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 We use this protocol and it's working

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MARVICS: A Robust and Safe Magnetic Nanoparticle based RNA Extraction Method Compatible with Phenol-chloroform Inactivated Infectious Samples V.1

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ABSTRACT

Diagnosis and surveillance of emerging pathogens such as SARS-CoV-2 depend on nucleic acid isolation from clinical and environmental samples. Under normal circumstances, samples would be processed using commercial proprietary reagents in Biosafety 2 (BSL-2) or higher facilities. A pandemic at the scale of COVID-19 has caused a global shortage of proprietary reagents and BSL-2 laboratories to safely perform testing. Therefore, alternative solutions are urgently needed to address these challenges. We developed an open-source method called Magneticnanoparticle-Aided Viral RNA Isolation of Contagious Samples (MAVRICS) that is built upon reagents that are either readily available or can be synthesized in any molecular biology laboratory with basic equipment. Unlike conventional methods, MAVRICS works directly in samples inactivated in acid guanidinium thiocyanate-phenol-chloroform (e.g., TRIzol), thus allowing infectious samples to be handled safely without biocontainment facilities.

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Keywords: SARS-CoV-2, MAVRICS, COVID-19, RNA extraction, Magnetic nanoparticle synthesis, Silica magnetic nanoparticles (SiMNP)

MATERIALS

- Bis-Tris **Gold Biotechnology Catalog #B-020**
- TRIzol™ Reagent **Thermo Fisher Catalog #15596018**
- SuperScript™ IV Reverse Transcriptase **Thermo Fisher Catalog #18090010**
- TaqMan™ Fast Advanced Master Mix **Thermo Fisher Catalog #4444556**
- Hydrochloric Acid **Fisher Scientific Catalog #A144S**
- Sodium hydroxide **Sigma-aldrich Catalog #306576**
- Ethanol absolute ≥99.8% AnalaR NORMAPUR® ACS Reag. Ph. Eur. analytical reagent **Contributed by users Catalog #20821.330DP**
- Tetraethyl orthosilicate ≥99.0% (GC) **Sigma-aldrich Catalog #86578**
- Iron (III) chloride anhydrous Extra Pure **Fisher Scientific Catalog #10224390**
- Guanidine Hydrochloride **Fisher Scientific Catalog #BP178-500**
- RNaseOUT™ Recombinant Ribonuclease Inhibitor **Invitrogen - Thermo Fisher Catalog #10777019**
- RNase H **New England Biolabs Catalog #M0297**
- 2019-nCoV RUO Kit **Contributed by users Catalog #10006605**
- Iron(II) chloride tetrahydrate ≥98% **Contributed by users**

1 Silica magnetic nanoparticles (SiMNP) synthesis.

SiMNP synthesis was done following the published protocols in [BOMB.bio: BOMB magnetic core nanoparticles synthesis](#) and [BOMB coating ferrite MNPs with silica oxide](#).

Overnight

2 COVID-19 patient samples.

Oropharyngeal or nasopharyngeal swabs were steeped in 1 mL acid guanidinium thiocyanate-phenol-chloroform (AGPC, e.g., TRIzol Reagent or TRI reagent).

Safety information

Follow CDC or institutional safety guidelines when handling potential infectious samples. AGPC, TRIzol and TRI reagent contain phenol. Follow local safety guidelines when handling and disposing these reagents.

00:01:00

3 Making Bis-Tris Buffer 50 mL

3.1 Dissolve 14.33 g guanidine hydrochloride and 104.6 mg Bis-Tris in 45 mL of 100% ethanol. 00:10:00

Note

If Bis-Tris is not available, it may be substituted by Tris Base (10 mM final concentration)

Note

Add 40 ml of 100% ethanol to the other chemicals, and wait for guanidine hydrochloride to completely dissolve and add the remaining volume of 100% Ethanol.

3.2 Adjust pH (<6.5) with HCl, and adjust the volume with H₂O to 50 mL .
00:05:00

4 Magnetic-nanoparticle-Aided Viral RNA Isolation of Contagious Samples.

4.1 In an Eppendorf tube add 200 µL clinical sample, 200 µL Bis-Tris buffer, mix well by vortexing. 00:01:00

Note

We recommend Lo-Bind Eppendorf tubes or similar low binding tubes.

Safety information

Samples contain phenol. Cap the tubes slowly. Make sure the tubes can be closed securely during vortexing.

4.2 Add 40 μL SiMNP, mix at 1300 rpm 00:05:00

Note

The stock SiMNP is composed of 1 volume of SiMNP in 1 volume of RNase-free water. The stock is further diluted to be used in RNA extraction. Typical dilution ranges from 1:4 to 1:10. The optimal ratio should be determined empirically.

4.3 Spin the tube for 2-3 seconds, settle the SiMNPs on a magnetic stand and remove the supernatant. 00:02:00

Safety information

The supernatant contains phenol. Follow local safety guidelines when handling and disposing these reagents.

4.4 Mix 200 μL of AGPC (TRIzol or TRI reagent) and 200 μL Bis-Tris buffer, add to the SiMNPs, mix well by vortexing. 00:01:00

4.5 Settle the SiMNPs on a magnetic stand and remove the supernatant. 00:02:00

Safety information

The supernatant contains phenol. Follow local safety guidelines when handling and disposing these reagents.

4.6 Add 400 μL 90% ethanol, spin for 2-3 seconds, settle the siMNPs on a magnetic stand and remove the supernatant. 00:02:00

4.7 Repeat Setp 4.6 three more times for a total of 4 ethanol washes 00:06:00

- 4.8 After removing the supernatant from the last ethanol wash, dried the SiMNP on a heat block at 50°C. Keep the lid open, no shaking. Do not elute before the SiMNP are dried.

⌚ 00:20:00

Note

Drying may take less than 20 min. Monitor the appearance of the SiMNP during drying. The SiMNP appear in a rusty brownish color when dried.

- 4.9 To elute the RNA, add 40 µL nuclease-free water, and mix at 1300 rpm

⌚ 00:05:00

- 4.10 Settle the SiMNP on a magnetic stand and transfer the eluted RNA to a new RNase-free tube.

⌚ 00:02:00

- 4.11 Analyze RNA concentration and purity using a Qubit fluorometer or Nanodrop. ⌚ 00:10:00

- 5 **Reverse transcription (RT):** use 4 µL of eluted RNA and follow the instructions for [SuperScript™ IV Reverse Transcriptase](#) adding the RNase H incubation step. ⌚ 01:00:00

Note

User should optimize the input RNA amount.

- 6 **Real-time PCR:** For each 10 µL qPCR reaction mix 1.5 µL cDNA, 0.5 µL [SARS-CoV-2 \(2019-nCoV\) CDC qPCR Probe Assay](#), 5 µL TaqMan Fast Advanced Master Mix, and 1.5 µL nuclease-free water. ⌚ 01:20:00