SOP Title	Extraction of nucleic acids from respiratory specimens for SARS-CoV-2 PCR
SOP no.	CGL-SOP-901
SOP version & status	v1-DRAFT
Scope	CGL Pre-PCR, Pathology Department

	NAME	TITLE	SIGNATURE	DATE
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1 PURPOSE

To extract RNA from 150 µL of Viral Transport Medium obtained from nasopharyngeal swabs for the detection of SARS-CoV-2 in suspected COVID-19 cases.

2 PROCEDURE DESCRIPTION AND BACKGROUND

VTM samples are lysed using Qiagen Buffer RLT in a 1:1 ratio. The RNA in the lysate is precipitated using Beckman Coulter AmpureXP solid-phase reverse immobilisation beads and washed with ethanol to remove impurities. RNA is eluted in 20 µL Illumina RSB for downstream RT-qPCR setup.

3 RESPONSIBILITIES

Role	Responsibility
Operator	Perform the procedure outlined herein Ensure that the instruments used are properly maintained Monitor consumable consumption and stock levels and report these to Supervisor Report non-conformances to Supervisor
Supervisor	Ensure appropriate stock levels Record non-conformances and initiate CAPA where appropriate

4 MATERIALS

4.1 INSTRUMENTS

Instrument	Location	SOP
Hamilton STAR X pre-PCR laboratory	C5.73162	-
BSL2 cabinet	C5.73162	-
VWR Standard Analog Rocker	C5.73162	-
Eppendorf Repeater E3	C5.73162	-
Eppendorf Reference 2 10-100 µL pipette	C5.73162	-
Eppendorf Reference 2 100-1000 µL pipette	C5.73162	-

Eppendorf Research Plus multichannel 1-10 µL	C5.73162	-
Vortex Stuart SA8	C5.73162	-
Sprout microcentrifuge	C5.73162	-
Eppendorf plate centrifuge	C5.73162	-

4.2 REAGENTS

Reagent / Kit Component	Item no.	Location
Qiagen Buffer RLT	79216	RT
Qiagen Carrier RNA	1081147	RT / -20°C
Ethanol 99.9% (Fisher Scientific)	BP2818-4	Flammable Cabinet
Illumina buffer RSB or TE 10 mM pH8.0		4°C
Beckman Coulter AmpureXP beads	A63882	4°C
Bleach 100% Clorox	196573.008IB	RT
Invitrogen UltraPure Distilled Water DNAse/RNAse free	10977-035	RT

4.3 CONSUMABLES

Consumable	Item no.	Location
Hamilton CO-RE 1000 µL w/o filters (1)	235904	
Hamilton CO-RE 300 µL w/o filters (3)	235902	
Hamilton CO-RE 50 µL w/o filters (2)	235966	
AbGene MIDI storage plate 0.8 mL (2)	AB0765	
Seahorse 250 mL reagent reservoir (3)	RRI-3014	
Hamilton 60 mL reagent reservoir with lid (1)	56694-01	
Eppendorf twin.tec 96 LoBind skirted PCR plate (1)	0030.129.512	
Eppendorf Combitips advanced 10 mL (1)	0030.089.820	
Axygen Reagent Reservoir (1)	RES-V-25-SI	
VWR Falcon tube 50 mL, sterile (2)	89039-656	
Eppendorf ep tips low retention dual filter 0.5-20 µL	0224.930.02	
Eppendorf epTips low retention 20-300 µL	0224.912.45	
Axygen Maxymum recovery 100-1000 µL	T-1000-C-L-R-S	
Invitrogen 1.5 mL non-stick tubes (1)	12450G	
Thermo Adhesive Sealing Sheets	AB-0558	

5 HEALTH & SAFETY

5.1 PPE

Wear the following PPE at all times when performing this procedure:

- Lab coat
- Nitrile gloves
- Closed shoes
- Surgical face mask
- Safety goggles

5.2 HAZARDOUS SUBSTANCES

Substance, contained in	Hazard	control
Sample VTM and supernatant		Process native sample in BSL2 cabinet, wear surgical face mask, lysed supernatant collected in 100% bleach
Buffer RLT		Wear gloves at all times when handling RLT. Immediate rinse exposed skin with running water.
Ethanol		Storage in flammable cabinet, use of limited amounts < 100 mL
Bleach		Wear gloves and safety goggles while handling bleach and the waste container

5.3 WASTE COLLECTION, STORAGE AND DISPOSAL

Solid waste is disposed in yellow bags, disposable tips are disposed in yellow sharps containers. All liquids are collected in the satellite biohazard liquid collection area.

6 WORKING GUIDELINES

Wear appropriate PPE at all times when handling infectious materials! The automation system poses a safety risk (pinching, crushing) – never reach into the liquid handler deck while it is running! If you need to interrupt a run, open the front cover, which will stop the robot. Make sure all liquids are well mixed and at the correct temperature before using them. When loading consumables onto the liquid handler, push all plates and reservoirs to the top left (well A1). Make sure all tip trays are clicked into position. Check that all liquid handler carriers and clicked into position

7 PROCEDURE

7.1 PREPARING RLT BUFFER PLATES

1. **prepare the following items in a BSL2 safety cabinet**
   - 1.1. Falcon tube 50 mL (2)
   - 1.2. Axygen Reagent reservoir (1)
   - 1.3. Eppendorf CombiTip 10 mL (1)
   - 1.4. Qiagen Carrier RNA (1)
   - 1.5. Invitrogen UltraPure Distilled Water DNAse/RNAse free, bottle, (1)
   - 1.6. Pipettes 10, 100, 1000, multichannel 10 µL + tips
   - 1.7. Eppendorf tube 1.5 mL (1)
2. **Make carrier RNA stock dilution 1 µg/µL**
   - 2.1. Add 1350 µL of water to the Qiagen carrier RNA tube.
   - 2.2. Vortex for 10 s
   - 2.3. spin 20 s using the microcentrifuge

3. **Prepare RLT buffer with Carrier RNA**
   - 3.1. Pour 35 mL of Buffer RLT into a 50 mL Falcon tube
   - 3.2. Add 117 µL (1 µg/µL) of stock carrier RNA to the Falcon tube
   - 3.3. Close the tubes

3.4. Shake on VWR Standard Analog Rocker for 10 min (set to speed 7, tilt 8, time 7)
3.5. This solution is sufficient for 2 MIDI plates.

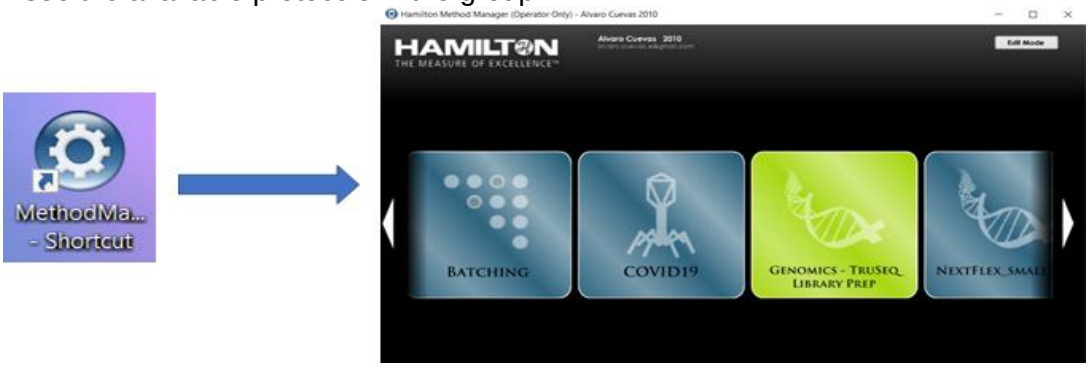
4. Prepare RLT MIDI plates
4.1. Pour the RLT-carrier RNA solution into the Axygen 8ch Reagent reservoir
4.2. Using the Eppendorf Repeater, dispense 150 µL of RLT-cRNA into each well of a 0.8 mL MIDI plate.
4.3. Seal using an adhesive plate seal and store at RT until further use.

7.2 PREPARING THE SAMPLES

1. Refer to Pathology Instructions how to handle VTM swabs of infectious samples.
2. Add 150 µL of sample VTM to each well of the RLT-MIDI plate in a BSL2 cabinet
3. Seal the plate using an adhesive plate seal

7.3 RUNNING THE EXTRACTION ON HAMILTON STAR

1. Start the liquid handler
1.1. If the instrument and control PC are already switched on, move to step 2.
1.2. Switch the PC on
1.3. Locate the power switch on the left side of the instrument's front panel and switch on.
1.4. Once the PC has finished booting, log in (User – Hamilton, password – hamilton1).
1.5. On the Desktop, look for "Method Manager Application" and double click to launch the method manager.
1.6. In the method manager, look for Method Group – COVID19. Click on COVID19 to see the available protocols in the group.



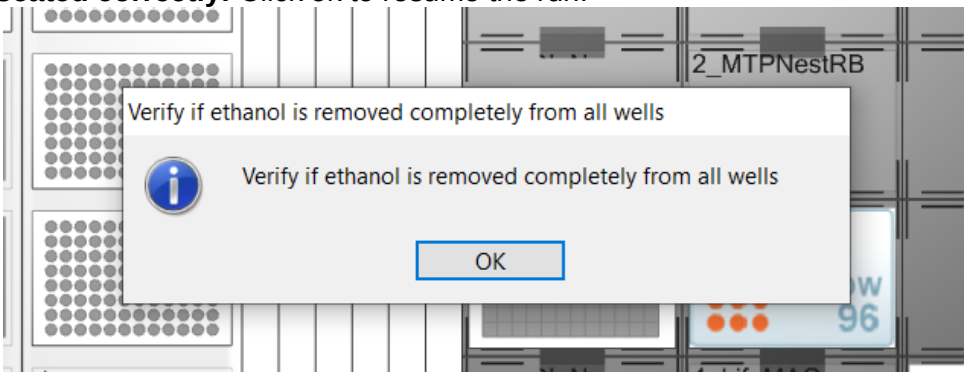
1.7. Based on number of samples to be processed (24 or 96) Click on respective Layout tab to see the labware positions and setup the deck layout.

1.8. Once the deck is set close the front cover and click on Run to start the method.

2. **Prepare reagents**
   2.1. Prepare an 80% Ethanol solution (100 mL)
   2.2. Bring Ampure XP to RT by rocking for 30 min
3. **Load tips**
   3.1. Place 1 tray of CO-RE 1000 µL tips into position 1 (back) of the carrier 1
   3.2. Place 3 trays of CO-RE 300 µL tips into positions 1-3 of carrier 2
   3.3. Place 2 trays of CO-RE 50 µL 1-3 of the carrier 3
4. **Load Reagents**

   4.1. Pour 80 mL of a freshly made 80% Ethanol solution into a Seahorse 250 mL reservoir and put it on carrier 4 position 3

   4.2. Pour 40 mL RSB into a Seahorse reservoir and put on carrier 4 pos 4
   4.3. Pour 30 mL bleach into a Seahorse reservoir and put on carrier 4 pos 5
   4.4. Pour 50 mL of AmpureXP beads into a Hamilton 60 mL reservoir and put the reservoir into carrier T1 position 5
5. **Load remaining labware**
   5.1. Make sure that the plate magnet is correctly seated in carrier 5 position 4. It needs to sit flat in the plate bed!
   5.2. Place one 0.8 mL MIDI plate into carrier 6 position 4
   5.3. Place one Eppendorf twin.tec 96w PCR plate into carrier 7 position 5
6. **Load samples**
   6.1. Spin the sample plate for 1 min at 2,000*g
   6.2. Carefully remove the plate seal from the sample plate
   6.3. Place the sample plate into carrier 7 position 4
7. **Starting the instrument run**
   7.1. On the PC in the Method Manager application, check that your deck layout matches the screen
   7.2. Once the deck is setup as mentioned in layout, click on RUN to start the method.
   7.3. After the 2nd Ethanol wash, there will be a pop-up dialogue, asking the operator to check if 1) the ethanol has been removed completely from all wells of the MIDI plate and 2) to assess the presence of a pellet in all wells. At this step, it is safe to open the front cover and take out the sample plate for verification. Once the above points are verified, place the sample plate back onto the magnet position **and make sure it is seated correctly!** Click ok to resume the run.



8. **Finishing the instrument run**
   8.1. Remove the extracted sample Eppendorf twin.tec 96w PCR plate from carrier 7 position 4. Seal it with a foil seal and place it on a cooling block, ice or at 4°C
   8.2. Check the MIDI ethanol waste plate for any bead pellets. If there are no bead pellets present, discard, otherwise retain the plate and manually remove the residual ethanol from the wells having pellet. Let the wells airdry for 7min at RT and add 22ul of RSB and resuspend the pellet with the help of plate shaker by shaking it 1800rpm

for 2min. Incubate the plate for 4min at RT and magnetize the plate for 3min. Collect the clear supernatant and transfer it back to correct well position in extracted sample plate.

8.3. Dispose the liquid waste in the biohazard satellite liquid collection area.
8.4. Dispose the tips, sample and waste plate in the solid Biohazard collection area.
8.5. The reservoirs for beads, ethanol and RSB can be reused if needed.

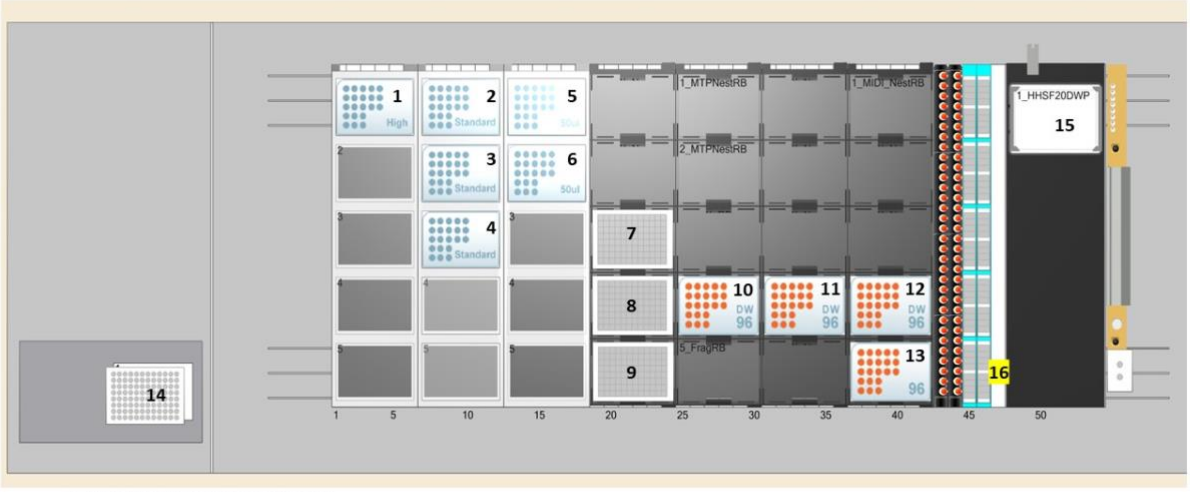
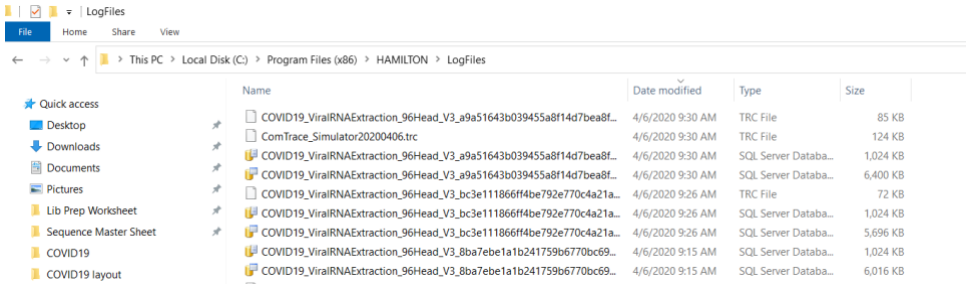


Figure 1: Deck layout before starting the run

8 SAMPLE TRACKING AND DATA RECORDING

The instrument is recording a sample tracking log that can be found in this location: C:\Program Files (x86)\HAMILTON\LogFiles



9 CHANGE HISTORY

Version	Changes	by	Date
1	Initial version	--	--

10 REFERENCES

11 RELATED DOCUMENTS

12 APPENDICES