

1st version of a poi S.O.P for sample NA purification

Before start checklist

- Magnetic rack or magnet with rubber band
- MPLC Total Nucleic Acid Isolation Kit (Roche)
- Disposable pipettes
- 50 mL collection tube.
- Two 1.8 ml tubes
- Two 4.5 ml tubes
- One 3.6 ml tube

The following SOP is based on the protocol by Rosenstierne *et al*: Rapid, safe, and simple manual bedside nucleic acid extraction for the detection of virus in whole blood samples. Journal of clinical microbiology, 2018(136). This protocol is developed to be used in a field-setting with no pipettes available, but this can be done depending on availability.

Preparation of Buffers for NA Extraction

To prepare the buffers for an NA extraction, place two 1.8 mL, two 4.5 mL, and one 3.6 mL tubes in a rack and allot as seen in Table 1. If different initial inactivated sample material is available, use the volume ratio to calculate your specific volumes.

Table 1. Buffer preparation for an inactivated sample mixture of 3.2 ml

Tube	Reagent	Volume	Volume ratio to 3.2 ml
1.8 ml	Magnetic glass particles (MGPs)	960 μ L	0.3
1.8 ml	Elution buffer	100 μ L	1.25
4.5 ml	Wash buffer I	4 mL	0.46875
3.6 ml	Wash buffer II	1.5 mL	0.9375
4.5 ml	Wash buffer III	3 mL	0.03125

NOTE: The tubes are stable for at least 1 month after their preparation at room temperature.

NA Extraction

1. Pour the prepared aliquot of MGPs directly into the inactivated sample mixture
2. Place a new lid on the collection tube on the tube containing the sample and mix the contents of the tube by flipping the blood collection tube by hand 5 - 10 times.
3. Place the tube in the magnetic holder or strap a magnet to the tube with a rubber band and keep a finger on the lid to ensure the tube is tightly closed.
4. Flip the magnetic holder with the tube a few times by hand to make sure that all the MGPs are collected at the side of the tube with the magnet.
5. Remove the lid of the tube and discard the contents of the tube either by using a disposable pipette or simply by pouring the contents into a 50 mL collection tube.

NOTE: Avoid aerosols from the 50 mL collection tube by closing the tube with a lid.

6. Pour the prepared aliquot of Wash buffer I directly into the sample tube.

7. Place the lid on the tube and place a finger on the lid to ensure the tube is tightly closed.
8. Remove the tube from the magnetic holder or unstrap the magnet, keeping the lid securely tightened with a finger.
9. Resuspend the MGPs by flipping the sample tube by hand 5 - 10 times.
10. Repeat steps 3-5
11. Pour the prepared aliquot of Wash buffer II directly into the sample tube.
12. Place the lid on the tube and remove the tube from the magnetic holder or unstrap the magnet.
13. Place a finger on the lid to ensure the tube is tightly closed.
14. Resuspend the MGPs by flipping the blood collection tube for a few seconds by hand.
15. Repeat step 3-5
16. Pour the prepared aliquot of Wash buffer III directly into the sample tube.
17. Place the lid on the tube and remove the tube from the magnetic holder or unstrap the magnet.
18. Place a finger on the lid to ensure the tube is tightly closed.
19. Resuspend the MGPs by flipping the blood collection tube by hand 5 - 10 times.
20. Repeat steps 3.5
21. Pour the prepared aliquot of Elution buffer directly into the sample tube.
22. Place the lid on the tube and remove the tube from the magnetic holder or unstrap the magnet.
23. Resuspend the MGPs in the Elution buffer by tapping the blood collection tube 5 - 10 times with a finger.

NOTE: The protocol can be paused here, and the tubes can be stored at -20 °C. Any downstream diagnostic NA amplification assay such as LAMP/RT-LAMP or qPCR/RT-qPCR assays can be used.

NOTE: The NAs will stick to the MGPs, so be sure to use the MGPs in the downstream NA amplification reaction. Mix the MGP suspension before use. After mixing, the MGPs will collect at the bottom of the tube.

Link:

Roche. MPLC Total Nucleic Acid Isolation Kit, Material Safety Data Sheet. Version 5.0. Revision Date: 24.12.2020. Date of last issue: 12.08.2019.

https://lifescience.roche.com/en_dk/products/magna-pure-lc-total-nucleic-acid-isolation-kit.html#documents. 23.03.2021