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Viral RNA extraction low-cost protocol optimized for SARS-Cov2 at AGROSAVIA

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Alejandro Caro-Quintero[1], Camilo Ruiz-Avila[1], Andrea Navarrete[1], Johan Bernal[1], Giuliana Daza[1], Roxana Yockteng[1]

[1]AGROSAVIA/Universidad Nacional de Colombia

Alejandro Caro-Quintero
AGROSAVIA/Universidad Nacional de Colombia

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We use this protocol and it's working

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Abstract

Here we present the second version of an RNA extraction protocol for SARs-cov2 using magnetic beads. This protocol was optimized in AGROSAVIA laboratories and is presented as an alternative to the limitations of commercial viral RNA extraction kits. The viral RNA extraction protocol was adapted from the protocol entitled “SARS-CoV-2 RNA purification from nasal / throat swabs collected in Viral Transfer Media” at https://bomb.bio/ and which is based on methods previously reported by the same group (Oberacker et al 2019). The optimized version here achieves Ct results like the commercial kits and reduces the cost of RNA extraction per sample by 90%, that is, 2000 Colombian pesos or 50 cents per sample. We share this protocol in the hope that it can ensure the sustainability of the diagnosis in Colombia and other countries where it is difficult and expensive to import commercial kits.

REFERENCES

Oberacker, P., Stepper, P., Bond, D. M., Höhn, S., Focken, J., Meyer, V., ... & Hore, S. R. (2019). Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid extraction and manipulation. PLoS biology, 17(1), e3000107.

Yu, F., Qiu, T., Zeng, Y., Wang, Y., Zheng, S., Chen, X., & Chen, Y. (2018). Comparative evaluation of three Preprocessing Methods for extraction and detection of Influenza A Virus Nucleic Acids from sputum. Frontiers in Medicine, 5, 56.

He, H., Li, R., Chen, Y., Pan, P., Tong, W., Dong, X., ... & Yu, D. (2017). Integrated DNA and RNA extraction using magnetic beads from viral pathogens causing acute respiratory infections. Scientific reports, 7, 45199.

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T.,Brünink, S., Schneider, J., Schmidt, M.L. and Mulders, D.G., 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 25(3).

Kumar, M., Mazur, S., Ork, B.L., Postnikova, E., Hensley, L.E., Jahrling, P.B., Johnson, R.and Holbrook, M.R., 2015. Inactivation and safety testing of Middle East respiratory syndrome coronavirus. Journal of virological methods, 223, pp.13-18

Attachments

DOCX

English translation ...

1,013KB

Guidelines

Here we present the second version of an RNA extraction protocol for SARs-cov2 using magnetic beads. This protocol was optimized in AGROSAVIA laboratories and is presented as an alternative to the limitations of commercial viral RNA extraction kits. The viral RNA extraction protocol was adapted from the protocol entitled “SARS-CoV-2 RNA purification from nasal / throat swabs collected in Viral Transfer Media” at https://bomb.bio/ and which is based on methods previously reported by the same group (Oberacker et al 2019). The optimized version here achieves Ct results like the commercial kits and reduces the cost of RNA extraction per sample by 90%, that is, 2000 Colombian pesos or 50 cents per sample. We share this protocol in the hope that it can ensure the sustainability of the diagnosis in Colombia and other countries where it is difficult and expensive to import commercial kits.

Materials

Reagent List

	Reagent
	Guanidine Thiocyanate (GITC)
	Sarkosyl
	Magnetic beas GE Healthcare Sera-Mag Carboxylate-Modified
	Isopropanol
	EDTA
	Ethanol
	Proteinase K (20 mg/mL)

Preparation of reagents

3.1 Paramagnetic beads

a. Take 1 ml of Sera-Mag pearls, be sure to mix well before you start.

b. Wash the beads 3 times in 1 ml of TE buffer (this is to remove sodium azide from the solution), use the magnet to separate the beads from the washes, remove the TE.

c. Re-suspend in 50 ml of TE buffer.

3.2 GITC Lysis Buffer

a . 35.46 g of GITC.

b. 2.5 mL of 1 M Tris HCl pH 7.6-8.0 stock solution

c. 1 g of Sarkosyl.

d. 2 ml of 0.5 M EDTA stock solution.

e. Fill to 50 ml with sterile distilled water.

Reagent prices

The price of the reagents was quoted with national suppliers and the average prices for the commercial houses are presented.

Table 1. Reagents and prices

	Reagent	Quantity	Approximate Price * (pesos)/USD	Number of samples	Price per sample * (pesos)/USD
	Guanidine Thiocyanate (GITC)	500 g	$2 000 000/500	70oo	$286/0.07
	Sarkosyl	25 g	$ 1 470 000/367.50	12500	$117/0.02
	Magnetic beas GE Healthcare Sera-Mag Carboxylate-Modified	15 mL	$3 000 000/750	18750	$160/0.04
	Isopropanol	500 mL	$300 000/75	1250	$240/0.06
	EDTA	250 g	$ 250 000/62.5	1000	$250/0.06
	Ethanol	2500 mL	$200 000/50	6250	$ 32/0.008
	Proteinase K (20 mg/mL)	6 mL	300 000/75	500	$600/0.15
				Total	$1685/0.45 (pesos/USD)

* price in Colombian pesos

Before start

The adjusted protocol presented here is susceptible to other improvements such as those reported for the detection of respiratory viruses (He et. al 2017). 2. Similar protocols and how to build the magnetic racks can be found on the page of the original Authors of the method (https://bomb.bio/). 3. We are not responsible for any accident, infection, or legal implication resulting from the use of protocols adjusted here or hosted at BOMB.bio.

1 **Pre-processing of mucous samples.**

1.1 Dilute 100 µL of the sample in 300 µL of Saline Solution [[M] 0.8 Mass / % volume , vortex mix 00:00:10 . Use only 100 µL of this dilution to star protocol. In step 2.3 add 540 µL isopropanol after incubation.

2 **Sample Processing (in 1.5 ml tubes)**

2.1 To 100 µL of GITC buffer add 12.5 µL of Proteinase K [M] 20 mg/mL], 200 µL of the sample and mix by vortex for 00:00:10 .

2.2 Incubate the sample for 00:10:00 at 60 °C , vortexing every 00:02:00 .

2.3 Place the tube in a rack and add 270 µL of Isopropanol and 40 µL of magnetic beads (see preparation below). Mix by inverting 8 times.

2.4 Place the tube (s) in the magnetic rack for 00:10:00 without moving.

2.5 Without moving the tube from the magnetic rack, withdraw approximately 610 µL of the solution

2.6 Without moving the tube from the magnetic rack, add 150 µL of Isopropanol and wait 30 seconds and remove the isopropanol from the tube.

2.7 Without moving the tube from the magnetic rack, add 200 µL of [M] 70 % (v/v) Ethanol, and remove from the tube.

2.8 Without moving the tube from the magnetic rack, repeat previous washing 2.7 and extract the Ethanol to the maximum.

2.9 Without moving the tube from the magnetic rack, allow drying for approximately 00:05:00 .

2.10 Add 30 µL l of molecular grade water to the tube trying to discharge the liquid on top of the tube walls where the beads are located.

2.11 Remove the tube from the magnetic rack and vortex 00:00:10 to allow the beads to enter the solution and incubate 00:01:00 at room temperature in a normal rack

2.12 Place the tube back in the magnetic rack and for 00:03:00 to 00:05:00 , and transfer 20 µL of the bead-free elution to a new tube and store at -80 °C .