

## SOP for Biosafety in Laboratories testing SARS CoV-2 by Real Time PCR



**Developed By**

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**Aim:** To ensure that the staff working at VRDL, Department of Microbiology, KGMU are adopting appropriate Biosafety Practices.

**Scope:** Laboratory performing Real Time PCR for screening or confirmation of SARS CoV-2 on samples from patients meeting the suspect case definition.

**Principle:** BSL-2 facilities are sufficient for Real Time PCR for SARS CoV-2 (as per WHO laboratory biosafety guidance related to the novel coronavirus).

**General Instructions:**

- All procedures will be performed only by identified designated persons
- Perform initial processing (before inactivation) of all specimens in a validated biological safety cabinet (BSC).
- Earphones, mobile phones are not allowed in the lab.
- Never store food or drink, or personal items such as coats and bags in the laboratory.
- No eating, drinking, smoking and/or applying cosmetics are allowed
- Never put materials, such as pens, pencils or gum in the mouth while inside the laboratory, regardless of having gloved hands or not.
- Thoroughly wash hands after handling any biological material before leaving the laboratory, and any time contamination is known or suspected to be present on the hands.
- Ensure that coverings are placed over any cuts or broken skin prior to entering the laboratory.
- Ensure prior to entry into the laboratory, supplies of laboratory equipment and consumables, including reagents, PPE and disinfectants, are sufficient and appropriate for the activities being performed.
- Ensure that supplies are stored appropriately according to storage instructions and safely to reduce the chance of accidents and incidents such as spills, trips or falls for laboratory personnel.

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- Ensure proper labeling of all biological and chemical agents.
- Ensure work is performed with care, in a timely manner and without rushing. Working when fatigued should be avoided.
- Keep the work area tidy, clean and free of clutter and materials not necessary for the work being done.
- Avoid contact of gloved hands with eyes, mouth and face
- Handle all sharps and needles, if necessary, with care so as to prevent injury and injection of biological agents
- Dispose of any sharps materials (for example, needles, needles combined with syringes, blades, broken glass) in puncture-proof or puncture-resistant containers fitted with sealed covers
- No written document from the BSL3 should come out.

**Sample Receipt Area:**

- 3 ply surgical masks to be worn by all staff in the sample receipt area at all times.
- Ask the person carrying samples to place the thermocol box/ Vaccine carrier on the table kept in area labeled “Sample receipt area”.
- Immediately spray the outside of container with 70% methanol with a spray bottle.
- Receive forms only with gloved hands. It will be better if e-copy of the forms is accepted.
- Sanitize your hands multiple times during the day.
- Do not allow the patients to clutter around within the area.
- Transfer Sample boxes to BSC-II A2 wearing gloves and gowns.

**Sample Collection:**

- Wear full PPE including N-95 masks, gowns, gloves, goggles during sample collection.

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- Collect nasal swab and throat swab only in area labeled “Sample Collection Area”.
- After collecting the nasal and throat swabs (follow SOP for sample collection), immediately place the swabs inside the VTM, close the container tightly and wipe outside of the container by 70% methanol/ 1% sodium hypochlorite.
- Transfer the samples to sample processing area.
- Sanitize your hands after every sample collection and patient handling.

**Sample Processing and Nucleic Acid Extraction:**

- Wear full PPE including N-95 masks, gowns, gloves, goggles during sample processing.
- Spray the BSC-II A2 with 1% sodium hypochlorite, leave it for 15-20 minutes, wipe with 70% alcohol thoroughly and switch on the UV light for 15 minutes.
- Switch off the UV light and open all the boxes within the biosafety cabinet.
- Arrange all necessary items within the BSC after wiping them with 1% sodium hypochlorite followed by 70% alcohol.
- Remove the triple layer packaging from the samples received from various districts and discard it into the autoclavable plastic bag.
- Disinfect the outer surface of the VTM with 70% alcohol/ 1% sodium hypochlorite.
- Match the labeling on VTM with that on the CRF.
- Place the VTMs in a rack and transfer them in a box to BSL-3. If there is no BSL-3, proceed for nucleic acid extraction of the samples for SOP for “nucleic acid extraction”.
- Segregate all waste according to table 1.
- Carry out all aerosol-generating procedures within the BSC.
- After the work is over spray every thing present in the BSC with 1% sodium hypochlorite before discarding.

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- Wipe the BSC with 1% sodium hypochlorite, leave it for 15-20 minutes, wipe with 70% alcohol thoroughly and switch on the UV light for 15 minutes.
- Sanitize your hands multiple times during the process and change your gloves whenever necessary.

**Master mix preparation:**

- Done in clean area designated for master mix preparation.
- Ensure that the person who has handled the samples or has done nucleic acid extraction is not entering the master mix room.
- Don the PPE (gowns, gloves, masks)
- Clean the laminar flow with 1% sodium hypochlorite, leave it for 15-20 minutes and wipe with 70% alcohol thoroughly.
- Prepare the master mix following SOP for “Real Time PCR of nCoV”.
- Dispense the master mix into PCR plates.
- Transfer the loaded plate to template addition room.

**Template addition:**

- Add the extracted Nucleic acid to the loaded plate, noting down the sequence of samples in a worksheet.
- Seal the plate with a plate sealer.
- Transfer the plate to Amplification room.

**Amplification:**

- Set the program in a Real Time PCR machine as per the SOP for “Real Time PCR of nCoV”.
- Enter the plate and run the test.
- Note down the results in a work sheet after the run is over.

**Waste Discarding:**

- Segregate all waste as per Table 1.

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- The waste generated during different steps, how to disinfect them on site and the final method of disposal is given in Table 2.
- **All waste must be autoclaved on site in an autoclave dedicated to waste, before handling to the biomedical waste collector.**
- Wear appropriate PPE.
- Pack all discarded waste in autoclavable non-chlorinated plastic bags and fasten the bag loosely so as to allow steam to enter into it.
- Stick a chemical indicator strip on each packet.
- Check the water levels of the autoclave and look for any signs of corrosion in it.
- Place bags in the autoclave ensuring sufficient space between bags so that steam can pass through the load (**Avoid overloading**).
- Start the autoclave and monitor the cycle parameters. These should be entered into log book.
- Wait for the cycle to be complete, allow the load to cool down, open the autoclave and store it at a secured place unreachable to animals and strangers, till it is carried away by the BMW carriers for final disposal.
- Stick the CI on the log book.
- The BMW should be removed from the BSL-3 atleast twice a day.

#### **Autoclave Maintenance**

- Once weekly use 4 Biological indicators in an autoclave cycle at 4 different places. Allow it to undergo the autoclave cycle. Place the strips into culture broth and incubate for 48 hours at 56°C.
- If sterile: Autoclave is OK
- If growth occurs: Contact the service engineer immediately.
- Ensure that the autoclave is always under Comprehensive Maintenance Contract.

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**Instructions Regarding Use of PPE and N95 masks:**

- Full PPE and N-95 mask are required and will be given only to person involved in SARS-COV2 testing and TB testing.
- 3 play masks are required only for doctors and staff involved with direct patient contact.
- Masks should also be worn by people who have fever and/ or respiratory symptoms.
- There is no additional risk to persons working in other labs.
- People working in offices, on computers, laptop or in their chambers are not on additional risk and hence do not require masks until sick.
- However it is advised that all people after touching things of common use as door knobs etc. should wash their hands with soap and water. Hand sanitizers to be used only sparingly.
- People using PPE/N-95 Mask/surgical Mask are responsible for its disposal. No item should be found lying in open within the Hospital premises otherwise strict action will be taken against them.

**Cleaning and Disinfection of sample collection area/ laboratory area:**

- Wear PPE before doing disinfection and cleaning procedure.
- It is to be done twice in a day or whenever surfaces are visibly soiled or when contamination of the environment is suspected (such as after doing aerosol generating procedure).
- For floor and surface cleaning use 3 bucket system. Clean with detergent and water followed by cleaning with 0.5% hypochlorite solution (prepare by mixing 1 part of 5-6% sodium hypochlorite to 9 part of water) or with bleaching powder solution (prepare by mixing 4 teaspoon in 1 litre of water).
- For metal surfaces this should be followed by wiping with 70% isopropyl or ethyl alcohol.
- Computer surfaces to be wiped with alcohol wipes multiple times a day.
- No case sheets to be handled with bare hands.

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- Fogging of the room/corridors is to be done with 7.35% H<sub>2</sub>O<sub>2</sub> and 0.23% peracetic acid daily (add 25 ml of solution in 1 litre of water).

**Spill management:**

- Vacate the area
- Wear PPE
- Cover the spill with absorbent cotton or a cloth.
- Pour bleach (For small spill- use 1% hypochlorite; For Large spill- use 10% hypochlorite) on the absorbent covering spill for 10-15 minutes.
- Discard it in the yellow/ red bag with the help of scoop. Finally do the mopping with detergent and water.
- Wash hands with soap and water after cleaning/ spill management.
- Fogging of the area should be done with 7.35% H<sub>2</sub>O<sub>2</sub> and 0.23% peracetic acid daily (add 25 ml of solution in 1 litre of water).

**If BSL-3 facilities are available follow (use for sample processing and nucleic acid extraction):**

**Entering the BSL-3**

- Enter password and get into the lab. **No jewelry, watches are allowed in the BSL-3 lab. (Please clean shave)**
- Don on PPE in the “Donning Area”.
- Keep the sample box to be transferred inside the BSL-3 in the UV pass box and allow it to remain for 20 minutes before taking it inside the inner lab.
- Likewise all supplies should go inside through the UV pass box.

**Exiting the BSL-3 lab:**

- Doff all PPE in the shower area and pack all material in an autoclavable bag.
- Take a shower before coming out.
- Autoclave all the PPE on exit.

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**Table 1: Waste Segregation:**

<b>Yellow (Non chlorinated plastic bags, autoclave safe plastic bags or containers)</b>	<b>Red (Non chlorinated plastic bags or containers)</b>	<b>Black</b>	<b>Translucent white box (Puncture, leak, tamper proof)</b>
Swab sticks from VTM	Used VTM tubes	Not to be used for the time being	Needles
Used Cotton swabs, alcohol wipes, tissue papers	PCR Tubes, Wells		Discarded & contaminated metal sharps
PPE Items including masks, gowns, shoe covers, head covers	Plate sealers		
Trashed papers	Vacutainers, Used Microcentrifuge tubes, tips		
Any non plastic item that is soiled with sample	Gloves		
Biodegradable General waste	Packaging material removed from triple layered packed samples		
	Non biodegradable general waste		

**Table 2: Summary of waste material produced at different steps and their treatment**

<b>S. No</b>	<b>Steps</b>	<b>Waste generated</b>	<b>Where to discard/ What to do</b>	<b>On site Treatment</b>	<b>Final Disposal</b>
1	Sample receiving and opening the triple layer packaging	Thermocol boxes	Sprayed with 1% sodium hypochlorite from outside and inside	Stick "To be autoclaved before disposing"	Autoclave
		Vaccine carriers	Sprayed with 1% sodium hypochlorite from outside and inside Ensure that the straps are also sprayed.	Stick "To be autoclaved before disposing"	Autoclave
		Gel Packs	Sprayed with 1% sodium hypochlorite	Can reuse/ dispose	
		Triple layer packaging	To be removed only inside a certified BSC	Autoclave	Autoclave

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			II A2. All packing material to be discarded in autoclavable plastic bags within the BSC Fasten the bag within the BSC		
		Cotton/ wipes after disinfecting the outer surface of VTMs	Autoclavable plastic bags Fasten the bag within the BSC	Autoclave	Incineration
2	Sample Processing	Swab sticks	To be done only in a certified BSC II A2 Discard in Autoclavable plastic bags Fasten the bag within the BSC	Autoclave	Incineration
		VTM tubes (left after aliquot preparation)	To be done only in a certified BSC II A2 Discard in Autoclavable plastic bags	Autoclave	Autoclave
3	Nucleic Acid Extraction	Microcentrifuge tubes Tips Columns	Discard in Autoclavable plastic bags	Autoclave	Autoclave
4	PCR	Microcentrifuge tubes, Tips, PCR tubes/ wells, Plate sealers	Discard in Autoclavable plastic bags	Autoclave	Autoclave
5	Used PPE	Gloves	Autoclavable plastic bags	Autoclave	Autoclave
		Remaining PPE	Spray the entire PPE with 1% sodium hypochlorite	Autoclave	Incineration
6		Worksheets and Papers used during any step	Autoclavable plastic bags	Autoclave	Incineration

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