Usefulness of the extraction-free RT-PCR methods for the SARS-CoV-2 diagnostics: An Italian experience

Alexander Domnich

Hygiene Unit, San Martino Policlinical Hospital - IRCCS for Oncology and Neurosciences

Vanessa De Pace

Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences

Beatrice M. Pennati

Department of Health Sciences, University of Genoa Studi di Genova Dipartimento di Scienze della Salute

Patrizia Caligiuri (✉ patrizia.caligiuri@hsanmartino.it)

Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences

<https://orcid.org/0000-0001-6485-2841>

Serena Varesano

Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences

Bianca Bruzzone

Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences

Andrea Orsi

Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences; Department of Health Sciences, University of Genoa

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Abstract

The extraction-based real-time reverse transcription polymerase chain reaction (RT-PCR) is currently the “gold standard” for the SARS-CoV-2 diagnostics. However, some extraction-free RT-PCR techniques have been recently developed. In this study we compared the performance of heated and unheated extraction-free methods with the traditional extraction-based SARS-CoV-2 RT-PCR. The unheated extraction-free showed a perfect agreement with the standard extraction-based RT-PCR. By contrast, the heat-treated technique was associated with an 8.2% false negativity rate. The unheated extraction-free RT-PCR for the SARS-CoV-2 molecular diagnostics is a valuable alternative to the traditional extraction-based methods and may accelerate turnaround times by about two hours.

Introduction

The ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has implied the largest socioeconomic and health emergency worldwide. The availability of accurate COVID-19 diagnostic tests is a cornerstone measure to tackle the pandemic [1–5].

Nowadays, the real-time reverse transcription polymerase chain reaction (RT-PCR) is considered the “gold standard” assay for diagnosis of both symptomatic and asymptomatic cases [6] and included in the protocols issued by several international institutional bodies [7, 8]. These commonly used protocols have a ribonucleic acid (RNA) extraction step that may be seen as an important bottleneck in the routine laboratory testing process [9].

Since March 2020, some manufacturers reported supply shortages for RNA extraction kits driven by the sudden increase in their global demand. This situation called for alternative protocols with similarly high diagnostic accuracy in order to ensure the continuity of testing [10–13]. Consequently, different direct approaches that avoid RNA extraction have been suggested, including heat-processed methods [9, 14].

The objective of this study was to evaluate the diagnostic performance of two different extraction-free RT-PCR techniques and to compare their performance to the traditional extraction-based method in the real-world setting.

Methods

In this study a total of 98 confirmed nasopharyngeal swabs from COVID-19 symptomatic individuals were analysed. These samples were routinely collected in October 2020 and were eluted in the universal transport medium (UTM™, Copan Diagnostics Inc, US) and processed at the regional reference laboratory for COVID-19 diagnostic of the San Martino Policlinico Hospital, Hygiene Unit, Genoa, Liguria, Northwest Italy.

All samples were processed in parallel by using three methods: (i) standard extraction-based (EB) method performed with STARMag 96x4 Viral DNA/RNA 200C Kit (Seegene Inc., South Korea); (ii) RNA extraction-free (EF) method with a heating step (EFh+) and (iii) unheated RNA extraction-free technique (EFh-). For the EB method, total RNA was extracted and set up for RT-PCR by means of the Nimbus IVD Seegene platform using the Allplex™ 2019-nCoV Assay kit (Seegene Inc., South Korea), according to the manufacturer's instructions. For the EFh + method, thermolysis was first performed at 95°C for 5 min and at 55°C for 3 min on Biorad CFX96™ thermal cycler (Bio-Rad Laboratories, US). EFh- method was identical to EFh + unless the thermolysis step.

The material thus obtained was tested for the identification of SARS-CoV-2 by means of a one-step real-time multiplex RT-PCR quantitative assay on Biorad CFX96™ thermal cycler, targeting the nucleoprotein region (N), RNA-dependent RNA-polymerase (RdRp)/spike (S) proteins and envelop (E) region. Samples showing a cycle threshold (Ct) value < 40 for at least two genes were considered positive.

The standard EB technique was considered a reference method against both EFh + and EFh- . A sensitivity with 95% confidence intervals (CIs) was calculated. As the observed Ct values were approximately normally distributed, the average target-specific difference in Ct values between the three techniques was computed and compared by applying the repeated measures analysis of variance (ANOVA) and follow-up Tukey honestly significant difference *post-hoc* test. Data were analysed in R stats packages, v. 4.0.3 [15].

Results

On considering the traditional EB technique as a “gold standard”, EFh- method displayed a perfect accordance with no false negative results [sensitivity of 100% (95% CI: 95.3–100%)]. By contrast, a total of eight (8.2%) swabs treated as per EFh + methodology were deemed false negative, giving a sensitivity of 91.8% (95% CI: 84.1–96.2%).

Figure 1 and Table 1 report the summary distributions and mean differences of Ct values according to the technique used and gene target. The omnibus ANOVA test rejected ($P < 0.001$) the null hypothesis that Ct values figured out by the three methods used had identical means; moreover, all the pairwise *post-hoc* comparisons were also statistically significant ($P < 0.01$). Indeed, the average Ct value for any gene was the lowest in EB group and the highest in the EFh+. Moreover, the highest between-method difference concerned RdRp/S gene, while the lowest one N gene (Table 1). Of note, all the eight false negatives determined by EFh + method had a Ct value > 30, independently from gene target.

Table 1
Mean absolute difference in cycle threshold values, by method used and gene target*

Method	Genet target		
	E	RdRp/S	N
EFh + vs EB	4.92 (4.44–5.40)	5.29 (4.74–5.85)	4.10 (3.71–4.48)
Efh– vs EB	2.26 (1.89–2.63)	2.87 (2.48–3.26)	2.53 (2.13–2.93)
Efh + vs Efh–	2.66 (2.34–2.98)	2.42 (1.99–2.86)	1.56 (1.23–1.90)
*Results are reported as mean difference (95% CIs for paired samples)			

Discussion

In the COVID-19 pandemic context it is crucial to obtain rapid and reliable results to diagnose patients in order to take timely public health decisions. Here, we demonstrated that the EF methods may be valuable alternatives to the traditional EB techniques. This study adds to the growing body of evidence on the utility of extraction-free methods to detect SARS-CoV-2 and to the best of our knowledge it is among the first European studies on the purpose.

In our study the RT-PCR readout obtained by the direct EFh– method had a full agreement with the traditional EB technique. Although there were significant differences between Ct values provided by these two methods, no disagreement in the overall output interpretation was found. On the other hand, we found some negative effect of the heat inactivation on virus detection; this, however, affected only samples with low viral loads ($Ct > 30$). Zou et al. [16] have similarly established that following the heat treatment about 13.0% of positive swabs turned negative and all these false negatives had a Ct value ≥ 37 . Analogously, Grant et al. [17] have observed lower Ct values in unheated samples. Finally, our results are also in line with a recent study by Burton et al. [18] who observed that the heat treatment at 95°C for 5 min (like in our study) has increased an average Ct by 4.5–6 points. The application of lower heating regimens may be therefore useful in order to increase the sensitivity of EFh + method and should be further investigated. For instance, Burton et al. [18] have not registered any significant differences at heating regimens of 56/60°C.

To conclude, our data suggest that EF methods (especially unheated) are a useful tool in providing RT-PCR results in a shorter time, speeding up clinical diagnostics and the subsequent burden on molecular labs and hospitals. Indeed, the average processing time in our study was 270, 163 and 156 min for the standard EB, EFh + and EFh–, respectively. These EF methods therefore urge an internationally recognized protocolization.

Declarations

Availability of data and materials

The whole raw dataset used may be available from the corresponding author on reasonable request and following approval from the San Martino Policlinico Hospital

Funding

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Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki. Ethical review and approval were waived for this retrospective analysis because it was based on the routine COVID-19 testing

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Conflict of interest

The authors declare no conflict of interest regarding this publication.

References

1. Park M, Cook AR, Lim JT, Sun Y, Dickens BL (2020) A systematic review of COVID-19 epidemiology based on current evidence. *J Clin Med* 9:967. <https://doi.org/10.3390/jcm9040967>
2. Pradhan D, Biswasroy P, Kumar Naik P, Ghosh G, Rath G (2020) A review of current interventions for COVID-19 prevention. *Arch Med Res* 51:363-374. <https://doi.org/10.1016/j.arcmed.2020.04.020>
3. Tang YW, Schmitz JE, Persing DH, Stratton CW (2020) Laboratory diagnosis of COVID-19: Current issues and challenges. *J Clin Microbiol* 58:e00512-20. <https://doi.org/10.1128/jcm.00512-20>
4. McArthur L, Sakthivel D, Ataide R, Chan F, Richards JS, Narh CA (2020) Review of burden, clinical definitions, and management of COVID-19 cases. *Am J Trop Med Hyg* 103:625-638. <https://doi.org/10.4269/ajtmh.20-0564>
5. Venter M, Richter K (2020) Towards effective diagnostic assays for COVID-19: A review. *J Clin Pathol* 73:370-377. <https://doi.org/10.1136/jclinpath-2020-206685>
6. Böger B, Fachi MM, Vilhena RO, Cobre AF, Tonin FS, Pontarolo R (2021) Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. *Am J Infect Control* 49:21-29. <https://doi.org/10.1016/j.ajic.2020.07.011>
7. World Health Organization (WHO). Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: Interim guidance, 19 March 2020.

- <https://apps.who.int/iris/handle/10665/331501>. Accessed 5 March 2021
8. European Centre for Disease Prevention and Control (ECDC). COVID-19 testing strategies and objectives. https://www.ecdc.europa.eu/sites/default/files/documents/TestingStrategy_Objective-Sept-2020.pdf. Accessed 5 March 2021
 9. Smyraki I, Ekman M, Lentini A, Rufino de Sousa N, Papanicolaou N, Vondracek M, Aarum J, Safari H, Muradrasoli S, Rothfuchs AG, Albert J, Högberg B, Reinius B (2020) Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. *Nat Commun* 11:4812. <https://doi.org/10.1038/s41467-020-18611-5>
 10. Rhoads DD, Cherian SS, Roman K, Stempak LM, Schmotzer CL, Sadri N (2020) Comparison of Abbott ID Now, DiaSorin Simplexa, and CDC FDA emergency use authorization methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from individuals diagnosed with COVID-19. *J Clin Microbiol* 58:e00760-20. <https://doi.org/10.1128/jcm.00760-20>
 11. Wang X, Yao H, Xu X, Zhang P, Zhang M, Shao J, Xiao Y, Wang H (2020) Limits of detection of 6 approved RT-PCR kits for the novel SARS-Coronavirus-2 (SARS-CoV-2). *Clin Chem* 66:977-979. <https://doi.org/10.1093/clinchem/hvaa099>
 12. Moran A, Beavis KG, Matushek SM, Ciaglia C, Francois N, Tesic V, Love N (2020) Detection of SARS-CoV-2 by use of the cepheid Xpert Xpress SARS-CoV-2 and Roche cobas SARS-CoV-2 assays. *J Clin Microbiol* 58:e00772-20. <https://doi.org/10.1128/jcm.00772-20>
 13. Waggoner JJ, Stittleburg V, Pond R, Saklawi Y, Sahoo MK, Babiker A, Hussaini L, Kraft CS, Pinsky BA, Anderson EJ, Roupahel N (2020) Triplex real-time RT-PCR for severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis* 26:1633-1635. <https://doi.org/10.3201/eid2607.201285>
 14. Fomsgaard AS, Rosenstjerne MW (2020) An alternative workflow for molecular detection of SARS-CoV-2 - escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020. *Euro Surveill* 25:2000398. <https://doi.org/10.2807/1560-7917.es.2020.25.14.2000398>
 15. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>. Accessed 5 March 2021
 16. Zou J, Zhi S, Chen M, Su X, Kang L, Li C, Su X, Zhang S, Ge S, Li W (2020) Heat inactivation decreases the qualitative real-time RT-PCR detection rates of clinical samples with high cycle threshold values in COVID-19. *Diagn Microbiol Infect Dis* 98:115109. <https://doi.org/10.1016/j.diagmicrobio.2020.115109>
 17. Grant PR, Turner ME, Shin GY, Nastouli E, Levett LJ. Extraction-free COVID-19 (SARS-CoV-2) diagnosis by RT-PCR to increase capacity for national testing programmes during a pandemic. <https://www.biorxiv.org/content/10.1101/2020.04.06.028316v1.full.pdf>. Accessed 5 March 2021
 18. Burton J, Love H, Richards K, Burton C, Summers S, Pitman J, Easterbrook L, Davies K, Spencer P, Killip M, Cane P, Bruce C, Roberts ADG (2021) The effect of heat-treatment on SARS-CoV-2 viability and detection. *J Virol Methods* 290:114087. <https://doi.org/10.1016/j.jviromet.2021.114087>

Figures

Figure 1

Distribution of the cycle threshold values by method used and gene target