**DECON RNA Extraction Protocol**

**Purpose**

To isolate viral RNA from clinical environmental swabs of SARS-Cov-2 positive patient rooms for future RT-qPCR analysis.

*This protocol is modified from the QIAamp Viral RNA Mini Kit to minimize aerosols during processing of samples potentially containing SARS-Cov-2 virus. Swabs are collected and stored in Buffer AVL (supplied from kit) to inactivate virus immediately. Additional viral inactivation prior to starting this protocol is not necessary.*

**Materials & Equipment**

* QIAamp Viral RNA Mini Kit
* Sterile 2mL tubes
* 100 µL & 1000 µL pipettors
* Sterile 100 µL & 1000 µL tips
* Microcentrifuge (in BSC)
* LabVantage Labels

**Preparation Steps**

1. Turn on biosafety cabinet (BSC) and allow to run for 15 minutes prior to start of extraction
2. Remove samples from –80C and allow to thaw at room temperature.

*Note:* ***do not vortex*** *samples at any point in the protocol as this can generate aerosols*

1. Clean BSC with RNase ZAP and 70% ethanol
2. Check all reagent bottles for precipitate
3. Prepare buffers if necessary by adding the indicated amount of 100% ethanol on the bottle

*Note: only 100% 200 proof ethanol should be used for any reagent preparation in this protocol, not to be confused with cleaning ethanol which is also stored in the flammables cabinet.*

**Documentation Procedure:** *See Infectious Disease LabVantage Training PowerPoint for detailed instructions*

1. While samples thaw, look up the External Participant ID listed on the samples in LabVantage (ex. PR\_0001)
2. Select the Environmental Swab samples in LabVantage for RNA extraction

*Note: we are no longer processing ES15 & ES16 samples, these are to be filed in the appropriate “DECON EXTRA SAMPLES” box and should not have RNA child samples produced.*

1. Select “Create Child Samples” and generate RNA child samples from Environmental Swab parent samples
   1. Check consume parent sample and auto-confirm child sample
2. Select the newly created RNA samples
   1. Print labels
   2. File samples into appropriate RNA box
3. Take labels to BSC and pre-label 2mL tubes for RNA elution

**RNA Extraction Modified Protocol:** *See QIAmp Viral RNA Mini Kit protocol for detailed explanation of procedures*

* *The following steps are to be performed in a Class II Biosafety Cabinet (BSC).*
* *Lab coat and gloves are required at all times when handling SARS-Cov-2 samples.*
* *Gloved hands are to be routinely cleaned with 90% ethanol between steps while at the BSC.*
* *When leaving the BSC to another area of the lab gloves are to be disposed of and changed before continuing the procedure. This is to prevent contamination of the lab with residual SARS-Cov-2 from samples.*

1. While waiting for samples to finish thawing, prepare the appropriate number of spin columns, collection tubes, and prelabeled elution tubes for the entire RNA extraction procedure. Label spin columns before beginning procedure.
2. Briefly spin down all samples to ensure no liquid is on the inner lid.
3. Add 560ul of Ethanol (95- 100%) to the sample and thoroughly mix by gently pipetting up and down 3-4 times.
4. Add 630ul of the sample to the matching prelabeled spin column.
5. Spin the columns at 6000 x g (8000 rpm) for 1 min.
6. Place spin column into a new collection tube. Empty contents of collection tube into liquid waste container and discard collection tube.

*Note: it is very important to not contaminate the bottom of the spin column filter. Should any remaining liquid splash up onto the bottom of the column, pulse spin the spin column apparatus again to remove the liquid.*

1. Add the remaining sample to the column and spin the samples at 6000 x g (8000 rpm) for 1 min.
2. Repeat step 6.
3. Add 500ul of AW1 to the sample and spin the samples at 6000 x g (8000 rpm) for 1 min.
4. Repeat step 6.
5. Add 500ul of AW2 and centrifuge at **full speed (13,200 rpm) for 4 min.**
6. Repeat step 6, placing spin column into a prelabeled 2mL microcentrifuge tube.
7. Add 60ul of AVE, let the AVE sit in the column for approximately 1 min before spinning the samples at 6000 x g (8000 rpm) for 1 min.
8. Before disposing spin column, check that the RNA has been eluted into the 2mL tube.
9. Discard the spin column and place the 2mL tube with the extracted RNA into the appropriate –80C storage.
10. Clean the BSC with ethanol between processing sample sets. Deep clean with RNA zap and ethanol at the end of the day, and empty contents of waste bin from inside BSC.