

Supplementary Method 15 Chemical inactivation of clinical samples for work in Containment Level 2

This SOP is associated with Risk Assessment Diagnostic screening of clinical respiratory ACDP Hazard Group 3 SARS-CoV-2

Warning

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. 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 by ➤ contact with droplets and contaminated fomites.

Restrictions

Access to CL2 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 Facility.



Location of clinical sample inactivation

Personal Protective Equipment (PPE) must be worn at all times in the CL2



Anyone entering the CL2 facility must wear the following PPE

- A 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

Liquid Sample Inactivation Protocol

Before starting

1. Check that all the required materials are in the MBSC
 - Pastettes in a holder
 - 2ml screw cap tubes containing the inactivation solution (henceforth referred to as “inactivation tubes”)
 - Eppendorf rack for barcoded inactivation tubes
 - FACS tube rack for sample vials
 - A suitable disinfectant
 - Liquid waste container: 10% solution of Surfanios/Distel in an ice cream tub with lid placed to the side of the MBSC (to close container prior to disposal)
 - i. To make 10% Surfanios/Distel, 8 pumps into tub (160ml) + 1.35L of 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
 - 100% Surfanios/Distel 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.

Liquid Sample Inactivation Protocol

Inactivation step

Warning

Exposure to SARS-CoV-2 can result in COVID-19

- All unsealed work must be undertaken in a Class II MBSC

! Single sample per cycle only

Working with multiple samples might lead to errors in sample identification

- Work with only one bagged sample at a time

1. Remove the liquid waste container lid and place it to the side in the MBSC
2. Pick up one sample bag.
3. Examine the sample within the bag to ensure no leakages in the bag, on the side of the tube etc
4. 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

5. Remove barcodes and place on the work area.
6. Take a tissue and spray with a suitable disinfectant so it is thoroughly soaked.
7. Spray the outer bag.
8. Open the outer bag and spray inside with a suitable disinfectant so inner bag is wet.
9. Remove inner bag and discard outer bag into the dry waste bag.
10. Open the inner bag
11. Spray inside with a suitable disinfectant

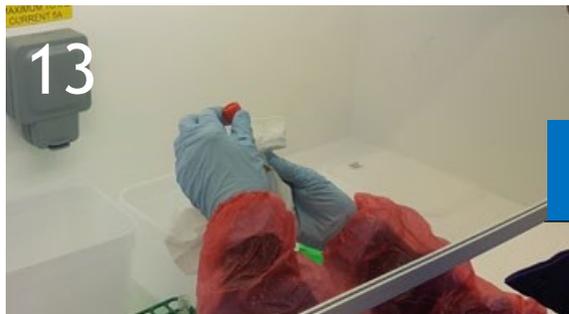


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! If two samples are present in one bag, take 50% of the liquid from each sample.

12. 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.
13. Wipe sample tube thoroughly with wet tissue and place into the rack.
14. Discard inner bag into dry waste bag.
15. Pick up a new inactivation tube
16. Check it contains inactivation liquid.
 - Discard any inactivation vial that does not contain inactivation liquid into the liquid waste container



! The ASF robot will only work if the labels are orientated vertically and as straight as possible!

17. Check provided barcodes for damage & use best-quality barcode (the others are spares)
18. Stick barcode label to the inactivation tube as shown.
19. Place the labelled inactivation tube into the rack.
20. Remove swab sample from the rack.
21. Open swab sample lid.
22. Place lid into liquid waste container.



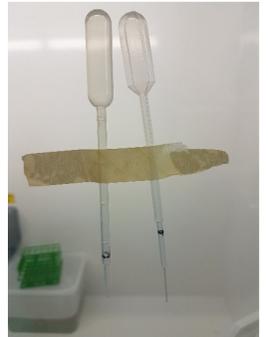
! If swab is still affixed to the lid, discard into liquid waste container

23. Holding the swab vial, place hands over the liquid waste container so the swab vial opening is slightly hanging above the liquid waste container to catch any drips.

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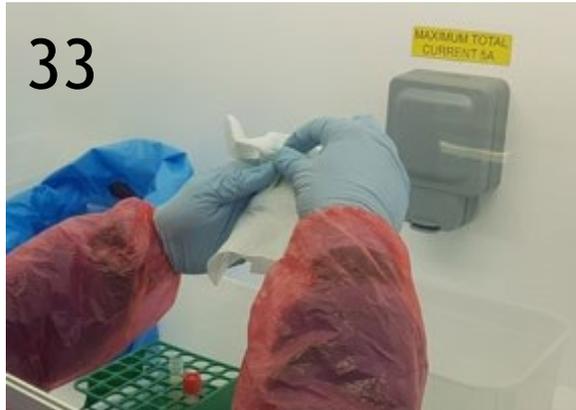


- a. Take care to not place the whole swab vial directly over the waste container in case it falls/slips/dropped accidentally.
24. Draw up ~100µl of swab vial contents into a fresh pastette.
 - a. Refer to example pastettes with marked 100µl level
25. Keep pastette hovering above liquid waste container.
26. Return sample vial to the rack.
27. Pick up inactivation vial.
28. Remove lid and either place it down or pinch between thumb and forefinger.
29. Dispense the pastette's content into the bottom of the inactivation vial (to minimise bubbling).
30. Seal inactivation vial.
31. Draw up some 10% Surfanios/Distel into the pastette.
32. Discard the pastette into the liquid waste container.
33. Wipe over inactivation vial with a suitable disinfectant-soaked paper towel.
34. Flick inactivation vial.
35. Place into Eppendorf rack.
36. Place open swab vial into liquid waste container.
37. Repeat steps 3-36 with the next sample



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 on page 10

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DRY SWAB PROCESS

i Important information regarding dry swabs

Dry swabs will arrive in a variety of containers e.g.

- Universal tubes with conical bottoms
- Universal tubes with flat bottoms
- Urine sample pot with flat bottoms

It is important that the SOP instructions are followed to prevent

- Cross contamination
- Maximise sample recovery

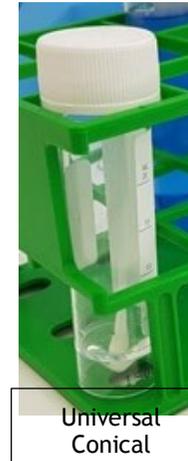
! Single sample per cycle only

Working with multiple samples might lead to errors in sample identification

- Work with only one bagged sample at a time

38. Pick up one sample bag.

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

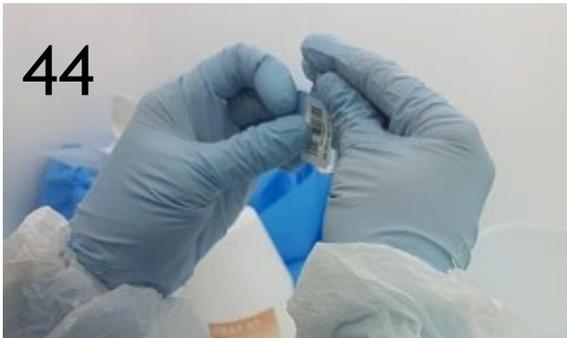


Universal
Conical



Urine pot

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43. Check the 2ml inactivation tube contains 1ml L6 lysis buffer



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

40. Check the barcodes stapled to the outer bag match the bar code on the swab vial within it.
41. Remove barcodes and place on the work area.

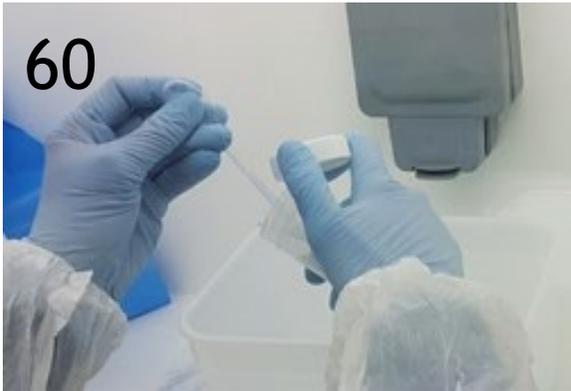


The robot will only work if the labels are orientated vertically and as straight as possible!

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

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44. Stick the barcode label to the inactivation tube.
45. Place the labelled inactivation tube into the rack.
46. Take a tissue and spray with A suitable disinfectant so it is thoroughly soaked.
47. Spray the outer bag
48. Open the outer bag
49. Spray inside the bag with a suitable disinfectant so inner bag is wet.
50. Remove inner bag and discard outer bag into the dry waste bag.
51. Open the inner bag
52. Spray the inner bag with A suitable disinfectant
53. Remove sample vial by either:



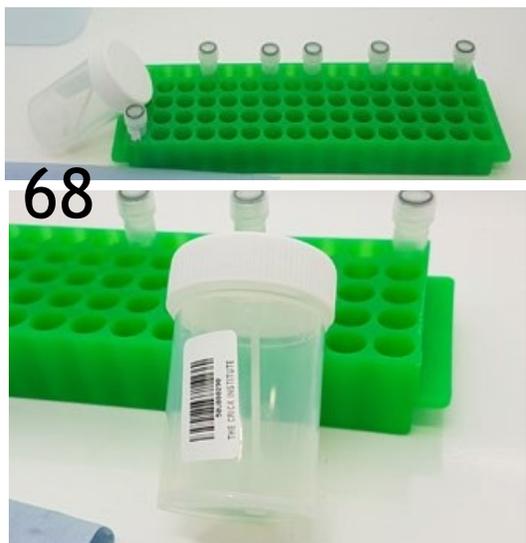
- a. Tipping sample onto wet tissue
 - b. Pinching the bottom of the sample tube through bag and scrunching up the bag to reveal the sample.
54. Wipe sample tube thoroughly with wet tissue and place into the rack.

55. Discard inner bag into dry waste bag.
56. Unscrew the swab container lid
57. Place the lid in front of the container
58. Unscrew barcoded 2ml inactivation tube containing the 1ml L6 lysis buffer
59. Draw up the lysis buffer into a fresh pastette
60. Aspirate the lysis buffer into opened swab vial



61. Draw up some 10% Surfanios/Distel into the pastette.
62. Discard the pastette into the liquid waste container.
63. Replace the lid on the swab container
64. Agitate the swab container to ensure the swabs are in contact with the lysis buffer
65. Replace the lid on the inactivation tube
66. If the swab pot is a flat-bottomed container follow steps 68 - 69

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67. Go to step 70 if the swab pot has a conical bottom.



Maximising sample recovery from flat bottomed containers

68. Place flat-bottomed containers on the angled rack as shown in the picture

69. Make sure the pot is rotated so that the swab is sitting in the lysis buffer

70. Leaving tubes two spaces apart on rack, proceed to the next sample



All swabs must have a minimum of 10 minutes contact with the lysis buffer

71. Follow steps 72 - 75 only after lysis buffer has been added to last of the sample set you have taken into the MBSC
(usually this will be a set of 12 swab samples).

72. Remove over-sleeves and outer gloves.

73. Set the timer outside the MBSC for 10 minutes.

74. Outside the MBSC put on a fresh pair of outer gloves

75. Outside the MBSC put on a fresh pair of over-sleeves



Single sample per cycle only

Working with multiple samples might lead to errors in sample identification

- Work with only one swab sample at a time
- Continue with the protocol ONLY after the 10-minute timer has sounded

76. Unscrew barcoded 2ml inactivation tube

77. Place 2ml inactivation tube lid down to the side

78. Pick up the corresponding swab container.

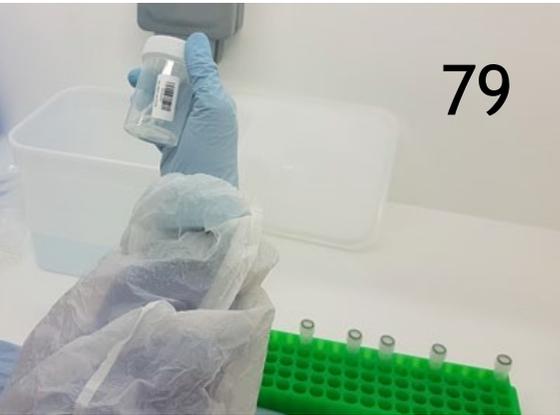


Correct barcode check

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- This is a vital check to ensure the right sample is processed back into its corresponding inactivation tube to avoid misdiagnosis of patient samples

79. Check the barcodes on the swab container and opened inactivation tube match



80. Once the match is verified, unscrew the swab container lid
81. Discard the swab container lid into the 10% Surfanios/Distel liquid waste tub
82. Holding the swab vial, place hands over the liquid waste container so the swab vial opening is slightly hanging above the liquid waste container to catch any drips.
 - a. Take care to not place the whole swab vial directly over the waste container in case it falls/slips/dropped accidentally

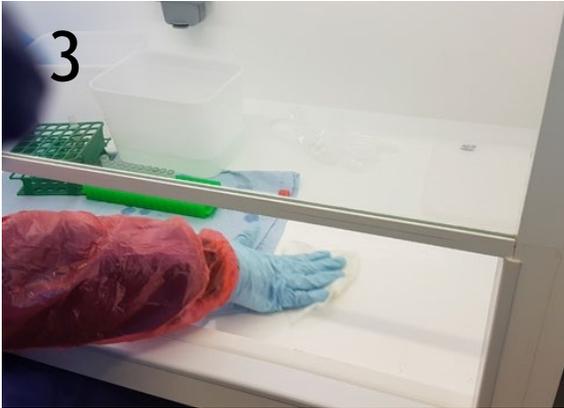
Important information: the dry swabs will have absorbed some of the lysis buffer

- Do not expect to recover the full 1 ml of lysis buffer

83. Draw up the lysis buffer from the swab container into a fresh pastette
84. Dispose of the swab container into the 10% Surfanios/Distel liquid waste tub.
85. Dispense the pastette's content into the bottom of the 2ml inactivation tube (to minimise bubbling).
86. Reseal the 2 ml inactivation tube.
87. Draw up some 10% Surfanios/Distel into the pastette.
88. Discard the pastette into the liquid waste container.
89. Replace the lid onto the 2ml inactivation tube.

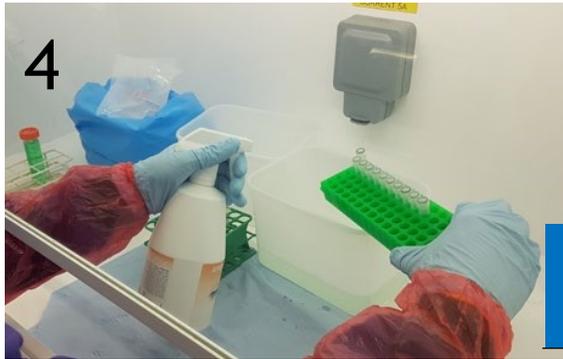
When a rack of inactivated samples is ready to be removed from the MBSC proceed to the **Removal of racks of inactivated samples from the MBSC** section below

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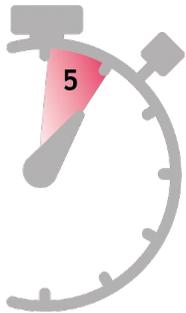


Removal of racks of inactivated samples from the MBSC

1. Visually inspect all the inactivation vials to ensure that all vials are capped.
2. Place lid loosely on liquid waste container. Dispose of all bags, barcodes and blue roll into the blue waste bag.
3. Set up a clean area large enough for rack to sit on top of for decontamination, either:
 - a. Spray suitable disinfectant onto tissue and wipe area.
 - b. Directly spray an area on the floor of the hood.
4. Spray the rack thoroughly with a suitable disinfectant and place on pre-cleaned area in MBSC.
5. Remove existing “dirty” second gloves and over sleeves. Discard at side of MBSC.
6. Start 5-minute timer.
7. When timer rings, remove the rack from the MBSC with clean orange or blue gloves.
8. Transfer completed inactivation vials to transport rack.
9. 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.



Robot processing in RNA extraction lab

The optimal number for robot processing is 94 tubes

- Fill each blue lid rack with 94 inactivated samples
- The final two spaces are required for a positive and negative control.

10. Once full, notify runner that samples are ready for collection.
11. Close box.
12. Spray box and transfer to corridor.

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13. Place a [removable] tape label on the box
14. Write the date and time and room on the box 15.
Place the rack in the transfer box and replace the lid.
16. Notify runners that samples are ready.
17. Restart sample processing.

Once a box is full to the required number of racks

- a) Place a “outgoing” laminated sign on the transfer box
- b) Spray and wipe the transfer box and remove it to the table in the corridor
- c) Phone to inform team there is a box ready for collection.

Full boxes of inactivated samples will be transported from the CL2 to RNA extraction lab by nominated staff (runners).

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Waste management

1. Ziploc bags, spare barcodes, paper towels, blue roll and blue gloves & oversleeves are disposed into the dry waste bag.
2. Pastettes and swab tubes are disposed into the ice cream tub containing 10% Surfanios/Distel.

The liquid level must be sufficient to cover them.

3. Spray MBSC surfaces, racks and other equipment and wipe with paper towel.
4. Place into dry waste bag.

When waste becomes full,

5. Take items out of dry waste bag and place into ice cream tub if there is still space.
6. Leave the rest of the dry waste items in the blue bag.
7. Spray inside of the dry waste bag.
8. Close the ice cream tub lid completely.
9. Spray the tub on all surfaces.
10. Place tub into the dry waste bag on top of the remaining waste (if any)
11. Spray internally around the opening of the bag and then all over outside of bag so that all exposed surfaces are wet after sealing.
12. Place 1 x rubber band on to seal the bag.
Do not twist the rubber band.
13. Open spare blue waste bag.
14. Place dirty waste bag inside spare waste bag.
15. Spray internally around the opening of the bag and then all over outside of bag so that all exposed surfaces are wet after sealing.
16. Place 1 x rubber band, doubled up, on waste bag.
17. Remove outer gloves and sleeves as per aseptic protocol.

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18. Leave gloves in MBSC.

19. Start 5-minute timer.

20. When timer rings, the waste is safe to remove from the MBSC with clean orange or blue gloves.

