University of Tübingen

Institute of Tropical Medicine

Clinical Trial Platform

Process Description

SOP010b-ITM CTP

Sampling, storage and viral RNA extraction of swab specimens

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| Applicability: | This Standard Operating Procedure (SOP) is applicable to all personnel involved in sampling and extraction of SARS-CoV-2 Viral RNA from oropharyngeal swab (OP) |

**Document History:**

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| **Version-No.** | **Description of changes (reasons for changes)** |
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# Purpose

The purpose of this Standard Operating Procedure (SOP) is to describe the purification of viral RNA from oropharyngeal swab that can be used as template for molecular detection and quantification of SARS-CoV-2 virus from COVID-19 patients.

# Definitions

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| BSC | Biological safety cabinet |
| CTP | Clinical Trial Platform; the unit of ITM that performs and manages ITM’s clinical trials activity |
| ITM | Institute of Tropical Medicine |
| OP | Oropharyngeal swab |
| PXXX | Pipette with indicator of maximal volume in µL |
| RNA | Ribonucleic Acid |
| SOP | Standard Operating Procedure |
| UKT | Universitätsklinikum Tübingen |

# Responsibilities

The procedure is intended for use by users trained in molecular biological techniques only. All personnel involved in handling COVID-19 patient samples and in the extraction of SARS-CoV-2 viral RNA is responsible to follow all aspects detailed in this SOP. Laboratory head, physicians, lab managers, and technicians are responsible to provide training on swab handling, storage, and RNA extraction procedure.

# Procedure

Ensure that there is no risk of contamination. Clean the working surface, pipette sets, tube racks centrifuge, heat block etc. with RNase decontamination solutions (RNase-Exitus Plus™ or equivalent). Always wear clean protective clothing, safety goggles, and change gloves frequently and whenever contamination with RNases is possible (e.g. after touching non-cleaned surfaces, door handles, keyboards). Use RNase & DNase-free certified disposable tubes and filter tips.

This SOP describes viral RNA extraction from oropharyngeal swab using QIAamp Viral RNA Mini Kit. Extraction should be performed from fresh specimen or specimen that has been stored in DNA/RNA Shield™ for later extraction. Samples should be equilibrated to room temperature (15-25°C) before starting the extraction. Specimen handling and pretreatment has to be performed in a space designated for DNA/RNA extraction work only. PCR and post-PCR activities are not allowed to be performed in the same room.

## Preliminaries

* Always refer to the kit instruction manual and the protocol for detail information on the principles of the procedure.
* Before starting the procedure, read carefully “QIAamp Viral RNA Mini Handbook, (January 2020)”.
* Nucleic acids should be eluted and stored in a 1.5 ml Eppendorf Safe-Lock Microcentrifuge Tube.
* Prepare a sample sheet in an excel spreadsheet with a proper labelling with the study code, volunteer identification number and the day of the sample collection (study visit)
* To validate the extraction efficiency of the extraction, extraction positive and negative controls should be included during the run.

## Specimen collection and storage

Oropharyngeal swab in 1ml of viral transport medium is received from the clinical team. If nucleic acids extraction is planned on the same day,140 µL is used for viral RNA extraction and an aliquot of 0.5 mL is mixed with 0.5mL DNA/RNA Shield (1:1) for storage or later processing.

### Materials

* 1.5ml Eppendorf Safe-Lock microcentrifuge tube (Eppendorf, cat# 0030120086)
* Screw cap micro tubes 2 mL (Sarstedt, cat# 72.693.005)
* DNA/RNA Shield™ (Zymo research, cat# R1100-250)

### Processing and storage of swab specimens

Specimens received in the laboratory for nucleic acid detection should be processed when received in the laboratory. In case of delay, please refer to the appropriate specimen storage conditions.

* Unscrew the cap, press the tip to the tube’s wall to drain most of the fluid from the tip.
* Discard the swab and transfer 140 µL of sample to a 1.5 ml Eppendorf Safe-Lock microcentrifuge tube for extraction.
* Aliquot 0.5 mL of remaining sample to 0.5 mL DNA/RNA Shield in a 2 mL tube (Sarstedt). Mix thoroughly by inverting the tube several times.
* Store samples mixed RNA Shield at –80° C (for long-term storage).

## Nucleic acids extractions

### Materials and Reagents

* QIAamp Viral RNA Mini Kit (Qiagen, cat# 52906)
* Disposable filter tips
* Pipettes (P1000, P100)
* Absolute Ethanol (AppliChem, cat# A1613,2500PE)
* Internal Control (RealStar SARS-CoV-2 RT-PCR Kit 1.0, Altona Diagnostics, cat# 821005)
* 1.5ml Eppendorf Safe-Lock microcentrifuge tube (Eppendorf, cat# 0030120086)

### Instruments

* Class II biological safety cabinets (BSCs)
* Microcentrifuge
* Vortexer

## Procedural description

### Preparation of carrier RNA, lysis buffer and Buffer AVL

* Add 310μl Buffer AVE to the tube containing 310μg lyophilized carrier RNA to obtain a concentration of 1μg/μl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –30 to –15°C. Do not freeze–thaw the aliquots of carrier RNA more than three times.

*Note: Addition of carrier RNA is critical for samples with low viral load.*

* Check Buffer AVL for precipitate, and if necessary incubate at 80°C until the precipitate is dissolved. Calculate the volume of Buffer AVL–carrier RNA mix needed per batch of samples by selecting the number of samples to be simultaneously processed from Table 1. For larger numbers of samples, volumes can be calculated using the following sample calculation:

**n** x 0.56 ml = **y** ml

**y** ml x 10 μl/ml = **z** μl

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| Where: | **n** = number of samples to be processed simultaneously |
|  | **y** = calculated volume of Buffer AVL |
|  | **z** = volume of carrier RNA–Buffer AVE to add to Buffer AVL |

* Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

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| **No. samples** | **Buffer AVL (ml)** | **Carrier RNA–AVE (μl)** | **No. samples** | **Buffer AVL (ml)** | **Carrier RNA–AVE (μl)** |
| 1 | 0.56 | 5.6 | 13 | 7.28 | 72.8 |
| 2 | 1.12 | 11.2 | 14 | 7.84 | 78.4 |
| 3 | 1.68 | 16.8 | 15 | 8.4 | 84.0 |
| 4 | 2.24 | 22.4 | 16 | 8.96 | 89.6 |
| 5 | 2.80 | 28.0 | 17 | 9.52 | 95.2 |
| 6 | 3.36 | 33.6 | 18 | 10.08 | 100.8 |
| 7 | 3.92 | 39.2 | 19 | 10.64 | 106.4 |
| 8 | 4.48 | 44.8 | 20 | 11.20 | 112.0 |
| 9 | 5.04 | 50.4 | 21 | 11.76 | 117.6 |
| 10 | 5.60 | 56.0 | 22 | 12.32 | 123.2 |
| 11 | 6.16 | 61.6 | 23 | 12.88 | 128.8 |
| 12 | 6.72 | 67.2 | 24 | 13.44 | 134.4 |

Table 1. Volumes of Buffer AVL and carrier RNA–Buffer AVE mix required for the QIAamp Viral RNA Mini procedure

* Buffer AVL–carrier RNA should be prepared fresh and is stable at 2–8°C for up to 48 hours. This solution develops a precipitate when stored at 2–8°C that must be re-dissolved by warming at 80°C before use. Do not warm Buffer AVL–carrier RNA solution more than six times. Do not incubate at 80°C for more than 5 minutes.

### Preparation of Wash Buffer 1: Buffer AW1

* Buffer AW1 is supplied as a concentrate. Before using for the first time, add the 130 ml of ethanol (96–100%).

### Preparation of Wash Buffer 2: Buffer AW2

* Buffer AW2 is supplied as a concentrate. Before using for the first time, add the 66 ml of ethanol (96–100%).

## Nucleic Acids extraction

* Pipette out 560μl prepared Buffer AVL containing carrier RNA into a 1.5 ml microcentrifuge tube.
* Add 6μl of Internal Control (RealStar SARS-CoV-2 RT-PCR Kit 1.0) to the tube.
* Add 140μl of swab sample to the above mixture (containing 560µl of AVL+6µl of Internal control). Mix by pulse-vortexing for 15 s.
* Incubate at room temperature (15–25°C) for 10 min.
* Briefly centrifuge the tube to remove drops from the inside of the lid.
* Add 560μl ethanol (96–100%) to the sample and mix by pulse-vortexing for 15s. After mixing, briefly centrifuge the tube to remove drops from inside the lid.
* First apply 630μl of the 1260µl of the mix to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 ml collection tube, and discard the tube containing the filtrate. Carefully open the QIAamp Mini column and repeat again with the rest of the 1260µl volume.
* Carefully open the QIAamp Mini column and add 500 μl Buffer AW1. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
* Carefully open the QIAamp Mini column and add 500 μl Buffer AW2. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
* Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube. Discard the old collection tube containing the filtrate. Carefully open the QIAamp Mini column and add 60 μl Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min.
* Centrifuge at 6000 x g (8000 rpm) for 1 min.
* Store purified nucleic acids at -80°C
* If reagent is partially used, seal and storage in suitable conditions.
* Empty waste bottle, discard used sample tubes, waste consumables.
* Wipe the internal surface area of the BSC with RNase-away and perform UV decontamination for 20 minutes.
* Sign Log book

# Safety

Swab tube is potentially contagious and needs to be handled as such. Always wear protective clothing, gloves, mask and other protection commensurate with universal precautions. Samples processing must perform in a Class II biological safety cabinet.

When needle stick or cut injuries occur safety regulations of the UKT apply. Instructions and emergency numbers and contact detail of the respective safety officer are displayed near the entry door of all laboratories at ITM where infectious material is handled.

# References

* QIAamp Viral RNA Mini Handbook, (January 2020)