RNA extraction of COVID-19 suspected samples using QIAamp Viral RNA kit.

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SARS-CoV-2 detection and diagnosis relies on high quality viral RNA extraction from nasopharyngeal swab specimens (commonly containing high viral loads) followed by viral nucleic acid amplification using reverse transcription PCR (RT-qPCR) [1]. The World Health organization (WHO) has produced interim laboratory guidelines directed towards decreasing the risk incurred by laboratory personnel while processing SARS-CoV-2 samples for diagnosis and research activities[2]. Viral nucleic acid extraction methods (as well as most other RNA or DNA extraction methods) normally involve the use of a cell lysis buffer during the initial steps of the procedure which effectively render most samples non-infectious (inactivation step). SARS-CoV-2 nucleic acid extraction should always be performed within a class Ii type A2 certified biological safety cabinet located in a closed-off area of the lab used exclusively for this purpose and one capable of being sealed with tape for decontamination purposes until samples have been inactivated, using BSL2 areas and employing BSL3 discipline [3]. Work with inactivated samples can proceed in BSL2 areas and with BSL2 discipline. QIAamp Viral RNA Mini Kits (Qiagen Cat Number 52906 [4]) provide a fast and easy way to purify viral RNA for use in nucleic acid amplification technologies.

It is essential to ensure that laboratory personnel adhere to biosafety practices. Any testing for the presence of SARS-CoV-2 or of clinical specimens from patients meeting the suspected case definition should be performed in BSL-2 laboratories, and ideally, by staff trained in technical and safety aspects of BSL-3 discipline.

All steps of the QIAamp Viral RNA protocol should be performed quickly and at a room temperature between 15–25°C. **Cell Biology Lab ambient temperature must be set at 22 ºC!**

Biological safety considerations and personal protective equipment (PPE)

Initial processing (before inactivation) of specimens should take place in a class II type A2 certified biological safety cabinet (BSC).

Appropriate disinfectants with proven activity against enveloped viruses should be used (for example, hypochlorite, alcohol, povidone□iodine, chloroxylenol, chlorhexidine, benzalkonium chloride).

Non-propagative diagnostic laboratory work, including PCR, sequencing, and nucleic acid amplification testing (NAAT), on clinical specimens from patients who are suspected or confirmed to be infected with COVID-19, should be conducted according to BSL-2 practices, procedures and discipline in BSL-2 laboratory facilities. Propagative work (for example, virus culture or neutralization assays) should be conducted in a containment laboratory with inward directional airflow (heightened control measures/BSL-3) facilities[2].

Appropriate PPE for **droplet precaution** should be worn when laboratory personnel collect patient samples as oropharyngeal and nasopharyngeal swabs. The minimum PPE for droplet precautions includes

medical mask, **eye protection** (goggles, face shield etc.), disposable **gloves** and solid-front or wrap-around or **back-fastening gown**.

Airborne precautions may be in effect for the collection of nasopharyngeal wash/aspirate, sputum, tracheal aspirate, bronchioalveolar lavage fluid and pleural fluid as well as for propagative work with clinical isolates derived from COVID-19 suspect cases. The minimum PPE for airborne precautions should include **gloves**, **long-sleeved gowns**, **eye protection**, and **fit-tested particulate respirators**.

CDC interim guidelines for the work with risk group 3 agents should be followed for the recollection of nasopharyngeal swab samples (or other respiratory samples) from COVID-19 suspected patients or general population in the case of community sampling.

Procedure

1. QIAmp Viral RNA kit is stored in the RT-PCR area of the Viral & Human Genomics Laboratory.

2. COVID-19 suspect case samples are processed in the Cell Biology area of the Viral & Human Genomics Laboratory.

3. **Vortex** 15 mL conical tubes containing the nasopharyngeal swab and to 2 ml of viral transport media (VTM) for 30 seconds.

4. **Transfer the entire VTM** volume to an appropriately labelled 1.5 mL microcentrifuge tube for permanent storage at -80ºC (Main Lab BSL2 Freezer #2).

5. Before storing the respiratory specimen, **transfer either 140 µL of VTM** (if sample is to be processed individually) **or 20 µL of VTM** (if sample is to be extracted as a pool of 7 samples) to new 1.5 mL microcentrifuge tube labelled with sample or pool ID.

   NOTE: Final specimen pool volume should be 140 µL irrespective of pool size (from 4 specimens to 18 specimens are only recommended for SARS-CoV-2 testing).

6. Prepare enough AVL buffer + carrier-RNA solution for the number of samples/pools to be processed by adding 5.6 µL of **carrier-RNA** (stored in main lab -20ºC freezer, "CMV oligo" carboard box on door panels) to 560 µL of **AVL buffer** (stored in RT-PCR area fridge, inside QIAamp Kit Box) for each sample/pool to be extracted.

7. **Add 560 µL of carrier-RNA + AVL buffer** to each 140 µL of sample/pool and vortex for 15 seconds.

8. **Incubate** at room temperature for 10 minutes and spin down for 30 seconds at 6000 g.

9. **Add 560 µL of molecular grade 96-100% ethanol** and vortex for 15 seconds. Spin for 30 seconds at 6000 g.

10. **Transfer 630 µL** of this volume to a QIAamp column, place in elution microtube and spin at 6000 g for 1 minute.

11. **Discard** flow-through and elution microtube and **transfer an additional 630 µL** of step 9 volume to the same QIAamp column, place in new elution microtube and spin at 6000 g for 1 minute.

12. **Discard** flow-through and elution microtube. Add **500 µL of AW1 buffer** (supplied with kit) and spin at 6000 g for 1 minute.

13. **Discard** flow-through and elution microtube. Add **500 µL of AW2 buffer** (supplied with kit) and spin at **16300 g for 3 minutes**.

14. **Discard** flow-through but **keep elution microtube** and spin again at **16300 g for 1 minute**.

15. **Discard** flow-through and elution microtube. Place spin column on a new pre-labelled 1.5 mL microcentrifuge tube, add **60 µL of AVE buffer** (supplied with kit) and **incubate** at room temperature for 1 minute.

16. Spin column and 1.5 mL microcentrifuge tube at **6000 g for 1 minute**.

17. Use extracted viral RNA immediately or store at -80ºC (Main Lab BSL2 freezer #2) until future use.

Notes

1. Buffers AVL and AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. In case of spills, clean with detergent and water, and then with 1% (v/v) sodium hypochlorite.

2. Viral RNA can be purified from plasma (treated with anticoagulants other than heparin), serum and other cell-free body fluids. Samples may be fresh or frozen. If samples have been frozen these should not be thawed more than once as repeated freeze–thawing of samples leads to reduced viral

titters compromising detection sensitivity. Cryoprecipitates accumulate when samples are subjected to repeated freeze–thaw cycles. This may lead to clogging of the QIAamp membrane when using the vacuum protocol.

3. All steps of the QIAamp Viral RNA protocol should be performed quickly and at a room temperature between 15–25°C. Cell Biology Lab **ambient temperature must be set at 22 ºC**!

4. The QIAamp Viral RNA method isolates RNA molecules larger than 200 nucleotides, recovery of smaller RNA molecules is less efficient and not recommended.

5. The standard source specimen for QIAamp Viral RNA kit are respiratory swab samples. Other specimens may be compatible with the kit. Please refer to the product page for up-to-date information on sample materials that have been tested by QIAGEN[4].

References

1. Esbin, M. N. *et al.* Overcoming the bottleneck to widespread testing: A rapid review of nucleic acid testing approaches for COVID-19 detection. *RNA* vol. 26 771–783 (2020).

2. *Laboratory biosafety guidance related to coronavirus disease (COVID-19): Interim guidance, 28 January 2021*. https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1 (2021).

3. Pastorino, B. *et al.* Evaluation of chemical protocols for inactivating SARS-CoV-2 infectious samples. *Viruses* **12**, (2020).

4. QIAamp Viral RNA Kits. https://www.qiagen.com/us/products/diagnostics-and-clinical-research/sample-processing/qiaamp-viral-rna-kits/.

Revision history

1.0 Original document.