May 16, 2020

Viral inactivation of clinical samples

In 1 collection

Tammy Krylova[1], Tim Budd[1], Jerome Nicod[1], Rupert Beale[1], Simon Caidan[1]

[1]The Francis Crick Institute

1 Works for me dx.doi.org/10.17504/protocols.io.bfadjia6

Crick COVID-19 Consortium

Jerome Nicod
The Francis Crick Institute

ABSTRACT

This protocol is part of the Crick COVID-19 RT-PCR Testing Pipeline collection.

This work involves handling and processing of clinical nasal or throat Swab samples from NHS staff or patients who are suspected of being infected SARS-CoV-2.
➢ This SOP is to be followed in order to avoid infection exposure to the virus

Purpose of examination / Clinical relevance

At the end of 2019, several pneumonia cases were reported in Wuhan, China and the pathogen was confirmed as a new viral strain. World Health organization has named the newly identified coronavirus as 2019-nCoV, also known as SARS-Cov-2. The disease developed into a dangerous pandemic, posing major challenges to the NHS. Although more research is necessary to better understand the virus, in response to the emergency, simple and rapid testing is essential to identify the virus in infected individuals. This will aid the implementation of efficient interventions to contain the spread, and distinguish healthcare workers who have been infected, and are required to self-isolate, from those showing similar symptoms but which are not 2019-nCoV associated. The latter category may continue to work, alleviating stress on hard-pressed healthcare resources. 2019-nCoV is an RNA virus, and the diagnostic tests detect viral RNA in swabs from patient airways using a reverse transcriptase PCR assay. Samples are submitted to HSL, an accredited reporting laboratory, and transferred to the Crick for testing. The first step of the process is sample receipt at the Crick. This SOP describes the inactivation of the virus.

SAFETY WARNINGS

This work involves handling and processing of clinical nasal or throat Swab samples from NHS staff or patients who are suspected of being infected SARS-CoV-2.

Work with live viruses is usually performed in CL3 labs but there is a derogation to allow diagnostic work with samples of unknown status to be carried out at CL2. The CL2 area must be isolated and secured from unauthorised access.

➢ This SOP is to be followed in order to avoid infection exposure to the virus

Safety Information – routes of infection

Person-to-person spread is thought to occur mainly via

- respiratory droplets produced when an infected person coughs or sneezes or
- contact with droplets and contaminated fomites.

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Restrictions

Access to CL2/3 facility is restricted to authorised personnel only.

- Only those with health clearance and have been signed off as trained and competent by both their manager and SHS are allowed to undertake this work within the CL2/3 Facility.

Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) must be worn at all times in the Containment facility.

Anyone entering the CL2/3 facility must wear the following PPE

- A Purple Howie style lab coat which must be worn at all times.
- Orange nitrile or neoprene disposable gloves

Staff processing samples will wear additional PPE whilst working within the Microbiological safety cabinets (MBSC)

- A second pair of blue nitrile or neoprene disposable gloves
- Over-sleeves

Safety Information – Procedures for dealing with accidents and incidents

Injury and/or personal contamination

- *Needlestick:* Encourage to bleed. Wash affected area with soap and warm water. Report immediately by dialling emergency number at any time.
- *Eye splash:* Irrigate thoroughly with warm water and report immediately by dialling emergency number at any time.
- *Mouth:* Wash out thoroughly and report by dialling emergency number at any time.
- *Skin:* Surface contamination - wash affected area with soap and water. Report by dialling emergency number at any time.
- *Clothing:* Remove carefully to avoid creating aerosols at exit to room, leave contaminated clothing at the door margin. Report by dialling emergency number at any time.

Warning: Spillages generate aerosols.

Safety Information – dealing with spillages

Minor, i.e. within a MBSC - with little or no aerosol release:

1. Cover with paper towels
2. Slowly pour 100% Surfanios/Amphospray over towels and allow a 10 min contact time, meanwhile supervise spillage area. Clean and repeat procedure (dispose of materials in CL2 container).

Serious spillage and/or production of significant aerosols (including personal contamination):

1. Instruct all personnel to leave.
2. Ensure the lab door is shut.
3. Prevent further entry.
4. Deal with any contamination involving skin, clothing etc. (see above).
5. Dial emergency number and inform the Health & Safety Team, who will deal with the spillage.

BEFORE STARTING

1. *Check that all the required materials are in the Microbiological Safety Cabinet (MBSC)*

- Pastettes in a holder
- 2 ml screw cap tubes containing the inactivation solution (L6 Inactivation Buffer, henceforth referred to as “inactivation tubes”)

- Eppendorf rack for barcoded inactivation tubes
- FACS tube rack for sample vials
- Amphospray disinfectant

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- Liquid waste container: [M]**10 % solution of Surfanios** in an ice cream tub with lid placed to the side of the MBSC (to close container prior to disposal)
  → To make [M]**10 % Surfanios** , **8 pumps** into tub (**160 ml**) + **1.35 L tap water**
- Paper towels
- 1 section of blue roll laid out over main working area
- A prepared blue bag for dry waste disposal by rolling the top to hold the bag open. This is for direct disposal of sample bags, paper towels, gloves and over sleeves
- A second blue bag, unopened. This is the secondary bag for waste disposal process
- 2 x rubber bands
- [M]**100 % Surfanios** in falcon tube for potential spillage

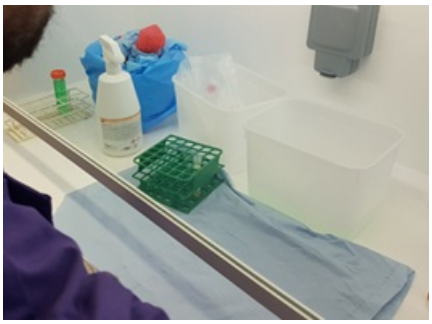
2. *Check items that you need outside the hood*
- Sample submission 96 well rack with lid
- Spare blue bags
- Paper towels
- Blue roll
- Timer

3. *Collect a single ice cream tub with samples from the table in the corridor*
- Each tub will contain 10 or 12 samples

4. *Take the sample ice cream tub into the room you are working in*

5. *Put on the second pair of gloves and over-sleeves*

6. *Open the tub and transfer all double-bagged samples inside into your hood. You are now ready to start working in the MBSC*



Set Up

Inactivation step

1

Warning! Exposure to SARS-CoV-2 can result in COVID-19.
➢ **All unsealed work must be undertaken in a class I or Class II Microbiological Safety Cabinet (MBSC).**

Single sample per cycle only!

Working with multiple samples might lead to errors in sample identification!

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➢ Work with only one bagged sample at a time

Step 1 includes a Step case.
Wet Swabs
Dry Swabs

step case

Wet Swabs

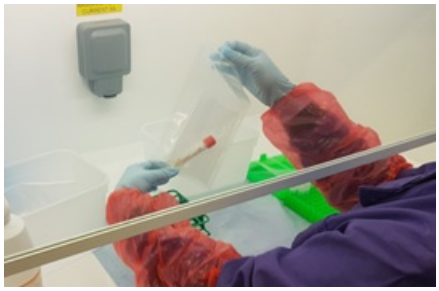
2 Remove the liquid waste container lid and place it to the side in the MBSC.

3 Pick up one sample bag.

4 Examine the sample within the bag to ensure no leakages in the bag, on the side of the tube etc.

5 Check the barcodes stapled to the outer bag match the bar code on the swab vial within it.

If barcodes do not align, DO NOT PROCESS. Spray bag out at end of work to be removed and rescanned at scanning station.



6 Remove barcodes and place on the work area.

7 Take a tissue and spray with Amphospray so it is thoroughly soaked.

8 Spray the outer bag.

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9 Open the outer bag and spray inside with Amphospray so inner bag is wet.

10 Remove inner bag and discard outer bag into the dry waste bag.

11 Open the inner bag.

12 Spray inside with Amphospray.

13

If two samples are present in one bag, take 50% of the liquid from each sample.

Open inner bag and remove sample vial by either:
a. Tipping sample onto wet tissue

b. Pinching the bottom of the sample tube through bag and scrunching up the bag to reveal the sample.

14 Wipe sample tube thoroughly with wet tissue and place into the rack.

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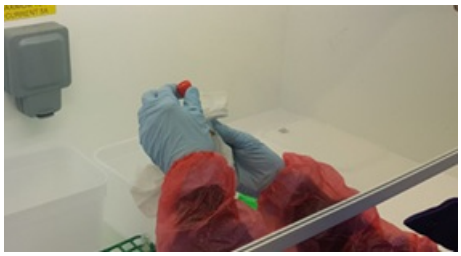

15 Discard inner bag into dry waste bag.

16 Pick up a new inactivation tube.

17 Check it contains inactivation liquid.
Discard any inactivation vial that does not contain inactivation liquid into the liquid waste container.

18 Check provided barcodes for damage & use best-quality barcode (the others are spares).

Downstream automation will only work if the labels are orientated vertically and as straight as possible!

19 Stick barcode label to the inactivation tube as shown.

20 Place the labelled inactivation tube into the rack.

21 Remove swab sample from the rack.

22 Open swab sample lid.

23 Place lid into liquid waste container.

If swab is still affixed to the lid, discard into liquid waste container.

24 Holding the swab vial, place hands over the liquid waste container so the swab vial opening is slightly hanging above

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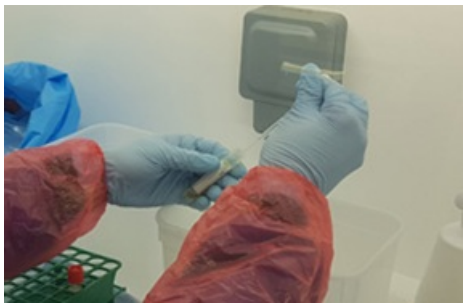
the liquid waste container to catch any drips.

Take care to not place the whole swab vial directly over the waste container in case it falls/slips/dropped accidentally.



25 Draw up ~ **100 µl swab vial contents** into a fresh pastette.

Refer to example pastettes with marked 100 µl level.

26 Keep pastette hovering above liquid waste container.

27 Return sample vial to the rack.

28 Pick up inactivation vial.

29 Remove lid and either place it down or pinch between thumb and forefinger.

30 Dispense the pastette's content into the bottom of the inactivation vial (to minimise bubbling).

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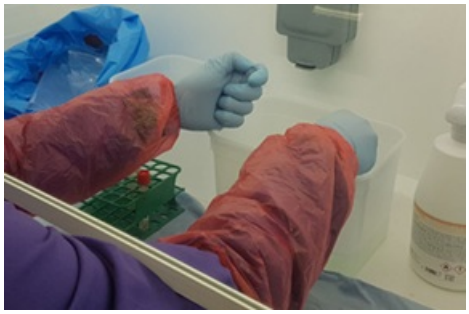
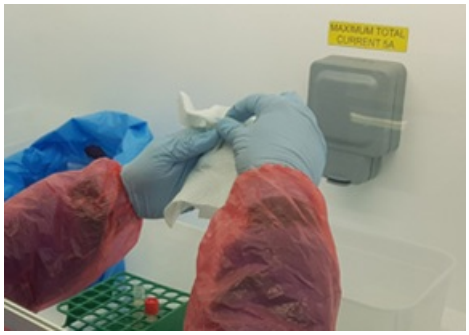
31 Seal inactivation vial.



32 Draw up some [M]**10 % Surfanios** into the pastette.

33 Discard the pastette into the liquid waste container.

34 Wipe over inactivation vial with Amphospray-soaked paper towel.



35 Flick inactivation vial.

36 Place into Eppendorf rack.

37 Place open swab vial into liquid waste container.

38 Repeat steps 3 - 36 with the next sample.

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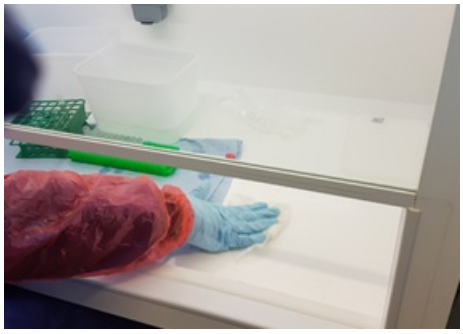
39 When a rack of inactivated samples is ready to be removed from the MBSC proceed to the 'Removal of racks of inactivated samples from the MBSC' section below.

Removal of racks of inactivated samples from the MBSC

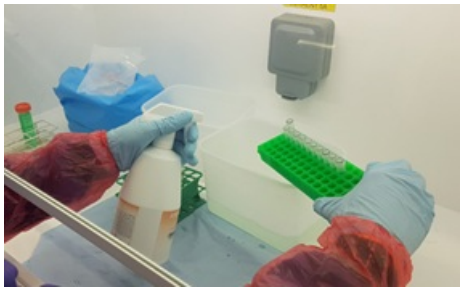
40 Visually inspect all the inactivation vials to ensure that all vials are capped.

41 Place lid loosely on liquid waste container. Dispose of all bags, barcodes and blue roll into the blue waste bag.

42 Set up a clean area large enough for rack to sit on top of for decontamination, either:
a. Spray Amphospray onto tissue and wipe area
b. Directly spray an area on the floor of the hood



43 Spray the rack thoroughly with Amphospray and place on pre-cleaned area in MBSC.



44 Remove existing “dirty” second gloves and over sleeves. Discard at side of MBSC.

45 Start 5-minute timer.

46 When timer rings, remove the rack from the MBSC with clean orange or blue gloves.

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47 Transfer completed inactivation vials to transport rack.

48 If processing another set of samples, start again by collecting a fresh tub from the corridor.

To prevent fatigue and operator error, work only in pre-arranged 1-hour shifts. If you are in the middle of a batch, stop and hand over to the next person.

49

Batching of tubes for downstream automation processing
The optimal number for plate transfer is 93 tubes
➢ Fill each blue lid rack with 93 inactivated samples
➢ The final three spaces are required for one positive and two negative controls (added during later steps).

Once full, notify runner that samples are ready for collection.

50 Close box.

51 Spray box and transfer to corridor.

52 Place a [removable] tape label on the box.

53 Write the date and time and room on the box.

54 Place the rack in the transfer box and replace the lid.

55 Notify runners that samples are ready.

56 Restart sample processing.

Once a box is full to the required number of racks
a) Place a “outgoing” laminated sign on the transfer box

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b) Spray and wipe the transfer box and remove it to the table in the corridor
c) Phone sample reception area to inform SHS there is a box ready for collection.

Movement of full transfer boxes to RNA extraction lab

57

Full boxes of inactivated samples will be transported from the CL2/3 to RNA extraction lab by Health & Safety or other nominated staff (runners).

Runners' tasks entering CL3 facility:

58 Put on a pair of gloves in the gowning room.

59 Enter the inner corridor.

60 Put on a purple lab coat.

61 Go to the relevant room.

62 Spray the outside of the transfer box with Amphospray.

63 Wipe down the outside of the box with tissue.

64 Discard the tissue into the waste bin in the room.

65 Spray your gloves with Amphospray.

66 Leave the room with the transfer box.

67 Place the transfer box on the table by the exit.

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68 Remove and hang up your lab coat.

69 Remove your gloves.

70 Dispose of the gloves in the blue autoclave bin under the table.

71 Take the transfer box into gowning room.

72 Wash your hands.

73 Leave the gowning room.

74 Deposit the samples at the RNA extraction lab reception area.

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