Laboratory decontamination following COVID-19 sample RNA extraction.

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Much research has been conducted to demonstrate airborne transmission of SARS-CoV-2. However, fomite transmission of respiratory droplets that have settled on surfaces have also been considered one of the main sources of viral spread. The stability of SARS-CoV-2 has been shown to be similar to that of SARS-CoV-1. SARS-CoV-1 and SARS-CoV-2 have shown to remain viable in aerosols for up to 3 hours, with a nominal reduction in infectious titre from $10^{4.3}$ to $10^{3.5}$ TCID50 and $10^{3.5}$ to $10^{2.7}$ TCID50 per liter of air, respectively without decontamination. SARS-CoV-2 has shown to be more stable on plastic (titre reduction from $10^{3.7}$ to $10^{0.6}$ TCID50 per ml of medium after 72 hours) and stainless steel (identical titre reduction in only 48 hours) in comparison to copper and cardboard. No viability has been detected in copper surfaces after 4 hours (SARS-CoV-2) and after 8 hours (SARS-CoV-1). On cardboard, no viable SARS-CoV-2 has been detected after 24 hours and no viable SARS-CoV-1 was detected after 8 hours [1]. Aerosol and fomite transmission of SARS-CoV-2 is plausible, as the virus can remain viable and infectious in aerosols for hours and on surfaces up to days (depending on the inoculum). Cross contamination by conventional surface-wiping methods, and its excessive time and labour requirement, have made disinfectant spray methods to become a common decontamination practice for hospitals and laboratories. Various types of disinfectant spray methods have been used to decontaminate frequently contacted surfaces as a daily routine. Decontamination by fumigation using a gas, vapour, or fine mist has been shown to be effective for whole room, all surface decontamination of SARS-CoV-2 [2].

This document describes the decontamination protocol used at the Viral & Human Genomics Laboratory after processing COVID-19 suspected samples.

Prior to decontamination

1. Maintain Biological Safety Cabinet of Cell Biology Area on and open during the entire decontamination procedure.

2. Verify that the extractor fan located in the Cell Biology Area is turned off.

3. Open polystyrene ice box used for sample transportation and remove cold-packs, place cold packs on paper towels and exposed for fogging.

4. Verify that fog machine has sufficient decontamination liquid (red arrow on back) and that the wireless receiver is plugged in correctly. See picture below.

5. Turn machine on (verify that the led indicator is lit).

6. Turn the Cell Biology Area air-conditioning unit off and leave remote control OUTSIDE of the lab.

7. Close door and if necessary tape a seal around door to prevent fog leaks.

Decontamination procedure

1. Allow fog machine at least 5 minutes to warm up before activating it.

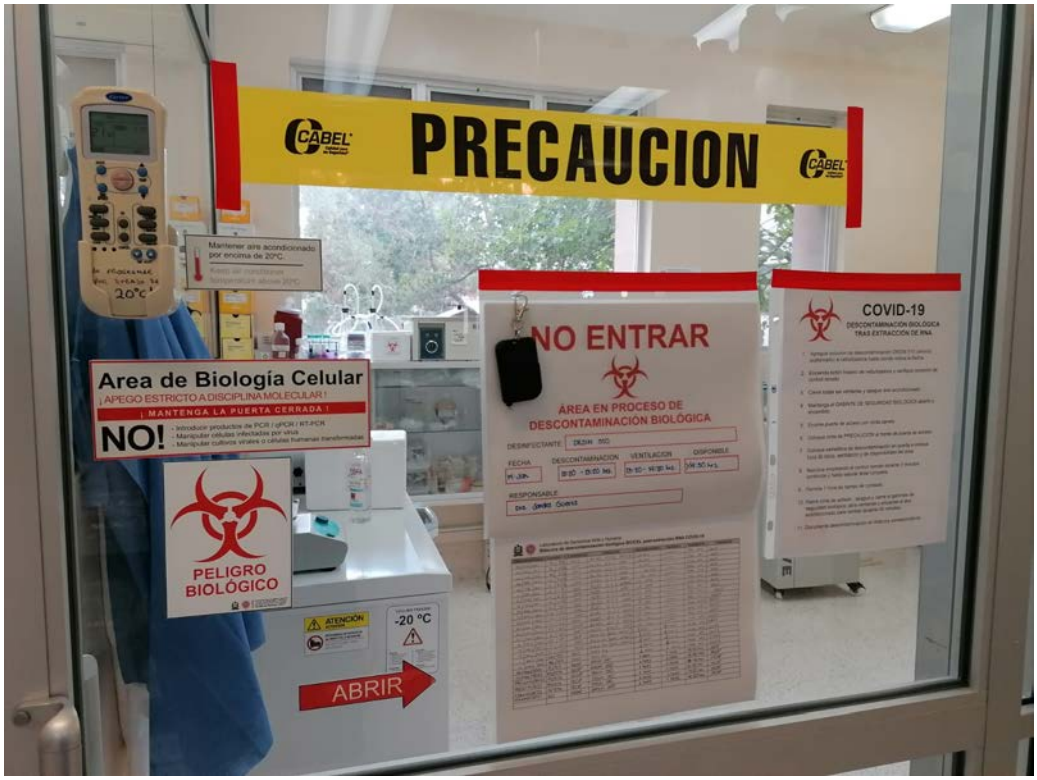
2. Press either button A or B on the wireless remote control until fog machine stops dispensing.

3. Wait 1 to 2 minutes and repeat fogging.

4. Continue fogging until room is completely obscured by thick fog and light coming in through the windows takes on a brownish tint.

5. Allow fog to decontaminate room for at least 1 hour.

6. Place warning sign and CAUTION flag on main door of Cell Biology Area.



7. Main warning sign should provide information regarding the type of disinfectant being used, the time at which decontamination was initiated, how long it is to be left fogging, how long to ventilate and when the area will again be available for use.

Ventilation procedure

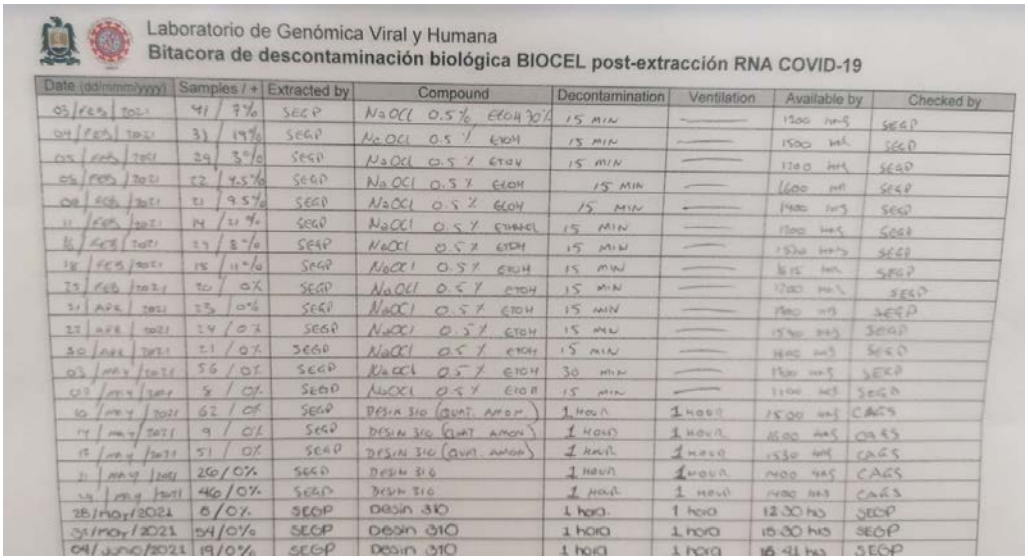
1. Activate Cell Biology Area extraction fan and turn air conditioning unit on, ventilate for at least 15 minutes.

2. After purging Cell Biology Area air, remove doors seals (if used) and turn both Biological Safety cabinet and fog machine off, close the Biological Safety Cabinet's front window.

3. Turn air conditioning unit off.

Logging of decontamination procedure

1. Annotate decontamination procedure in the corresponding log.



Laboratorio de Genómica Viral y Humana
Bitacora de descontaminación biológica BIOCEL post-extracción RNA COVID-19

Date (dd/mmm/yyyy)	Samples / +	Extracted by	Compound	Decontamination	Ventilation	Available by	Checked by
03/FEB/2021	41 / 7%	SEGP	NaOCl 0.5% ETOH 70%	15 MIN	—	1500 hrs	SEGP
04/FEB/2021	31 / 19%	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1500 hrs	SEGP
05/FEB/2021	29 / 3%	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1200 hrs	SEGP
06/FEB/2021	22 / 4.5%	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1600 hrs	SEGP
08/FEB/2021	21 / 9.5%	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1400 hrs	SEGP
11/FEB/2021	14 / 21 %	SEGP	NaOCl 0.5 % ETHANOL	15 MIN	—	1500 hrs	SEGP
16/FEB/2021	23 / 8 %	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1530 hrs	SEGP
18/FEB/2021	18 / 11 %	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1615 hrs	SEGP
23/FEB/2021	20 / 0%	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1700 hrs	SEGP
31/APR/2021	23 / 0%	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1500 hrs	SEGP
27/APR/2021	24 / 0 %	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1530 hrs	SEGP
30/APR/2021	21 / 0 %	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1400 hrs	SEGP
03/MAY/2021	56 / 0%	SEGP	NaOCl 0.5 % ETOH	30 MIN	—	1300 hrs	SEGP
03/MAY/2021	8 / 0%	SEGP	NaOCl 0.5 % ETOH	15 MIN	—	1200 hrs	SEGP
10/MAY/2021	62 / 0%	SEGP	DESIN 310 (QUAT. AMON.)	1 HOUR	1 HOUR	1500 hrs	CAGS
14/MAY/2021	9 / 0%	SEGP	DESIN 310 (QUAT AMON)	1 HOUR	1 HOUR	1500 hrs	CAGS
15/MAY/2021	51 / 0%	SEGP	DESIN 310 (QUAT. AMON)	1 HOUR	1 HOUR	1530 hrs	CAGS
21/MAY/2021	260/0%	SEGP	DESIN 310	1 HOUR	1 HOUR	1400 hrs	CAGS
24/MAY/2021	460/0%	SEGP	DESIN 310	1 HOUR	1 HOUR	1400 hrs	CAGS
28/MAY/2021	8/0%	SEGP	DESIN 310	1 hora	1 hora	12:30 hs	SEGP
31/MAY/2021	54/0%	SEGP	DESIN 310	1 hora	1 hora	15:30 hs	SEGP
04/JUNE/2021	19/0%	SEGP	DESIN 310	1 hora	1 hora	16:45 hs	SEGP

2. Remove al warning signs and flags.

3. Use a 70% ethanol-soaked tissue to clean any remaining residue from surfaces and instruments.

Notes

1. Desin 310 corresponds to quaternary ammonium compound for use in fog machines.

2. High-level (BSL3) decontamination [3] of laboratory areas by fumigation/fogging also include the following CDC approved formulation (to prepare the 1 litre minimum volume for fog machine):

    a. Prepare Chemical Decontamination Mix by adding 18 to 54 grs of Peracetic Acid to 600 mL of reverse osmosis/distilled water as well as 30 to 72 grs of Acetic Acid, 0.6 to 12 grs of Sulfuric Acid, between 0.3 and 12 % w/v silver nitrate as ion stabilizer, 0.3 and 12 grs of surfactant sodium dodecyl (laureate) sulphate (SDS).

    b. Prepare Fog Solution by adding 50 mL of food grade glycerol to 300 mL of reverse osmosis/distilled water.

    c. Mix the 600 mL of Chemical Decontamination Mix with the ≈ 350 mL of Fog Solution.

3. Mid-level (BSL2) decontamination of laboratory areas by fumigation/fogging may use a Hydrogen Peroxide solution using 350 mL of the aforementioned glycerol-based Fog Solution plus 350 mL of a 60% (v/v) food-grade Hydrogen Peroxide in reverse osmosis/distilled water supplemented

with 60 ppm (21 mg) of silver nitrate. This results in a 30% Hydrogen peroxide vapor. Ideal concentrations for laboratory area decontamination vary from 33 to 35% has they have shown to provide 6 Log reduction in microbial load. The use of Hydrogen Peroxide solutions above 60% is considered toxic to humans.

References

1. van Doremalen, N. *et al.* Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N. Engl. J. Med.* **382**, 1564–1567 (2020).

2. Cutts, T., Kasloff, S., Safronetz, D. & Krishnan, J. Decontamination of common healthcare facility surfaces contaminated with SARS-CoV-2 using peracetic acid dry fogging. *J. Hosp. Infect.* **109**, 82–87 (2021).

3. Krishnan, J. *et al.* Evaluation of a Dry Fogging System for Laboratory Decontamination. *Appl. Biosaf.* **17**, 132–141 (2012).

Revision history

1.0 Original document.

2.0 Modified ventilation procedure to incorporate use of air purge extractor fans recently installed in lab.