SARS-CoV-2 RNA purification from nasal/throat swabs collected in Viral Transfer Media

Dr Kylie Drake (Canterbury Health Laboratories), Dr Tim Hore (University of Otago, BOMB.bio)

This protocol is for the purification of RNA from the SARS-CoV-2 virus collected from nasal/pharangeal swabs collected in 2.5 mL of Viral Transfer Media (VTM).

Following collection of swabs from suspected carriers, and storage in 2.5 mL of viral transfer media, virus is lysed using added "GITC lysis buffer". This buffer consists of 6M guanidine isothiocyanate plus detergent. Viral RNA is then precipitated onto solid phase reversible immobilisation paramagnetic beads, with lysis buffer and extraneous cellular/viral components removed by isopropanol and 70% ethanol washes.

This protocol is based upon the Bio-On-Magnetic-Beads (BOMB) system (Oberacker et al., 2019, https://bomb.bio) with minor modifications to suit collection of viral swabs and RNA purification from them. Although commercial carboxylate coated paramagnetic beads were used in this protocol, other paramagnetic bead types are likely to work as well.

Because of increased volumes, this protocol can only be performed using **0.8 mL deep well** plates.

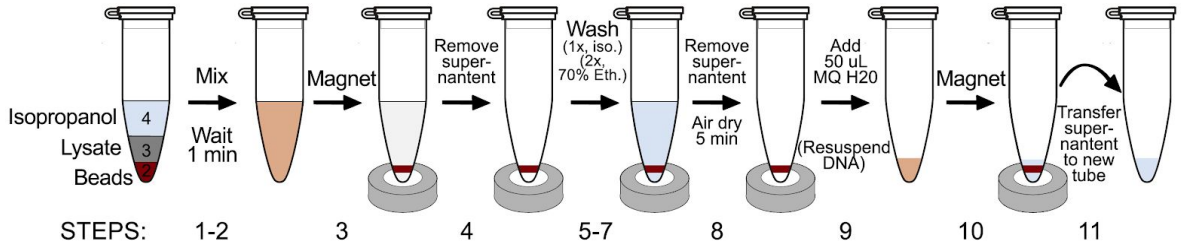
Important safety notes, (1) The GITC lysis buffer contains GUANIDINE ISOTHIOCYANATE, a chemical which can cause burns/sensitivity and produces hydrogen cyanide when mixed with acid (MSDS). **(2)** While GITC + detergent buffers are expected to inactivate coronaviruses (e.g. MERS-CoV, Kumar et al., 2015), this has not been definitively tested for SARS-CoV-2. **(3)** Isopropanol (2-propanol) has been shown to inactivate SARS-CoV-2 (Kratzel et al, 2020) at a minimal final concentration of at least 30 %.

Note: 6M GITC lysis buffer may precipitate at room temperature.

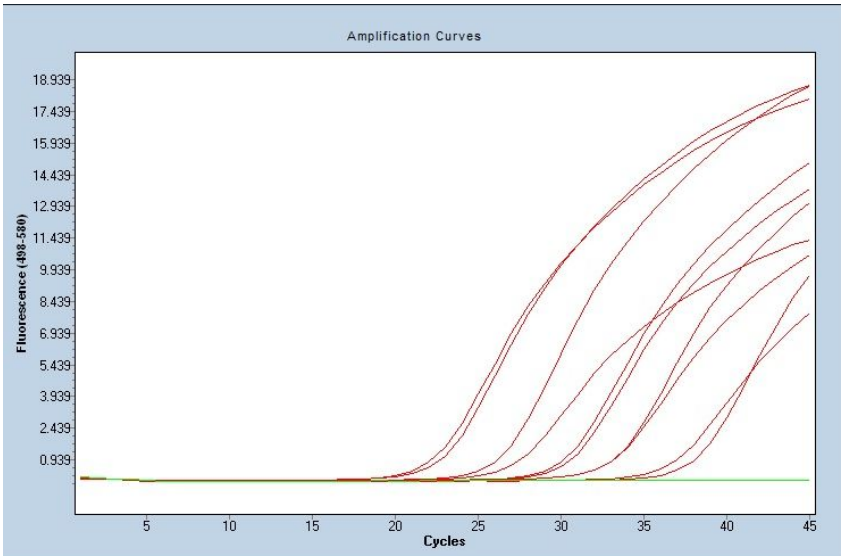
Protocol

1. **Remove** 200 uL of VTM containing nasal/throat swab, and combine with 100 uL of GITC lysis buffer
2. **Add** 40 uL of paramagnetic beads + 270 uL of isopropanol, mix 4-6 times with pipette, wait for 1 min allowing RNA to bind beads
    a. NB, beads and isopropanol can be premixed to improve workflow
3. **Place** plate on magnetic rack for ~10 min, or until solution clarifies
    a. Clarification can be accelerated by slowly pipetting bead solution up and down (thus ensuring beads come in close proximity to magnets)
4. **Remove supernatant** (leave plate on magnet)
    a. NB, when removing supernatant, put pipette tip right the bottom of the plate so all liquid can be aspirated

5. **Wash** with 150 uL of isopropanol
    a. NB, washing means first **add** isopropanol to plate (while on the magnet) and let it sit for ~30 sec before **removing** isopropanol again
6. **Wash** with 200 uL of 70% ethanol (leave plate on magnet)
7. **Repeat** step 6
8. **Leave on magnet to dry** for 5 mins (but do not leave it for much longer as fully dry/flaky beads are difficult to resuspend)
9. **Resuspend** in 20 uL of MilliQ (MQ H2O) and take off magnet, leave for 1 min
10. **Magnet** once more until solution clarifies
11. **Transfer** 5 uL (containing template RNA) to a new plate for reverse transcription and qPCR



REPRESENTATIVE DATA



A verified positive SARS-CoV-2 sample was diluted 10^{-2}- 10^{-7} in VTM and extracted in duplicate using the BOMB method described above. 5 uL of TNA was then amplified using Superscript III one step RT-PCR system with Platinum Taq Polymerase and the 2019-nCoV E-gene real-time PCR assay developed by Corman et al (2020) to assess extraction efficiency. Amplification was achieved from the 10^{-2}- 10^{-6} dilution samples, but not from the 10^{-7} dilution samples.

Reagents

GITC Buffer preparation

GITC Lysis Buffer (stable for at least several weeks at RT, 100 µL per sample, 10 mL for a 96-well plate)

Reagent	Concentration	For 50 mL
GITC	6 M	35.46 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

Viral Transfer Media

Gibco Hanks	860 mL
10% BSA	100 mL (final conc. 1%)
Amphotericin B	10 mL
Penicillin	10 mL
pH 7.2-7.4	

Gibco Hanks Solution

10x Gibco Hanks balanced Salt Solution	200 mL
MilliQ H2O	770 mL
0.4% phenol red	5 mL

Paramagnetic Bead preparation

1. Take 1 mL of beads (SeraMag - details below)
    a. Ensure well mixed before starting
2. Wash 3x in 1 mL of TE buffer (this is to remove the Sodium Azide from storage solution)
3. Resuspend in 50 mL of TE buffer

GE Healthcare Sera-Mag™ Magnetic SpeedBeads™ Carboxylate-Modified Dia.: 1 µm; 3 EDAC/PA5; 15 mL	GEHE451521 05050250	Global Science	$483.00

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