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Short communication

A fast and cheap in-house magnetic bead RNA extraction method for COVID-19 diagnosis

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ABSTRACT

COVID-19 has posed a worldwide public health challenge affecting millions of people in different countries. Rapid and efficient detection of SARS-CoV-2 is essential for pandemic control. Reverse Transcription quantitative PCR (RT-qPCR) of nasopharyngeal swabs is the gold standard method for the virus detection, but the high demand for tests has substantially increased the costs and reduced the availability of reagents, including genetic material purification kits. Thus, the present study aimed to compare two bead-based RNA extraction methods (an in-house and a commercial kit) from nasopharyngeal swabs and RT-qPCR detection of SARS-CoV-2. Twenty-five positive and five negative nasopharyngeal swab samples were subjected to extraction of nucleic acids using both methods in an automated platform. Both protocols revealed a high correlation between Cycle Quantifications (Cqs) ($r = 0.99$, $p < 0.0001$). In addition, the in-house kit was 89.5 % cheaper when compared to the mean cost of commercial RNA extraction kits. The results show that the in-house protocol is an affordable and reliable option for RNA extraction for SARS-CoV-2 detection from nasopharyngeal swabs.

Coronaviruses (CoVs), a subfamily of the Coronaviridae family, are single-stranded, non-segmented, positive RNA viruses. On December 30, 2019, four cases of pneumonia were reported to the CDC (Center for Disease Control) in Hubei province in Wuhan, China and the causative agent, a new type of coronavirus, was isolated and sequenced, the seventh type reported in humans until then, called SARS-CoV-2 (WH-Human_1) (Contini et al., 2020; Helmy et al., 2020; Weston and Frieman, 2020; Zheng, 2020).

Coronavirus Disease 2019 (COVID-19) has an incubation period of 1–14 days, during which time the infected individual is contagious. The most common symptoms are fever, cough, fatigue, dyspnea, sore throat and headache, but the individual may not have any symptoms (asymptomatic) and still spread the virus (Contini et al., 2020; Guo et al., 2020). Most adults and children infected with SARS-CoV-2 have mild flu-like symptoms that last up to two weeks. Some individuals may develop the severe form of the disease, which lasts three to six weeks and progresses with acute severe respiratory syndrome, pneumonia, renal failure, multiple organ failure and death (Guo et al., 2020; Helmy et al., 2020). As of December 12, 2021, SARS-CoV-2 had already infected 270,238,909 people worldwide and resulted in the death of 5,320,878 (Dong et al., 2020).

SARS-CoV-2 has high infectious rate and transmissibility (Yamada et al., 2009; Zhang et al., 2020). For this reason, rapid and accurate diagnostic methods are needed to efficiently identify, isolate and treat positive people to reduce the risk of infection and the mortality caused by the disease (Long et al., 2020). In the current situation, there is a worldwide demand for tests to identify SARS-CoV-2 with reduced costs that also grant fast and accurate results to assist in monitoring outbreaks (Kriegova et al., 2020).

The most used diagnostic method for COVID-19 is the RT-PCR (RT-qPCR) using nasopharyngeal swabs, throat swabs or saliva samples. This method is considered the gold standard for COVID-19 diagnosis by detecting viral RNA in respiratory samples. A variety of genomic regions have been used to detect the virus, including the envelope protein gene

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(E gene) and the nucleocapsid protein gene (N gene) (Sethuraman et al., 2020).

The isolation of nucleic acid from other elements of the sample is a key step for molecular diagnosis, improving efficiency by removing potential PCR inhibitors. For nucleic acid purification, commercial RNA extraction kits are most used. MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific™, Walthan, MA, USA) is a widely used, highly effective magnetic beads based kit (Eisen et al., 2020; Lázaro-Perona et al., 2021; Lungu et al., 2020). However, due to its high demand there is a difficulty in obtaining these materials in addition to the high acquisition costs. Therefore, an in-house extraction protocol was evaluated looking for a fast, easy to perform and repeatable purification under conventional laboratory conditions along with a reduced cost. The present study aimed to compare and evaluate the adapted extraction protocol in comparison with commercially available MagMAX™ CORE Nucleic Acid Purification Kit using samples of nasopharyngeal and throat swabs for COVID-19 diagnosis.

The in-house extraction protocol developed for SARS-CoV-2 detection was adapted from the Bio-On-Magnetic-Beads (BOMB) platform, based on Guanidine Isothiocyanate cell lysis and nuclease inactivation and magnetic beads purification (Drake and Hore, 2020; Oberacker et al., 2019). Information about this platform is available on the website (www.bomb.bio).

Oropharyngeal Rayon swab samples previously collected and stored in 15 mL conical tubes with 2 mL of 0.9 % saline solution were vortexed and transferred to 2 mL tubes. Twenty-five positive samples and five negative samples were selected by convenience and aiming to cover the largest Cq range. After a brief spin, 200 µL of sample was added into the first column of a previously prepared extraction plate for automated RNA extraction (UniXtractor™ deep well plates, Uniscience Corp., Miami, FL, USA). The remaining sample aliquots were stored at −80 °C. The presence of SARS-CoV-2 was verified with the Charité RT-qPCR protocol (Corman et al., 2020).

The wells that received the sample had 100 µL of "Guanidine Isothiocyanate (GITC) lysis buffer" (Table 1), 20 µL of paramagnetic beads solution, 270 µL of isopropanol 100 % and 10 µL of proteinase K (20 mg/mL) (totaling 400 µL).

The bead solution was prepared with GE Healthcare Sera-Mag™-Magnetic SpeedBeads™. To achieve the use concentration, an aliquot of 1 mL of the original bead solution had its buffer removed and beads were washed 3 times with 1xTris-EDTA (TE) Buffer on a magnetic rack and resuspended in 25 mL of 1xTE Buffer.

After lysis and binding step, two washes were performed: the first using 150 µL of isopropanol 100 % and the second one with 200 µL of 70 % ethanol. At the end of the process, RNA was eluted in 100 µL of the elution buffer (Table 2), as previously described (Jolivet and Foley, 2014).

Concomitantly, the same samples were extracted with a commercial kit (MagMAX™ CORE Nucleic Acid Purification, Thermo Fisher Scientific™), according to the manufacturer's instructions. The UniXtractor™ (Uniscience™) equipment was used to extract RNA from the samples by both methods simultaneously.

After the purification, RNA samples were submitted to the RT-qPCR reaction, using the KiCqStart™ One-Step Probe RT-qPCR ReadyMix™ kit (Sigma-Aldrich, San Luis, MI, USA). Cycling conditions included a reverse transcription step at 50 °C for 10 min, followed by denaturation at 95 °C for 3 min, and 45 cycles at 95 °C for 10 s and 60 °C for 30 s.

Cycle quantification (Cq) values for both RNA extraction methods were tabulated and evaluated for normality with graphical analysis (qq Plot and Histogram) and Shapiro-Wilk test. Mean, maximal and minimal Cqs were calculated for each method. Pearson Correlation Coefficient (R) was also calculated. P value lower than 0.05 was considered statistically different. The analysis was made with the aid of *Statistical Analytical Software – SAS Studio.*

The prices of five commercial RNA extraction kits were obtained from different suppliers, and the mean cost of each reaction in US dollars was calculated (Table 3). The cost of the in-house extraction kit was also determined (Table 4).

The mean Cq for the commercial extraction method was 23.14 (ranging from 13.20–33.29) and for the in-house kit was 23.71 (ranging from 13.9–31.38). High correlation of the Cq was found between both extraction methods ($r = 0.99$, $p < 0.0001$) (Fig. 1). No negative samples amplified. The costs of in-house protocol were 89.5 % lower when compared to the mean cost of commercial RNA extraction kits (US$ 6.42 for the mean price of commercial kits and US$ 0.68 for the in-house protocol).

The high demand of molecular diagnostic reagents to detect SARS-CoV-2 worldwide increased the costs and drastically reduced the global availability. On the other hand, massive testing of population is crucial for pandemic control efforts. Results showed that the in-house protocol is a robust and reliable alternative for RNA extraction of oropharyngeal swab samples for SARS-CoV-2 RT-qPCR detection, with an expressively lower cost and with commonly available reagents. Although the protocol in this study was carried out in an automated version, manual extraction can be made in ordinary laboratory conditions without quality losses. It must be emphasized that proper biosafety levels and laboratorial practices must be observed.

Table 1
GITC Lysis Buffer formulation used in oropharyngeal swab samples RNA extraction for SarsCov-2 qPCR detection.

Reagent	Concentration	For 50 mL
GITC	5.5 M	32.5 g
Tris HCl pH 7.6–8.0	50 mM	2.5 mL of 1 M stock
Sarkosyl	2%	1 g
EDTA	20 mM	2 mL of 0.5 M stock
Antifoam	0.1 %	50 µL
MilliQ H2O		25 mL

Oberacker et al., 2019.

Table 2
Elution Buffer formulation used in oropharyngeal swab samples RNA extraction for SARS-CoV-2 qPCR detection.

Reagent	Concentration	For 50 mL
Trisodium citrate	1 M	50 µL
Tween 20	10 %	250 µL
HCl	1 N	21 µL
Nuclease-free water		49.679 mL

Jolivet and Foley, 2015.

Author's contributions

FSP: Conceptualization, methodology, validation, formal analysis, data curation, writing original draft, writing review & editing, visualization.

LSU: Conceptualization, methodology, validation, investigation, writing original draft, writing review & editing.

Table 3
Costs of commercial RNA extraction kits used in SARS-CoV-2 molecular diagnostic.

Supplier	Kit Name	Reference Number	US$ per reaction*
Sigma-Aldrich	GenElute™ Total RNA Purification Kit	RNB100-50RXN	10.04
ThermoFisher	MagMAX™ CORE Nucleic Acid Purification Kit	A32702	4.00
Qiagen	RNeasy Mini Kit	74104	6.60
Promega	SV Total RNA Isolation System	Z3101	5.85
GE(Cytiva)	illustra™ RNAspin	GE25-0500-71	5.60
Mean Cost per Reaction (US$)			**6.42**

* Prices in December/2020.

Table 4
Reagent costs (excluding water) of an in-house protocol for RNA extraction of oropharyngeal swabs for RT-qPCR detection of SARS-Cov-2.

Reagent	Reference Number*	US$ per Reaction**
Guanidine thiocyanate	G9277-100G	0.2822616
Tris Buffer 1 M	648314-100ML	0.0138600
N-Lauroylsarcosine	61739-5G	0.2768000
EDTA 0,5M	324506-100ML	0.0092640
Antifoam 204	A6426-100G	0.0000155
SpeedBeads™ magnetic carboxylate modified particles	GE65152105050250	0.0342400
Ethanol	E7023-1L	0.0184000
2-Propanol	I9516-1L	0.0411600
Tween 20 10 %	11332465001	0.0001070
Trissodium Citrate 1 M	S1804-500G	0.0000005
HCl 1N	H9892-100ML	0.0000011
Total costs per reaction (US$)		**0.68**

* Reference number from Sigma-Aldrich catalogue.
** Prices in December/2020.

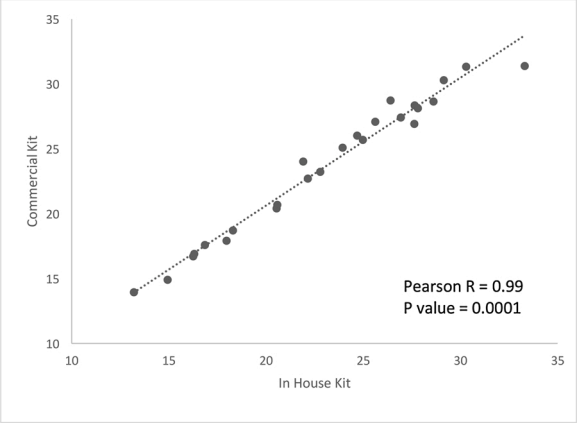

Fig. 1. Scatterplot for Cqs in SarsCoV-2 RT-qPCR positive samples processed by two RNA extraction methods.

CDM, GTM, ECS, EFM, ISB, IFP: validation, investigation, writing original draft

JPAJr: Conceptualization, methodology, resources, supervision, project administration, funding acquisition.

Data availability

Data will be made available on request.

Founding

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Declaration of Competing Interest

The authors declare no conflict of interest

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