Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Short communication

Proteinase K treatment in absence of RNA isolation classical procedures is a quick and cheaper alternative for SARS-CoV-2 molecular detection

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ABSTRACT

The World Health Organization (WHO) has declared a pandemic of COVID-19, the disease caused by the recently described SARS-CoV-2. The relevance and importance of mass diagnosis in order to find the asymptomatic individuals is widely recognized as a mandatory tool to reinforce the control measures for monitoring virus circulation and reduce the spreading of SARS-CoV-2. Here, we described quickness and cheaper strategies of direct RT-qPCR (in the absence of RNA isolation) and compared the results to those obtained using standard RNA isolation procedure. The tests varied using pure, diluted samples, combined with Proteinase K (PK) or Lysis Buffer. Our findings showed consistently that PK pre-treated samples in the absence of RNA extraction procedures presents similar results to those obtained by standard RNA isolation procedures. On average, 16 samples extracted with the MagMAX™ CORE Kit, take around 2 h, costing an average of USD 5, the pre-treatment of samples using PK, on the other hand, would cut the value to less than USD 0.30 and reduce the time of procedure in more than 1 ½ hours. The present study suggests the use of PK treatment instead of RNA isolation in order to reduce costs and time in processing samples for molecular diagnosis of SARS-CoV-2.

The COVID-19 pandemic is caused by SARS-CoV-2 (Gorbalenya et al., 2020) and has been responsible for infecting more than 116,521, 281 individuals and cause 2.589.548 deaths in more than 216 territories until March 8th, 2021 (WHO, 2021). The relevance and importance of mass diagnosis in order to find the asymptomatic individuals is widely recognized as a mandatory tool to reinforce the control measures for monitoring virus circulation and reduce the spreading of SARS-CoV-2 (Ulloa et al., 2020; Vandenberg et al., 2020). Molecular assays for viral genome detection using RT-qPCR is considered the "gold standard" method in detecting SARS-CoV-2 infection. However, it is a high-cost and labor technique, which requires time and skilled personnel. The RNA isolation step is laborious and expensive, thus hampering the diagnosis due costs and time for execution especially in developing countries. The use of several reagents and commercial kits and the high demand for COVID-19 diagnostics may induce a lack of chemical materials necessary for performing the tests. The American Society of Microbiology has already expressed concern about the shortage of reagents for COVID-19 testing as well as many other scientific societies and governments (Akst, 2020). The urgency to test more individuals leads to the need to find alternatives with low cost and that reduces the time of analyses. Therefore, the need to simplify procedures, reduce costs, and expand the number of samples analyzed are extremely important. In order to optimize the quickness and costs in the diagnosis process, three strategies of direct RT-qPCR (in the absence of RNA isolation) were evaluated in clinical samples, previously analyzed with a standard procedure using the commercial MagMAX™ CORE Nucleic Acid Purification Kit by Thermo Fisher Scientific. The tests varied using pure, diluted samples, combined with Proteinase K (PK) or Lysis Buffer, in times of three, five and eight minutes and temperatures of 70 °C and 98 °C.

Nasopharyngeal swabs were collected and submitted for routine diagnosis by the local municipalities from suspected patients (n = 135) living in Southern Brazil. All samples were delivered under refrigeration and eluted in 3–5 mL of saline solution, following CDC (USA) guidelines for storage of respiratory specimens (CDC, 2020). Personal data from the patients was not accessed in the present study, following standard ethical regulatory guidelines. Sixty-five out 135 (48.1 %) samples showed positive results for the presence of SARS-CoV-2 RNA using the standard method. For this, viral RNA extraction was performed manually using the MagMAX™ CORE Kit with magnetic racks. For the

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detection of SARS-CoV-2 genome, the one-step RT-qPCR targeting the E gene from the Charité protocol was used[6], as described in Table 1. The results are expressed in Cycle threshold (Ct) in supplementary tables one, two and three.

The first trial was conducted employing different strategies for direct RT-qPCR (in the absence of RNA extraction). These were evaluated in comparison with RT-qPCR results obtained with the standard procedure. Following Fomsgaard and Rosenstierne study (2020), twenty samples (17 positives and 3 negatives for the standard procedure) were tested. The samples were analyzed pure and diluted, 1:1 with PBS, and heated to 98 °C and 70 °C during 3 and 5 min, followed by incubation at 4 °C until the time of analysis, totaling eight trials to each sample. The first assay showed that heat the pure samples for 5 min at 98 °C had been the best parameter. The results showed 85 % and 90 % of agreement between the standard treatment using heat treatments of 70 °C and 98 °C, respectively. The results also showed that diluting the sample with PBS is not ideal, on average, the treatments showed only 86 % of the relation to the standard treatment. Thus, a second strategy was proposed. In the second trial, fifteen positive and two negative samples were submitted to eight trials. The tests were performed to 5 and 8 min, at 70 °C and 98 °C, followed by incubation at 4 °C until the time of analysis. The sample was tested combined with Proteinase K (PK), respecting a 1:21 ratio of PK to each sample (1 µL of PK and 20 µL of the specimen) and with Lysis Buffer, 1:4.5 (20 µL of specimen and 70 µL of Lysis Buffer) both obtained by the MagMAX™ CORE Kit. In the second strategy, heating of the samples combined with PK to 98 °C, SARS-CoV-2 was detected at both times, and Ct values were next to standard procedure. Otherwise, there was no detection with heating to 70 °C, just like in the trials where Lysis Buffer was added.

Considering that the best result of the second strategy was to use PK, the third test was performed using it and incubating for five minutes at 98 °C. Seventy-one positive and sixty-four negative samples were combined with PK. Subsequently, all the samples containing PK were submitted to 98 °C during 5 min, followed by incubation at 4 °C until the time of analysis. The strategy showed that of the fifty-seven positive samples analyzed, only four were not detected. This represents 94.4 % of agreement with standard procedure, presenting an average increase of 2.5 Ct in the RT-qPCR. The no detection of the four samples may suggest the presence of RT-qPCR inhibitors, since they showed a Ct bigger than 37.4 in standard procedure. The Ct mean of the results is expressed in Supplementary Table 3. Analyzing the negatives samples, three of the sixty-four showed positive results, meaning that the test showed 95.3 % of agreement to the standard procedure. The statistical analysis was performed using the Wilcoxon and normality tests. The results showed that the tests are similar concerning the general average, presenting an average result of 35.16 for PK and 33.84 for the standard method. However, the results of the PK test have a smaller standard deviation than the standard procedure, showing 6.58 and 7.96, respectively (Fig. 1).

Analyzing the two assays, the use of pre-treatment with PK in the absence of RNA isolation showed favorable results since it was similar to the classic RNA isolation assessed. Considering laboratories that receive high demand for tests and whose apply standard procedures, adherence to pre-treatment using PK would result in less need for reagents that are in worldwide demand, in addition to the mass reduction of plastics and waste disposal. Thinking about the massive diagnosis of the population, in a country or state, where more than 40 thousand tests are performed per day, there would be a reduction of more than USD 200,000 daily.

Table 1
Primers and probe sets targeting E gene according Charité protocol (Corman et al., 2020).

E gene	Sequence (5′- 3′)
Forward primer	GGAAGAGACAGGTACGTTAATA
Reverse primer	AGCAGTACGCACACAATCGAA
Probe	FAM- ACACTAGCCATCCTTACTGCGCTTCG-BHQ1

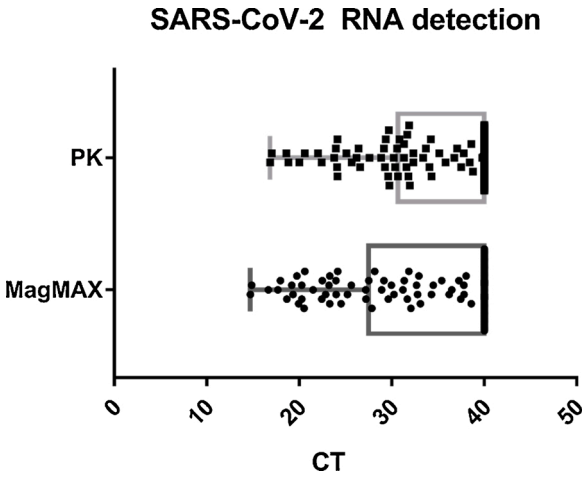

Fig. 1. Comparison between the Ct values for the Standard procedure and PK test (Wilcoxon and normality test).

Our findings showed consistently that PK pre-treated samples in the absence of RNA extraction procedures presents similar results to those obtained by standard RNA isolation procedures. PK is widely used in the purification and extraction of nucleic acids due to broad-spectrum enzymatic activity and can be readily usable for RT-qPCR under emergency situations, decreasing the time of sample treatment and the value. On average, 16 samples extracted with the MagMAX™ CORE Kit, take around 2 h, costing an average of USD 5, the pre-treatment of samples using PK, on the other hand, would cut the value to less than USD 0.30 and reduce the time of procedure in more than 1 ½ hours. The present study suggests the use of PK treatment instead of RNA isolation in order to reduce costs and time in processing samples for molecular diagnosis of SARS-CoV-2.

Author statement

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Ethical statement

Given the important impact from this study could have on Public Health, all nasopharyngeal samples from COVID-19 positive patients were unidentified and not considered as human samples.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest associated with this work.

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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.jviromet.2021.114131.

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