Manual Extraction of Viral RNA using HI-Media kit

Standard Operating procedure of National Public Health Laboratory Tripura Marg, Kathmandu Nepal

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SUMMARY

This SOP is part of a suit of SOPs that are set up to allow processing of COVID-19 samples in the National Public Health Laboratory (NPHL). Staff should be aware of associated Risk Assessments and have received sufficient training to be able to demonstrate competency before performing this task alone.

This SOP details procedures for **manual extraction of viral RNA using the HiPuraA viral RNA purification kit**. Samples are loaded onto Hielute Miniprep spin columns where the released viral RNA is bound to the spin column membrane. Contaminants are removed by washing before high-quality RNA is eluted, yielding purified intact RNA ready for downstream RT-PCR.

SAFETY

All sentences written in red bold text and denoted with ⚠, indicate a Safety Critical step or comment and as such extra attention must be given when undertaking them.

All staff should be familiar with Risk Assessments and have undertaken specific training

1.0 CROSS REFERENCES

NPHL/COVID-19/RA/001 – General COVID-19 Laboratory RA
NPHL/COVID-19/FORM/001 - Sample tracking form

Manual : HiPurA viral RNA Purification Kit (Himedia)

2.0 TRAINING

All staff should have undertaken specific training before carrying out this procedure.

3.0 REQUISITES and REAGENTS

3.1 Reagents

HiPurA viral RNA Purification Kit MB615

Ethanol (96-100%) Sigma #51976

3.2 Equipment

Collection tubes (2 ml)

* or generic equivalent

Eppendroff tube 1.5 ml

RNase- free pipette tips (aerosol barrier)

Tabletop Microcentrifuge

Vortex Mixer

3.3 Clinical samples

3.3.1 The first steps of this procedure include viral inactivation and **must be carried out in a Class II Biological Safety Cabinet.**

⚠**Before samples can be safely handled on the bench (i.e. out of containment), they must first be inactivated by a validated method (refer to pathogen/task-specific guidance).**

4.0 PROCEDURE

Perform all pipetting steps using **positive displacement or aerosol resistant pipette tips.**

Staff carrying out **viral inactivation in an isolator** should wear a disposable laboratory gown, a single layer of gloves and suitable footwear with dedicated lab footwear e.g. clogs.
For eye protection, wear safety glasses as a minimum; face shield/goggles are preferable.

⚠**Proper attention to the use of required/specified PPE is critical to mitigate the risk of accidental exposure to pathogenic material and chemical hazards.**

4.1 Advance preparation

4.1.1 Carrier RNA is supplied lyophilised with the HiPuraA Viral RNA Kit.

In advance, reconstitute carrier RNA as following with elution buffer (RNase free water) and store at -20 °C in convenient sized aliquots to avoid repeated freeze and thaw.

Number of Preps	Carrier RNA	Elution Buffer
250	3.5 mg	3.5 ml

4.1.2 **Prior to use:** Prepare AVL buffer containing carrier RNA and template internal control and water if required.

Select relevant internal control (IC) depending on downstream application (note, most PCR kits will contain their own IC – this should not be substituted).

Calculate the volume of lysis buffer as 560µl per sample.

Calculate the volume of carrier RNA as 5.6 µl/sample

4.1.3 Dilute Wash solution concentrate (Ws) (DS0012) supplied with HiPuraA Viral RNA Kit. Dilute 75 ml wash concentrate with 225 ml ethanol (96-100%). Record date reconstituted on bottle. Store closed at room temperature.

4.2 Procedure

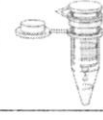
4.2.1 For each batch of patient samples, a **negative extraction control** can be added that is treated identically. For different sample matrices use the following controls:

- For plasma/serum, use pooled human serum/plasma or serum/plasma from known negative sample(s)
- For wet swabs, use VTM
- For dry swabs, use RNAse-free water

4.2.2 Lysis and virus inactivation:

Pre-extraction	1	Take aliquots of pre-prepared **AVL/internal control/carrier RNA mix.** *Choose the correct IC for the downstream RT-PCR assay* **label tube with lab-assigned sample ID number**. Transfer the aliquoted patient- samples (from the refrigerator where aliquots are stored) for PCR *AND aliquots of negative extraction controls (plasma, VTM or water) – if to be included.*	
Within Biosafety Cabinet Class II	2	Take 140 µl of sample (in 1.5ml eppendorff tube) and add 560µl of carrier RNA/Lysis solution (560µl AVL and 5.6µl carrier RNA/sample). **Mix thoroughly** by inverting several times Or Pµlse vortex for 15 seconds Briefly **centrifuge** tube to remove drops from inside of lid. **Incubate** 10 min at room temperature (15-25°C) ⚠CRITICAL STAGE FOR SAFE INACTIVATION OF SAMPLE ⚠ENSURE BUFFER USED IS **AVL** AND TIMINGS ARE ADHERED TO	
	3	Add 10µl of Extraction control, vortex and pµlse spin.	
	4	Carefµlly add 560µl of chilled ethanol (Molecµlar-grade if available, Absolute if required). **Mix** by gentle pipetting Briefly **centrifuge** tube to remove drops from inside of lid. ⚠ENSURE SAMPLES ARE ALLOWED TO REACT WITH ETOH	
	5	**Remove samples from cabinet** and transfer to RNA extraction area.	↓

4.2.3 RNA purification using QIAamp Mini spin columns in RNA extraction area of main lab:

In RNA extraction area	1	**Label** spin column caps with sample tracking number. Apply **630 µl of the solution from step 4 of 4.2.2** to a HiElute spin column (in a 2ml collection tube). Close cap *This marks end of ethanol inactivation.*	
	2	**Centrifuge** at ~8000 rpm (6000 x g) for 1 min.	
	3	Put HiElute spin column into clean 2ml collection tube. Discard filtrate. Add remaining **630 µl of the solution from step 4 of 4.2.2** to a HiElute spin column. Close cap.	
	4	**Centrifuge** at ~8000 rpm (6000 x g) for 1 min	
	5	Put HiElute spin column into clean 2ml collection tube; discard filtrate. Open the HiElute spin column. Add **500 µl** diluted wash solution(WS) (DS0012). Close cap.	
	6	**Centrifuge** at ~8000 rpm (6000 x g) for 1 min.	
	7	Place HiElute spin column in clean 2 ml collection tube; discard filtrate. Open the HiElute spin column. Add another **500 µl** diluted wash solution(WS) (DS0012). Close cap.	
	8	**Centrifuge** at fµll speed 14,000 rpm (20,000 g) for 3 min.	
	9	Put HiElute spin column into clean 2ml collection tube. **Centrifuge** at fµll speed 14,000 rpm (20,000 g) for 1 min.	
	10	Put HiElute spin column in clean **LABELLED 1.5 ml microfuge tube** and open lid. **Add 60 µl Buffer AVE (Elution Sol"). Close cap and incubate 1 min at room temperature.	
	11	Centrifuge at ~8000 rpm (6000 x g) for 1 min. Store eluate at 4 °C until PCR analysis (same day).	
	12	Proceed to PCR	
	13	For longer periods of storage store eluted RNA for up to 1 year at -20°C or -70°C .	

4.2.4 Discard all the sample preparation waste (tubes, tips, filtrate) into a leak-proof bag with absorbent material (e.g. tissue or absorbent spillage granµles) and seal. Treat as dry waste.

CAUTION

⚠**Buffers AVL and AW1 contain guanidine hydrochloride/thiocyanate.**

⚠**DO NOT mix with sodium hypochlorite solutions.**

In event of spillage wipe up with detergent, then rinse with water, then rinse with sodium hypochlorite.

5.0 RESPONSIBILITIES

All trained staff or new staff undergoing training must adhere to this SOP.