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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 (Cq) (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 Friedman, 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 (Kriegerova 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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The isolation of nucleocapsid protein gene (N gene) (Sethuraman et al., 2020).

The 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; Lazaro-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, Univiscence 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™ (Univiscence Corp.) 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 KigQStart™ 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 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 laboratory practices must be observed.

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 1

| 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 |
| Sarcosyl        | 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

| 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.

Table 3

| Supplier          | Kit Name             | Reference Number | US$ per reaction* |
|-------------------|----------------------|------------------|-------------------|
| Sigma-Aldrich     | GenElute™ Total RNA  | RNR100-50RXN     | 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.
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 of this manuscript.

Although we have no conflict of interest, the authors declare no conflict of interest.

References

Contini, C., Di Nuzzo, M., Barp, N., Bonazza, A., de Giorgi, R., Tognon, M., Robino, S., 2020. The novel zoonotic COVID-19 pandemic: an expected global health concern. J. Infect. Dev. 14, 254–264. https://doi.org/10.3855/jidc.12671.
Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T., Bruinskik, S., Schneider, J., Schmidt, M.L., Malsert, D.G., Haagmans, B.L., Van Der Meer, V., Van Den Brink, S., Wijmans, L., Godekersi, G., Romette, J.L., Ellis, J., Zambon, M., Peiris, M., Goossens, H., Reusken, C., Koopmans, M.P., Drosten, C., 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 25, https://doi.org/10.2807/1560-7917.es.2020.25.20.300445, 2000045.
Dong, E., Du, H., Gardner, L., 2020. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect. Dis. https://doi.org/10.1016/s1473-3099(20)30120-1.
Drake, K., Hore, T.A., 2020. SARS-CoV-2 RNA purification from nasal/throat swabs collected in Viral Transfer Media. ROMB.bio 1–4.
Eisen, A.K.A., Demoliner, M., Gularle, J.S., Hansen, A.W., Schallenberger, K., Mallmann, L., Hermann, B.S., Heldt, F.H., de Almeida, P.R., Fleck, J.D., Spilkis, F.R., 2020. Comparison of Different Kits for SARS-CoV-2 RNA Extraction Marketed in Brazil. BioRxiv. https://doi.org/10.1101/2020.05.29.122358.
Guo, Y.K., Cao, Q.D., Hong, Z.S., Tan, Y.Y., Chen, S.D., Jin, H.J., Tan, K.Sen, Wang, D.Y., Yan, Y., 2020. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Mil. Med. Res. https://doi.org/10.1186/s40779-020-00240-6.
Helmly, Y.A., Farey, M., Elaswad, A., Sobib, A., Kenney, S.P., Shehata, A.A., 2020. The COVID-19 pandemic: a comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. J. Clin. Med. 9, 1225. https://doi.org/10.3390/jcm9041225.
Jolivet, P., Foley, J.W., 2014. Solutions for purifying nucleic acids by solid-phase reversible immobilization (SPRI). Igars 2014, 1–5.
Kriegova, E., Fillerova, R., Kvapil, P., 2020. Direct-RT-qPCR detection of SARS-CoV-2 without RNA extraction as part of a COVID-19 testing strategy: from sample to result in one hour. Diagnostics 10, 605. https://doi.org/10.3390/diagnostics1006056.
Lázaro-Porona, F., Rodríguez-Antolin, C., Algaicil-Guillén, M., Gutiérrez-Arroyo, A., Mingorance, J., García-Rodríguez, J., SARS-CoV-2 Working Group, 2021. Evaluation of two automated low-cost RNA extraction protocols for SARS-CoV-2 detection. PLoS One 16, e0246302. https://doi.org/10.1371/journal.pone.0246302.
Long, C., Xu, X., Shen, Q., Zhang, X., Fan, B., Wang, C., Zeng, B., Li, Z., Li, X., Li, H., 2020. Diagnosis of the Coronavirus disease (COVID-19): RT-PCR or CT? Eur. J. Radiol. 126, 108961. https://doi.org/10.1016/j.ejrad.2020.108961.
Lungu, L.M., De Luca, A., Li, J., Bayani, J., Spears, M., Pugh, T.J., Bartlett, J.M.S., 2020. Abstract PO-075: Performance Comparison of Five Extraction Kits for SARS-CoV-2 RNA Extraction, in: Clinical Cancer research. American Association for Cancer Research (AACR). https://doi.org/10.1158/1557-3265.covid-19-po-075 p. PO-075-5.
Mallmann, L., Hermann, B.S., Heldt, F.H., de Almeida, P.R., Fleck, J.D., Spilkis, F.R., 2020. Comparison of Different Kits for SARS-CoV-2 RNA Extraction Marketed in Brazil. BioRxiv. https://doi.org/10.1101/2020.05.29.122358.
Ning, X., Zhong, X., Zhang, X., Wang, C., Zhang, B., Zhang, X., 2020. The novel zoonotic COVID-19 pandemic: an expected global health concern. J. Infect. Dev. 14, 254–264. https://doi.org/10.3855/jidc.12671.
Oberacker, P., Stepper, P., Bond, D.M., Hohn, S., Focken, J., Meyer, V., Schell, L., Sugrue, V.J., Jeunen, G.J., Moser, T., Hore, S.R., von Meyenn, F., Hipp, K., Hore, T.A., Jurkowski, T.P., 2019. Bio-On-Magnetic-Beads (BOMB): open platform for high-throughput nucleic acid extraction and manipulation. PLoS Biol. 17, e3000107. https://doi.org/10.1371/journal.pbio.3000107.
Sethuraman, N., Jeremiah, S.S., Ryo, A., 2020. Interpreting diagnostic tests for SARS-CoV-2. JAMA - J. Am. Med. Assoc. https://doi.org/10.1001/jama.2020.8259.
Weston, S., Friedman, M.B., 2020. COVID-19 knowns, unknowns, and questions. mSphere 5, https://doi.org/10.1128/mSphere.00203-20.
Yamada, Y., Liu, X.B., Fang, S.G., Tay, F.P.L., Liu, D.X., 2009. Acquisition of cell-cell fusion activity by amino acid substitutions in spike protein determines the infectivity of a coronavirus in cultured cells. PLoS One 4, e01310. https://doi.org/10.1371/journal.pone.0001310.
Zhang, T., Wu, Q., Zhang, Z., 2020. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Curr. Biol. 30, 1346–1351. https://doi.org/10.1016/j.cub.2020.03.022 e2.