stage, we selected nasopharyngeal swabs from four COVID-19/SARS-CoV-2 patients and four COVID-19 negative controls. For the validation stage, 90 independent samples (41 COVID-19 positives and 49 negatives) were subjected to the treatment providing the smallest cycle threshold deviations from the standard protocol in the exploratory stage. Sample manipulation and diagnosis, and alternative protocols are detailed in Supplementary materials.

Results

Exploratory stage

The non-template control did not show amplification in any of the protocols both for the SARS-CoV-2 or the internal control (**Table S1**). The positive control for the E-gene amplification yielded positive results in the RT-qPCR experiments of the three alternative protocols. Furthermore, all samples gave positive results for the internal control. When RT-qPCR was carried out on the same four positive samples treated using the alternative protocols (FAE, RNAsnap™ and Direct), we observed amplification of the E-gene in all three conditions, although with a displacement of the Ct values (Table 1). Compared to the standard RNA extraction, we observed an average (± SD) increase in the Ct of 6.9 (± 1.7), 7.8 (± 1.7), and 8.5 (± 1.1) for the Direct, RNAsnap™ and FAE treatments, respectively (**Figure S1**).

Validation stage

Based on these results, we assayed 90 independent VTM samples from 41 COVID-19 positives (**Table S2**) and 49 negatives using the Direct method. We verified that all samples gave positive results for the internal control (average Ct of 29.6 ± 2.5) although the amplification Ct was, on average, slightly larger than that obtained by the standard RNA extraction method in the same samples (average Ct of 27.0 ± 1.5).

Out of the 41 COVID-19/SARS-CoV-2 positive VTM samples, only five did not yield amplification for the E-gene with the Direct treatment. Regarding the internal control results on the extracted RNA of these five samples, we did not observe significant differences when compared with those from the other COVID-19/SARS-CoV-2 positive samples (average Ct of 27.6 ± 1.2 and 26.9 ± 1.6, respectively; p = 0.457). However, their Ct values for the E-gene were larger (average Ct of 34.0 ± 2.0 and 25.7 ± 4.9, respectively; p = 0.0007). Therefore, we considered these five samples as false negatives, corresponding to a false negative rate in the Direct treatment of 12% (95% confidence interval [CI] = 5-28). Considering the 36 samples that were COVID-19/SARS-CoV-2 positive by the two methods, there was an average increase in the E-gene Ct by the Direct method of 6.1 (± 1.6) compared to that obtained by a standard RNA extraction. None of the COVID-19/SARS-CoV-2 negative VTM samples was classified as positive by the Direct treatment. Therefore, the Direct method yielded a sensitivity, specificity and accuracy of 87.8% (95% CI = 73.8-95.9), 100% (95% CI = 92.8-100), and 99.9% (95% CI = 95.7-100), respectively.

Table 1
Main RT-qPCR results for SARS-CoV-2 E-gene amplification.

		Threshold cycle			
Sample	Diagnosis	RNA extraction	Direct	RNAsnap	FAE[a]
1	Positive	19.3	28.0	28.9	27.1
2	Positive	19.7	27.5	27.0	29.7
3	Positive	29.0	34.0	34.7	37.6
4	Positive	31.0	37.0	39.7	38.6
5	Negative	NA	NA	NA	NA
6	Negative	NA	NA	NA	NA
7	Negative	NA	NA	NA	NA
8	Negative	NA	NA	NA	NA
Non-template	-	NA	NA	NA	NA
Positive control	-	30.0	29.2	29.2	32.6

[a] FAE, formamide-EDTA; NA, not available.

Discussion

While the three heating treatments of the sample and direct use in the subsequent detection showed positive amplification of the SARS-CoV-2 E-gene, the Direct method provided the best results and were highly consistent with the COVID-19/SARS-CoV-2 infection diagnosis based on the standard RNA extraction (Fig. 1a) in nearly half of the time (Fig. 1b). We caution that the study was done with a limited number of samples and amplifications should be closely monitored to avoid increasing the false negatives above that of the standard diagnosis based on RNA extractions (Xie et al. 2020). Despite that, diverse empirical assessments of our protocol and that proposed by Fomsgaard & Rosenstierne (2020) revealed that the quantitative results are highly comparable (Calvez et al. 2020). Remarkably, SARS-CoV-2 and SARS-CoV-1 show comparable environmental stability (van Doremalen et al. 2020), and several evidences suggest that SARS-CoV-1 (Geller et al. 2012) and SARS-CoV-2 (Pastorino et al. 2020) lose infectivity above 56 °C within short periods of time, and without any significant effect on the number of viral gene-copies detected by RT-qPCR below 92 °C, even after 30-60 min of pre-treatment (Pastorino et al. 2020). Therefore, we postulate that the Direct protocol at 70 °C for 10 min may also help to diminish the infectiveness of the samples, without significant viral RNA degradation during manipulation.

Finally, we warn that the choice of RT-qPCR kits might have impact on the sensitivity of the Direct protocol. As an example, the average increase in the Ct by the Direct method compared to the standard RNA extraction was 3.5 (± 2.0) using the newly released TaqPath COVID-19 CE-IVD RT-PCR Kit using their ORF1ab assay (Thermo Fisher Scientific).

Authors' contributions

JAF, RGM and CF designed the study. JAF, RGM, AIC, DGM, HGC, TMTST and CF participated in data acquisition. JAF, RGM and CF performed the analyses and data interpretation. LC, AVF, RGM and CF wrote the draft of the manuscript. All authors contributed in the critical revision and final approval of the manuscript.

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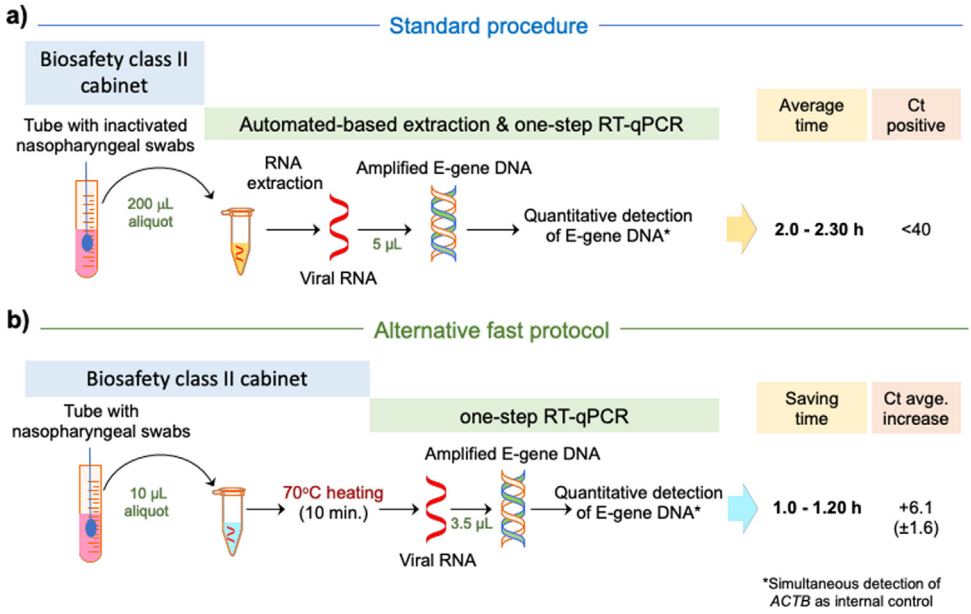

Fig. 1. Schematic diagram of the standard RNA extraction-based protocol (**a**) compared to the alternative fast protocol (**b**) and the timesaving estimates for RT-qPCR-based SARS-CoV-2 testing. Indicated times should be taken as conservative estimates as they will be dependent on the personnel skills and the number of samples being assessed on the experiment. Ct, cycle threshold.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical Approval

The University Hospital Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain) review board approved the study (ethics approval number: CHUNSC_2020_24).

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.05.099.

References

Calvez R, Taylor A, Calvo-Bado L, Fink C. Molecular detection of SARS-CoV-2 using a reagent-free approach. MedRxiv 2020;(May), doi:http://dx.doi.org/10.1101/2020.04.28.20083626.
Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25(Jan (3))2000045.
van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 2020;382(Mar (16)):1564–7.
Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. Overcoming the bottleneck to widespread testing: A rapid review of nucleic acid testing approaches for COVID-19 detection. RNA. 2020;(May), doi:http://dx.doi.org/10.1261/rna.076232.120.
Fomsgaard AS, Rosenstierne MW. An alternative workflow for molecular detection of SARS-CoV-2 – escape from the NA extraction kit shortage, Copenhagen, Denmark, March 2020. Euro Surveill 2020;25(Apr (14))2000398.
Geller C, Varbanov M, Duval R. Human Coronaviruses: Insights into Environmental Resistance and Its Influence on the Development of New Antiseptic Strategies. Viruses. 2012;4(Nov):3044–68.
Pastorino B., Touret F., Gilles M., de Lamballerie X., Charrel R.N. Evaluation of heating and chemical protocols for inactivating SARS-CoV-2. bioRxiv Apr; 10.1101/2020.04.11.036855.
Shedlovskiy D, Shcherbik N, Pestov DG. One-step hot formamide extraction of RNA from Saccharomyces cerevisiae. RNA Biol. 2017;14(Dec (12)):1722–6.
Stead MB, Agrawal A, Bowden KE, Nasir R, Mohanty BK, Meagher RB, et al. RNA snap ™: a rapid, quantitative and inexpensive, method for isolating total RNA from bacteria. Nucleic Acids Res. 2012;40(Jul (20)) e156–e156.0.
Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing. Radiology 2020;(Feb) 200343.