This SOP is associated with Risk Assessment Diagnostic screening of clinical respiratory ACDP Hazard Group 3 SARS-CoV-2:

<https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens>

WARNING: This work involves handling and processing of clinical nasal swab samples from individuals who are suspected of being infected with SARS-CoV-2.

Exposure to SARS-CoV-2 can result in COVID-19

Although viral samples have been heat inactivated, initial steps until addition of lysis buffer need to be performed in a BL3 laboratory in order to avoid exposure to the virus

Safety Information

* Person-to-person spread is thought to occur mainly via respiratory droplets produced when an infected person coughs or sneezes or by contact with droplets and contaminated fomites (need reference!).
* Personal Protective Equipment (PPE) must be worn at all times in the containment facility
* Anyone entering the containment facility from the lobby must wear the following PPE: green gloves, gown, googles, mask and orange gloves
* All unsealed work must be undertaken in a biological safety cabinet (Class I MBSC)
* To prevent fatigue and operator error, work only in pre-arranged 5-hour shifts. If you are in the middle of a batch, stop and hand over to the next person.

Restrictions

Access to the containment facility is restricted to authorised personnel only:

* Only those with health clearance and have been signed off as trained and competent are allowed to undertake this work within the CL3 Facility.

**Equipment, Consumables & Reagents Required**

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| **Equipment** |
| **Pipettes:** P1000 & P200 multi-channel (recommended) or P1000 & P200 Single Channel. |
| 96 Well Magnetic Plate Separator. |
| Multi-channel vacuum aspirator. |

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| **Consumables** | **Number Required** |
| **Pipette Tips:** STARLab 1000ul Barrier | ~ 4 Boxes |
| **Pipette Tips:** STARLab 200ul Barrier | ~ 4 Boxes |
| **Pipette Tips:** P200 No Barrier (for vacuum aspirator) | ~ 4 Boxes |
| 0.2 mL 96 well v-bottom plate | 1 |
| 96 well plate seal | 1 |
| STARLab Reagent Reservoir | 5 |

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| **Reagents** | **Amount Required** |
| RNAdvance Proteinase K Solution | 7ul / sample |
| RNAdvance Lysis LBF Buffer | 105ul / sample |
| RNAdvance Bind BBD | 3.5ul / sample |
| RNAdvance Wash WBE Soloution | 280ul / sample |
| 70% EtOH | 560ul / sample |
| Nuclease-Free Water | 80ul / sample |

**Notes**

* Example volumes in tables for solutions generate enough excess to account for pipetting error. If using a different number of samples, mix 10% excess volume.
* If using a multi-channel pipette, dispense solutions into reagent reservoirs prior to adding to plate.

**Procedure**

In the BL3 laboratory

1. Thaw samples (stored at -80º) inside the Class I MSC and pipette 140ul of sample into 96 deep well plate
2. Mix a volume of Proteinase K and LBF Lysis buffer enough for the number of samples.

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| Reagent | Volume per Sample | e.g. for 48 samples | e.g. for 96 samples |
| Proteinase K Solution | 7 uL | 350 uL | 700 uL |
| Lysis LBF Buffer | 105 uL | 5.25 mL | 10.5 mL |
| Final Solution Volume | 112 uL | 5.60 mL | 11.2 mL |

1. Add 112uL of Proteinase K/LBF mixture to each well and mix by pipetting up and down 5 times.
2. Incubate plate at room temperature for 10 minutes.
3. Seal the plate and place it in a plastic bag. Wipe bag with 70% ethanol
4. Place bag with plate in the tray for removal of inactivated samples (“transport tray”) located in the BL3 lobby

The samples can now be taken out of the CL3 laboratory and the rest of the work should be carried out in the “RNA extraction room”.

After receiving the samples brought by overseer from the BL3 lobby:

1. Mix a volume of BBD Bind and Isopropanol enough for the number of samples. Vortex BBD for 30 seconds to resuspend beads.

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| Reagent | Volume per Sample | e.g. for 48 samples | e.g. for 96 samples |
| Bind BBD | 3.5 uL | 175 uL | 350 uL |
| Isopropanol | 140 uL | 7 mL | 14 mL |
| Final Solution Volume | 143.5 uL | 7.175 mL | 14.35 mL |

1. Add 143.5ul if BBD/Isopropanol solution to each sample and mix by pipetting up and down 5 times.
2. Incubate plate at room temperature for 5 minutes.
3. Place plate on magnetic separator and wait for 5 minutes for beads to form a pellet.
4. Fully remove the supernatant with aspirator while keeping the plate on the magnet.
5. Remove the plate from the magnetic separator and add 280ml of Wash WBE to each well. Mix by pipetting up and down 5 times to resuspend the beads.
6. Place plate on magnetic separator and wait for 3 minutes for beads to form a pellet.
7. Fully remove the supernatant with aspirator while keeping the plate on the magnet.
8. Remove the plate from the magnetic separator and add 280ml of 70% EtOH to each well. Mix by pipetting up and down 5 times to resuspend the beads.
9. Place plate on magnetic separator and wait for 3 minutes for beads to form a pellet.
10. Fully remove the supernatant with aspirator while keeping the plate on the magnet.
11. Remove the plate from the magnetic separator and add 280ml of 70% EtOH to each well. Mix by pipetting up and down 5 times to resuspend the beads.
12. Place plate on magnetic separator and wait for 3 minutes for beads to form a pellet.
13. Fully remove the supernatant with aspirator while keeping the plate on the magnet. *Take extra care to remove all EtOH at this point to avoid contamination of RNA.*
14. Allow beads to air-dry at room temperature for 5 minutes.
15. Remove plate from magnetic separator and elute RNA by adding 80ml of nuclease-free water. Mix by pipetting up and down 5 times to resuspend beads.
16. Place plate on magnetic separator and wait 1 minutes for beads to form a pellet.
17. Transfer eluted RNA away from the beads into a 0.2mL 96 well plate.
18. Seal plate and store at -80º