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MAVRICS: A Robust and Safe Magnetic Nanoparticle based RNA Extraction Method Compatible with Phenol-chloroform Inactivated Infectious Samples V.3

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

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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

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COVID-19 patient samples.
Oropharyngeal or nasopharyngeal swabs are steeped in **1 mL** acid guanidinium thiocyanate-phenol-chloroform (AGPC, e.g., TRIzol Reagent or TRI reagent).

Follow CDC or institutional safety guidlines 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**

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3.1 Dissolve **14.33 g** guanidinium hydrochloride and **104.6 mg** Bis-Tris in **50 mL** of 90% ethanol. **00:10:00**

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

Add 90% ethanol to the chemicals to a final volume of 45-48 ml and wait for guanidinium hydrochloride to completely dissolve by mixing.

3.2 Adjust pH (<6.5) with HCl, and adjust the volume with 90% of ethanol 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**

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

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**

The SiMNP stock is further diluted with RNase-free water to be used for RNA extraction. Recommended dilution for swab samples mixed with TRIzol is 1 volume of fully resuspended SiMNP stock to 4 volumes of RNase-free water. The optimal ratio for other samples should be determined empirically; typical dilution ranges from 1:4 to 1:10.

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

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

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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**

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

4.6

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

It is highly recommended to prepare fresh 90% ethanol before use. Make sure the 90% ethanol container is closed tightly to prevent evaporation.

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, dry the SiMNPs on a heat block at 50°C. Keep the lid open, no shaking. Do not elute before the SiMNPs are dried. **00:20:00**

Drying may take less than 20 min. Monitor the appearance of the SiMNPs during drying. The SiMNPs 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** for **00:05:00** at room temperature.

4.10

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

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00:02:00

Pipet slowly, avoid taking up SiMNPs.

4.11

Analyze RNA concentration and purity using a Qubit fluorometer or Nanodrop. **00:10:00**

4.12 Store RNA at -80°C or use immediately.

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**

User should optimize the input RNA amount.

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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. Run qPCR on a Biorad CFX384 Touch Real-Time PCR Detection System (or similar instrument) using the following program: 50°C for 2 min, 95°C for 2 min followed by 45 cycles of 95°C for 5 sec and 59°C for 30 sec. **01:20:00**

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