Supplementary Method 1 Sample receipt, unpacking and barcoding

This SOP is associated with <u>Risk Assessment Diagnostic Screening of Clinical Respiratory ACDP Hazard Group 3 SARS-CoV-2</u>

⚠️ **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.   ⇒   This SOP is to be followed in order to avoid infection exposure to the virus

ℹ️ 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.

❗ The following Personal Protective Equipment (PPE) must be worn at all times in the sample receipt and processing area:

- ➤ A Howie style lab coat which <u>must be worn at all times</u>.
- ➤ nitrile or neoprene disposable gloves.

Supplementary Method 1 Sample receipt, unpacking and barcoding

> **i**   Location of sample receipt and processing area

Clinical samples will be delivered by courier and transferred to sample reception area by nominated staff

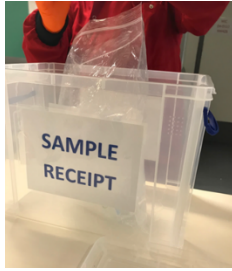**At the Receipt station, staff will act in the following capacities**

> ➢ Sample Checker (SC)
> ➢ Barcode Operator (BO)
> ➢ Barcode Label Operator (BLO)
> ➢ Sample Sorter (SS)

> **i**   Sample packaging

Incoming sample packs will arrive in a UN3373 medical carrier. They will then be transferred to a really useful box.

Supplementary Method 1 Sample receipt, unpacking and barcoding

Multiple samples will initially be triple bagged

  ➢ Each sample will be in 2 sample bags.

  ➢ The sample bags will grouped be in a larger outer bag (the sample pack).

Supplementary Method 1 Sample receipt, unpacking and barcoding

| A	Registering samples on Tube Tracker

Tube Tracker is a software system to track the whereabouts of samples received into the building.

1.   Logging into Tube Tracker

1.1   Open up the internet browser (Google Chrome) and search for the designated tube track website

1.2   Click on "Logon" in the top right hand corner of the screen.

1.3   All users must have an individual login for the system; generic accounts must not be used.

1.4   Log in using the username and desgnated password supplied.

1.5   Once a username and password have been entered the relevant location will be visible now click on "Sign In".

2.   Unpacking the contents from the bag

2.1   Once samples arrive into the laboratory they will need to be unpacked from each bag received. Click on the icon "Unpack Box" from the main menu on the left hand side of the screen.
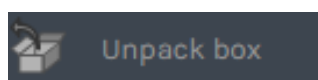
2.2   Scan the barcode label which should be on the outer bag into the field 'unpack box'. Always make sure the cursor is inside the field before scanning the bag barcode.


2.3   The contents of the box will appear to the right of the screen.

2.4   Begin scanning the barcodes of each individual sample to unpack and receive them. This will automatically update the sample audit trail to show when and where each sample has been received, and which user received it.

Supplementary Method 1 Sample receipt, unpacking and barcoding

2.5   When all samples have been unpacked from the box, a message will appear to the bottom right of the screen indicating that the box is now empty.
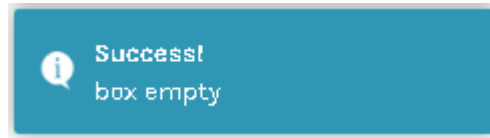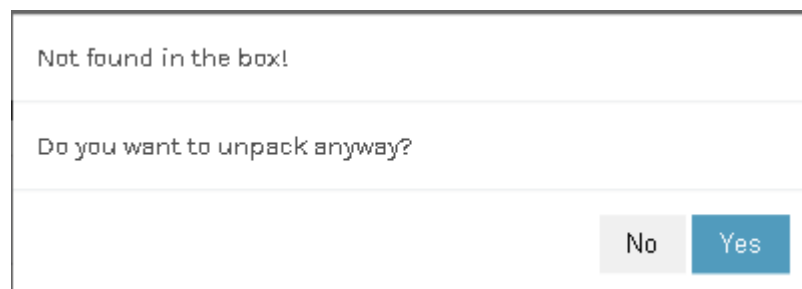

3.   **Missing samples**

3.1   If there are any missing samples, for example, a sample has been scanned into the bag but is not physically in the bag then this must be marked as "missing".

3.2   To mark samples as missing click on the following symbol that appears next to each sample number in the list. **Please ensure that the correct samples are marked as missing as once this has been clicked it cannot be undone.**

3.3   Notify the site that sent this sample so they are able to investigate what has gone wrong.

3.4   If there are any samples that are physically in the bag, but have not been tracked into the bag, the following message will appear:

3.5   Click "Yes" and the system will show on the audit trail for that sample that is has been unpacked. **Please ensure you have scanned the correct barcode before selecting yes, especially if there are other barcodes on the sample.**

Supplementary Method 1 Sample receipt, unpacking and barcoding

4.   Reporting System issues

4.1   There may be times when the system is slow, or error messages appear such as 'bad gateway'. This can be due to server issues. In most cases refreshing your browser and/or logging off and logging back on can resolve these. However, if the issue persists, please report using the contacts that have been provided for escalation.

B   Processing of "correct" clinical nasal or throat swab samples

Receipt

Sample Checker will;

    5. Open the really useful box of incoming samples

> ⚠️ **Warning Risk of exposure due to damaged or leaking materials**
>
> 6. Without opening a sample pack bag, visually inspect the samples within it for gross leakage.
>
>   If no leakage seen, proceed to step 7
>
> **If leakage is observed**
> i.   Do not open the sample pack or remove any sample bags
> ii.   If a member of health and safety (H&S) is not already in attendance, contact health and safety immediately
> iii. H&S will spray the sample bags with distel disinfectant
> iv.   Place the sample pack into a designated really usefull box labelled 'leaked samples'
> v.   Place the lid on the really useful box
> vi.   Transfer the samples to the CL3 facility to disinfect and recover any remaining usable samples.
> vii. Usable samples will start at receipt stage again
> viii. Unusable samples will be disposed of via autoclave process

    7. Open a sample pack

    8. Working with one sample bag at a time, remove a sample from the sample pack.

> ⚠️ **Warning Risk of exposure due to incorrect samples eg faeces, urine and blood**
>
>   9. Visually inspect the individual sample bag to ensure appropriate sample.
>
> If no leakage seen, proceed to step 11
>
> **If incorrect sample is observed**
> i.   Go to process C on page 12

Supplementary Method 1 Sample receipt, unpacking and barcoding

⚠️	**Warning Risk of exposure due to damaged or leaking materials**

10. Visually inspect the individual sample bag for leakage.

If no leakage seen, proceed to step 11

If leakage is observed

      ii.   Go to process D on page 15

i   **Sample check – process for <u>correct samples</u>**

It is important that the samples are clinical nasal or throat swab samples

    ➢ **Incorrect samples will need to be returned (see section B)**

11. Check that the sample is a clinical nasal or throat swab sample.

12. Check that sample has a barcode label. Note the barcode should start with a two digit number followed by a letter, commonly known as an EDTA number.

13. Tell the barocder that it is a correct sample.

Barcoding

The barcode operator will;

14. Scan the barcode though the sample bag twice,

    ➢ On barcode reader 1 to register sample on Clarity software

    ➢ On barcode reader 2 to allow the printing of 2 labels.

i   Barcode reader 1 scan check

    ➢ If the barcode has been scanned successfully, the following notification should be received:

Supplementary Method 1 Sample receipt, unpacking and barcoding

> If the barcode scanning has failed, the following notification should be received:

> Double check correct barcode has been scanned if an EDTA number matching sample 'force' the sample through for processing
> If non EDTA number entered check sample for an EDTA number anywhere on sample pack, if there is one scan if successful send sample through for processing. If failure 'force' sample through for processing
> If no EDTA number do not force but 'reset' and send sample through

process C page 12

| **i** | Barcode reader 2 scan check |

> Ensure that x2 barcodes (x3 barcodes if sample container has no barcode) have been produced.

15. Pass the sample to a barcode lable operator.

Supplementary Method 1 Sample receipt, unpacking and barcoding

<u>Labelling the sample bags</u>

The barcode label operator will;

> **16.** Advance the printer one label and cut using a scissors

⚠	**Warning Risk of exposure through damaging the sample bag integrity**

> ➤ **Do not staple through the main compartment of the sample bag**

17. Staple through the unbarcoded label onto the sample bag above the zip lock as shown here.

<u>Sample Sorting</u>

The sample sorter will;

ℹ	**Sample & the printed barcodes must match to prevent misdiagnosis**

> ➤ **The sample sorter must check the printed barcodes match** with the original one in the sample bag

> 18. Place all correct samples that have been correctly labelled in a really useful box

19. Fix the lid of the really useful box once full or all samples accounted for.

The samples are now ready for transport into the CL3 suite.

A runner will transfer full incoming sample transfer boxes to CL3 ready for staff processing the samples in CL3.

C	Processing of "incorrect" samples

⚠	**Warning Risk of exposure due to damaged or leaking materials**

20. Visually inspect the individual sample bag for leakage.

If no leakage seen, proceed to step 21

If leakage is observed

     iii.   Go to process D on page 15

---

i	**sample check – process for <u>incorrect samples</u>**

➢ <u>Incorrect samples include urine, faeces, blood</u>

21. Identify the sample as an incorrect sample (i.e. the sample is not a clinical nasal or throat swab sample).

22. Tell the barcode operator that it is an incorrect sample

Barcoding

The barcode operator will;

23. Scan the barcode though the sample bag twice,

➢ On barcode reader 1 to register sample on Clarity software

➢ On barcode reader 2 to allow the printing of 2 labels.

i	Barcode not readable

➢ If the barcode is unreadable pass to sample sorter for manual input onto paperwork

24. Advise the barcode label operator that it is an incorrect sample

25. Pass the sample to the barcode label operator

Labelling the sample bags

Supplementary Method 1 Sample receipt, unpacking and barcoding

The barcode label operator will;

26.   Advance the printer one label and cut using a scissors

⚠	Warning Risk of exposure through damaging the sample bag integrity

➢ **Do not staple through the main compartment of the sample bag**

27. Staple through the unbarcoded label onto the sample bag above the zip lock as shown here.

Sample Sorting

The sample sorter will;

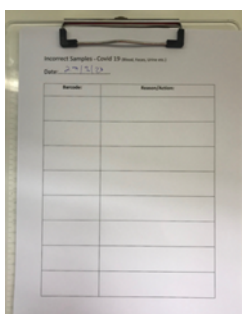| ℹ	**Sample & the printed barcodes must match to prevent misdiagnosis**

➢ **The sample sorter must check the printed barcodes match** with the original one in the sample bag

28. Record the details onto the two "Incorrect samples Covid19" form

Supplementary Method 1 Sample receipt, unpacking and barcoding

29. Place one of the printed barcodes onto the first "Incorrect samples Covid19" form.

    a. If barcode incorrect, hand write details on form
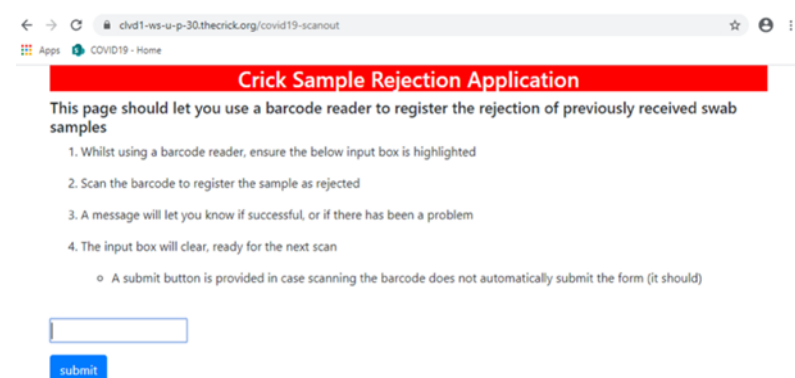
Place the second printed barcode onto the duplicate "Incorrect samples Covid19" form

30. Place all incorrect samples that have been correctly labelled in a really useful box labelled Return Samples

31. Fix the lid of the really useful box once full or all samples accounted for.

32. Place the sample into the box marked return/incorrect samples

33. Rescan the barcode in the sample rejection application to register the sample as rejected

D   Recording details of leaked/contaminated samples.

Sample Checking

The sample checker will;

34. Identify the sample as a leaked sample (i.e. the sample shows liquid in internal bag)

35. Tell the barcode operator that it is a leaked sample

Barcoding

The barcode operator will;

36. Scan the barcode though the sample bag twice,

   ➤ On barcode reader 1 to register sample on Clarity software
   ➤ On barcode reader 2 to allow the printing of 2 labels.

> **i**   Barcode not readable
>
>   ➤ If the barcode is unreadable pass to sample sorter for manual input onto paperwork

37. Advise the barcode label operator that it is an incorrect sample

38. Pass the sample to the barcode label operator

Labelling the sample bags

The barcode label operator will;

   39.   Advance the printer one label and cut using a scissors

> **⚠ Warning Risk of exposure through damaging the sample bag integrity**

> ➢ **Do not staple through the main compartment of the sample bag**

40. Staple through the unbarcoded label onto the sample bag above the zip lock as shown here.

Sample Sorting

The sample sorter will;

> **ℹ Sample & the printed barcodes must match to prevent misdiagnosis**

> ➢ **The sample sorter must check the printed barcodes match** with the original one in the sample bag

41. Record the details onto the two "leaked samples Covid19" form
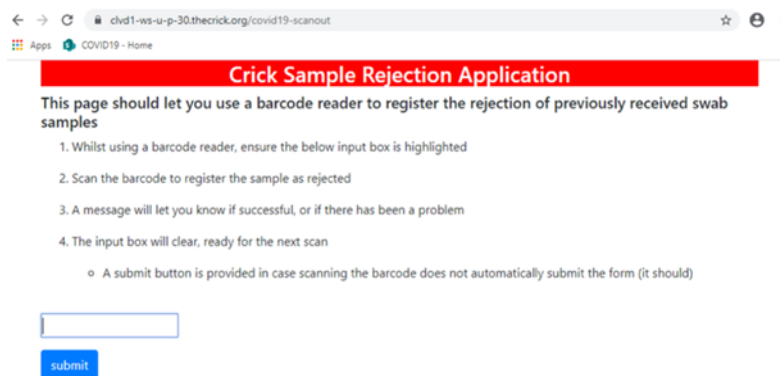
42. Place one of the printed barcodes onto the first "leaked samples Covid19" form.

   a. If barcode incorrect, hand write details on form

Place the second printed barcode onto the duplicate "Incorrect samples Covid19" form

43. Place all leaked samples that have been correctly labelled in a really useful box labelled Leaked Samples

44. Fix the lid of the really useful box once full or all samples accounted for.

45. Place the sample into the box marked <u>leaked samples</u>

46. Rescan the barcode in the sample rejection application to register the sample as rejected

Supplementary Method 2 Chemical inactivation of wet and dry swabs

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

⚠	**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.

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

ℹ	**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 the containment facility is restricted to authorised personnel only.

➢ Only those with health clearance and have been signed off as trained and competent are allowed to undertake this work within the containment Facility.

!	**Personal Protective Equipment (PPE) must be worn at all times in the containment facility**

Anyone entering the containment facility from the Gowning room must wear the following PPE

➢ A Purple Howie style lab coat which <u>must be worn at all times</u>.
➢ **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
   - Amphospray disinfectant
   - Liquid waste container: 10% solution of Surfanios 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, 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 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 I or 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 Amphospray so it is thoroughly soaked.
7. Spray the outer bag.
8. Open the outer bag and spray inside with Amphospray 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 Amphospray

Supplementary Method 2 Chemical inactivation of wet and dry swabs

> **!** **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 sequencing robot will only work if the labels are orientated vertically and <u>as straight as possible</u>!**

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.

Supplementary Method 2 Chemical inactivation of wet and dry swabs

28-29

30

33

    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 into the pastette.

32. Discard the pastette into the liquid waste container.

33. Wipe over inactivation vial with Amphospray-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.
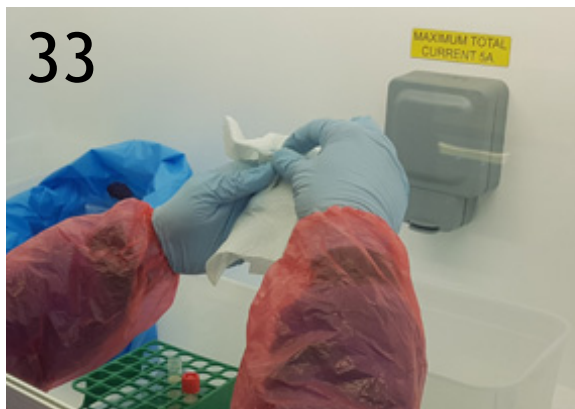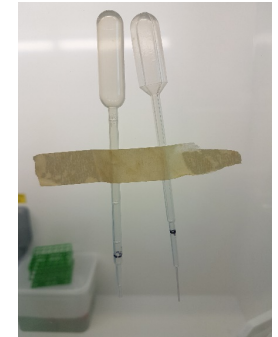
When a rack of inactivated samples is ready to be removed from the MBSC proceed to the **Removal of racks of inactivated samples from the MBSC** section on page 10

Supplementary Method 2 Chemical inactivation of wet and dry swabs

DRY SWAB PROCESS

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

Universal Conical

Urine pot

! **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.

! **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 sequencing 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)

Supplementary Method 2 Chemical inactivation of wet and dry swabs
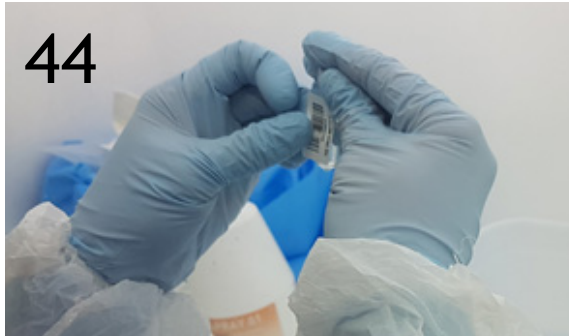
43. Check the 2ml inactivation tube contains 1ml L6 lysis buffer
44. Stick the barcode label to the inactivation tube.
45. Place the labelled inactivation tube into the rack.
46. Take a tissue and spray with Amphospray so it is thoroughly soaked.
47. Spray the outer bag
48. Open the outer bag
49. Spray inside the bag with Amphospray 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 Amphospray
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 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

Supplementary Method 2 Chemical inactivation of wet and dry swabs

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
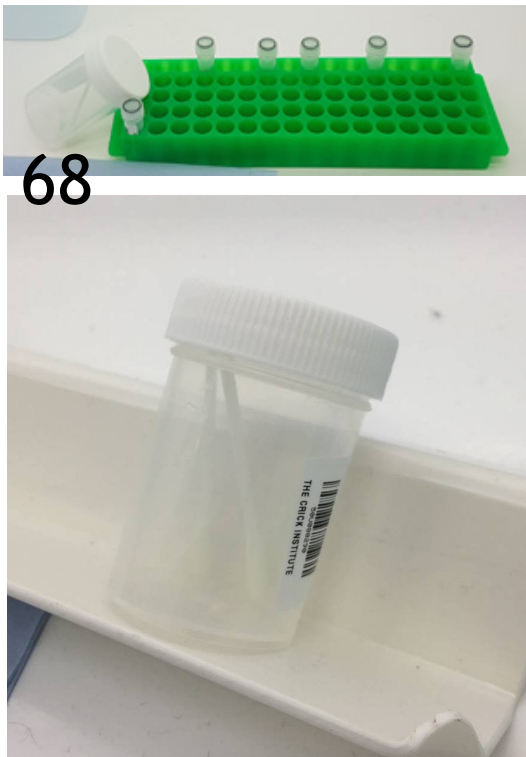
> ❗ **Single sample per cycle only**

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.

Supplementary Method 2 Chemical inactivation of wet and dry swabs

> **!** **Correct barcode check**

> ➤ 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 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

> **i** **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 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 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

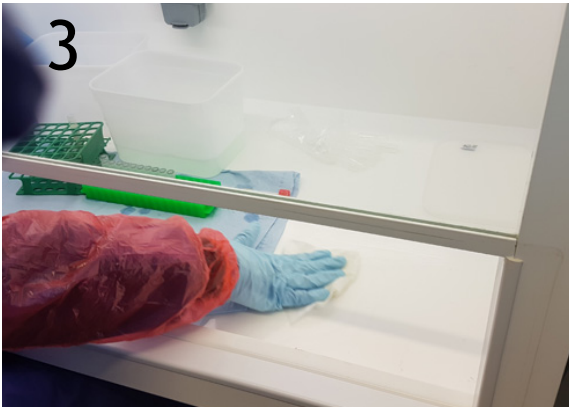**Supplementary Method 2 Chemical inactivation of wet and dry swabs**

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 Amphospray onto tissue and wipe area.
   b. Directly spray an area on the floor of the hood.
4. Spray the rack thoroughly with Amphospray 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.
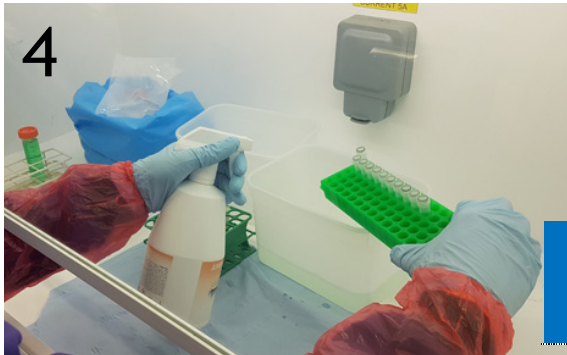
! **To prevent fatigue and operator error, work only in pre-arranged 1-hour shifts. If you are in the middle of a batch, stop and hand over to the next person.**
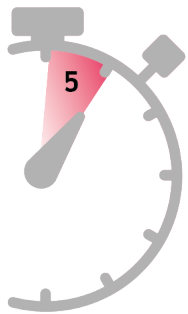
! **Robot processing in sequencing facility**

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.

Supplementary Method 2 Chemical inactivation of wet and dry swabs

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

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.

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.

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.

Supplementary Method 3 Aliquoting Inactivated Virus to 96-DeepWell Plates

Equipment / Consumables

- Hamilton Star or Starlet robot

- Hamilton rack with 2 mL tube inserts

- Nunc 96-Well Polypropylene DeepWell Storage Plates (Cat no. 260251)

- Hamilton 1000 μL Pipette tips

- Screw cap (coloured) for microtube (e.g. red - Cat no. 65.716).

Procedure - Transfer of inactivated virus to 96 well plates

1. Sample tubes will arrive from the Cat 3 virus inactivation lab at room temp. Working on a "**dirty**" bench, wipe outside of tube with alcohol wipe, invert tube twice, and place in centrifuge. Pulse spin tubes.

2. Remove tubes from centrifuge and place in new box on "**clean**" bench.

3. Unscrew 2 mL tubes containing sample and discard lid. Place open 2 mL tubes in Hamilton rack (Cat no. SMP_CAR_32_A00 with blue 2 mL tube inserts) with barcode facing forward as shown below:
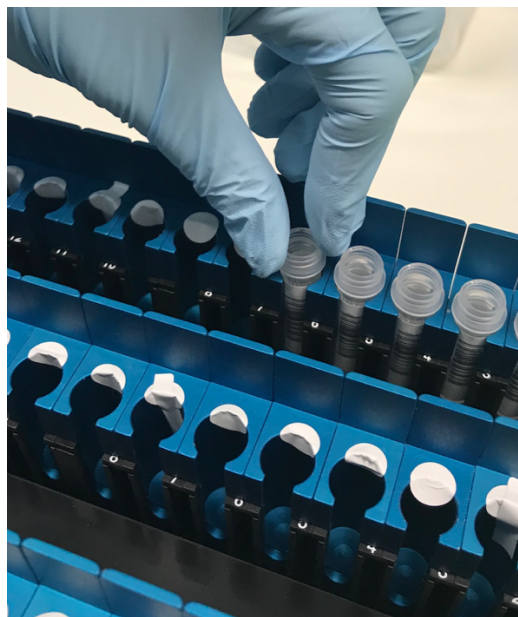
Supplementary Method 3 Aliquoting Inactivated Virus to 96-DeepWell Plates

4   Place rack in robot track position 45-47 (racks 1-3 respectively) depending on number of samples to process. Align the barcodes on the tubes to be visible in the inserts window. If processing 94 samples, leave rack 3, spaces 31 and 32 empty for controls to be added later.

5   Place an LPL barcode label on lower edge on the right-hand side of a 96-well Nunc (1.3 mL) plate (Cat no. 260251).
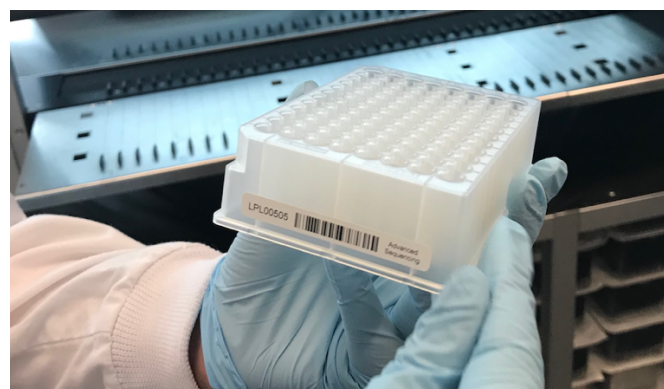
6   Scan barcode into label printer to make an additional identical barcode for archiving box. Place in plate carrier (Cat no. PLT_CAR_L5MD_A00) position 5, in track position 49-54 on Hamilton robot.

Supplementary Method 3 Aliquoting Inactivated Virus to 96-DeepWell Plates

7   Access sample transfer protocol from 'Hamilton protocol shortcut' button on Hamilton desktop.

8   A pop-up window will give the option to use loading help, always select **yes**.

9   Follow guide using tip carrier with a tip rack in all 5 positions (can be empty, full or part used. Carrier cat no. TIP_CAR_480_AOO). Tip carrier placed in track positions 37-42).

10   Check cover is closed as the final step, press **OK**, protocol will start and will transfer 150 µL of inactivated virus solution to a specified position in the barcoded 96-well plate.

11   When program is complete, and the carriers are back in initial position, remove 96-well plate.

12   Visually check volume in plate (this is easiest to observe from below):

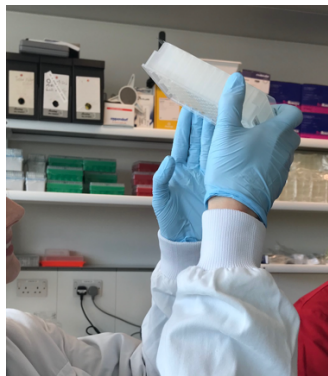13   Check .csv output for the transfer run for error messages. These should all be zero. If errors occur, report code to technical support. Upload .csv file into sample tracking app.

14   Either: Cover plate and handling very carefully, pass plate immediately to RNA extraction operators to be placed on BioMek FX.

Or: If plate is not to be used immediately, add plastic seal (Cat no. AB-0558) and store 96-well Nunc plate at room temperature in clear box labelled "to be extracted" until extraction robot is available.

15   Remove 2 mL tube containing residual sample from the Hamilton rack and cap with new coloured lid.

Supplementary Method 3 Aliquoting Inactivated Virus to 96-DeepWell Plates

Archiving

- The app that is used to perform the consolidation step will ask you to enter the barcode. Attach this barcode label to the box that will be used to archive the samples:

NOTE: This app is also used for archiving the RNA samples

- When this step is carried out in ClarityLims, a database table is updated with the information.

- This step results in an entry appearing on the dashboard to let you know the samples are ready to be archived:

- An archiving app then displays the boxes and plates that are waiting to be archived, and gives you the chance to enter the storage information (see below):

- Once logged, the record disappears from this screen, and the archiving information will appear in the archive search app (see below):

Supplementary Method 3 Aliquoting Inactivated Virus to 96-DeepWell Plates

Covid Sample Finder

Find a Sample Tube

Enter the sample barcode you're looking for

[   ]

[find]

Tube Box Archive

Use this table to find the storage location for an archived tube box

Use the search to the left to find out which box holds a particular sample

Box Barcode	Freezer	Slot	Date

Stock RNA Plate Archive

Use this table to find the storage location for an archived stock RNA plate

Use Clarity to find out which plate has a particular sample

Plate Barcode	Freezer	Shelf	Box	Date

- Place samples in freezer box labelled with printed barcode and store at -80°C.

Checklist – Aliquoting Inactivated Virus to 96 well plate

Name(s) of operator(s)	
Sample box barcode	LPL
RNA plate barcode	SPL
Bead Lot No.	
qPCR plate barcode	

Sample reception and Hamilton operation

Operator name: _____ **Date/time:** _____

- ❑ Collect samples from submission fridge and press the 'Collect' button.

- ❑ Verify correct number of tubes. Number counted:_____

- ❑ Wipe down the bench and sample box.

- ❑ Clean, invert and centrifuge tubes.

- ❑ Place tubes in new Wesbart box.

- ❑ Open tubes, discard cap and place in Hamilton rack.

- ❑ LPL barcode affixed to Nunc plate. Second person sign-off:_____

- ❑ Duplicate LPL barcode and affix to Wesbart box.

- ❑ Run Hamilton software to transfer samples from tubes to plate.

- ❑ Visually check volumes in plate.

- ❑ Check Hamilton error log.

- ❑ Complete consolidation step in Clarity.

- ❑ Seal the sample plate and hand to FX operator or place in 'to be extracted' container.

- ❑ Re-cap tubes with coloured lids and place in Wesbart box. Put box in 'to be archived' container.

- ❑ Wipe down the bench and Hamilton.

- ❑ Bring this sheet to the corresponding Page 2 with Biomek FX operation instructions.

Supplementary Method 3 Aliquoting Inactivated Virus to 96-DeepWell Plates

Notes – errors, variations on SOP etc

Supplementary Method 4 Automated RNA extraction using Biomek FX

Equipment & Consumables

Consumables	Used/ run
Eppendorf 96-well skirted plates LoBind (Eppendorf, Cat.no. 0030129512)	1
NUNC 96-well deep well plates (ThermoScientific, Cat.no. 260251)	3
Axygen squared 96-well deep well plates (Fisher Scientific Cat.no. P-2ML-SQ-C)	1
Reservoir (VWR, Cat. no. 613-1175)	3
Adhesive PCR Plate Seals (Thermo Fisher, Cat. no. AB0558)	1
BIOMEK FX 250 μL tips, Filtered, Sterile (Cat. no. 717253)	4
BIOMEK FX 50 μL tips, Filtered, Sterile (Cat. no. A21586)	3
Equipment	
BIOMEK FX workstation and associated equipment	1
Alpaqua Magnum FLX (Cat. no. A000400)	1

Supplementary Method 4 Automated RNA extraction using Biomek FX

<u>**Reagents**</u>

Reagents (note: some of these reagents are used to make the buffers below)
Water for HPLC (Fisher Scientific, 2.5 L, 270733-2.5L, 4 GBP)
1 M Tris-HCl pH 8.0 (AppliChem, 500 mL, A4577,0500, 47 GBP)
Guanidine hydrochloride (Sigma, 1 kg, G3272-1KG, 168 GBP) BATCH CERTIFIED
Isopropanol (e.g. Merck Millipore, 2.5 L, 1096342500, 13 GBP)
3 M sodium acetate pH 5.2 (Sigma, 500 mL, S7899-500ML, 47 GBP)
PE buffer (Qiagen, 100 mL, 19065, 53 GBP)
Ethanol (e.g. Merck Millipore, 1 L, 1009832500, 17 GBP)
Tween-20 (Sigma Aldrich, 100 mL, T2700-100ML, 23 GBP)
0.5 M EDTA pH 8.0 (Applichem, 1 L, A4892,1000, 72 GBP)
Silica beads (VWR--G-BioSciences, 5 mL, 786-915, 127 GBP) BATCH CERTIFIED

Supplementary Method 4 Automated RNA extraction using Biomek FX

Buffers

TET buffer [10mL] **See Supplementary Method 10**

PRE-PREPARED AND **BATCH CERTIFIED**

Reagent	Volume	Final concentration
Water	~49.4 mL	
0.5 M EDTA, pH 8.0	100 μL	1 mM
1 M Tris-HCl, pH 8.0	500 μL	10 mM
Tween-20	25 μL	0.05%

Binding buffer [BB] [50mL] See Supplementary Method 9

PRE-PREPARED AND **BATCH CERTIFIED**

Reagent	Volume/amount	Final concentration
Guanidine hydrochloride	23.88 g	5 M
Water	to 30 mL	
Isopropanol	to 50 mL	40%
Tween 20	25 μL	0.05%
3 M sodium acetate	2 mL	115 mM

PE-buffer, 60 ml needed for 1 BIOMEK FX extraction run

Prepare according to instructions by Qiagen

REMEMBER TO ADD ETHANOL TO QIAGEN REAGENT AS PER QIAGEN INSTRUCTIONS ON THE PE BOTTLE

Reagent	Volume
PE buffer by Qiagen	60 mL

Supplementary Method 4 Automated RNA extraction using Biomek FX

Procedure

RNA extraction using IN HOUSE protocol on BIOMEK FX
NB The deck layout is specific to the robot performing the extraction. Be sure to use the correct set-up for the correct robot!

1. Open the Biomek software, and select the relevant RNA extraction program "**(See protocol below)**
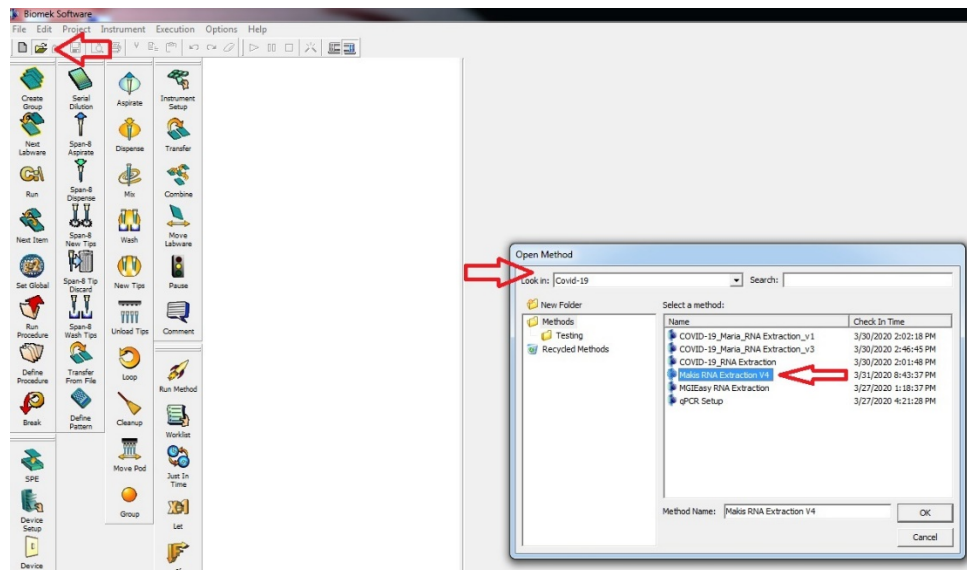
2. Click on "**Instrument Setup**" as shown below to view the deck layout:
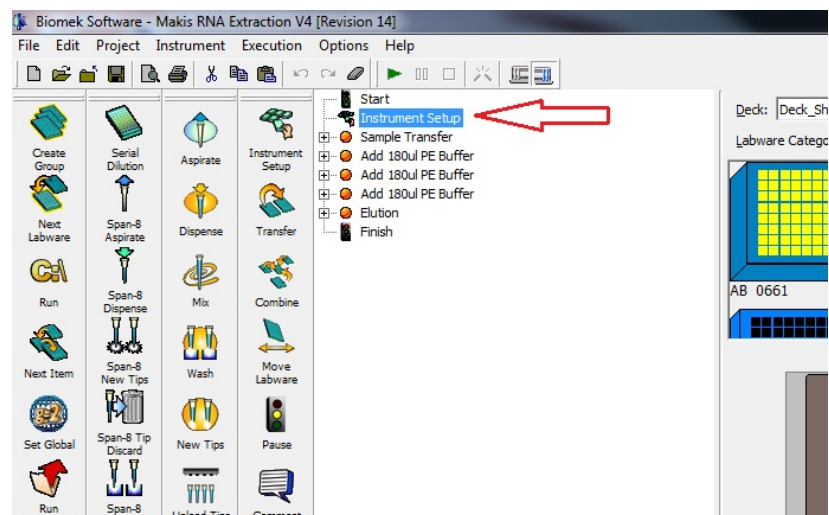
Supplementary Method 4 Automated RNA extraction using Biomek FX

FX1 DECK LAYOUT
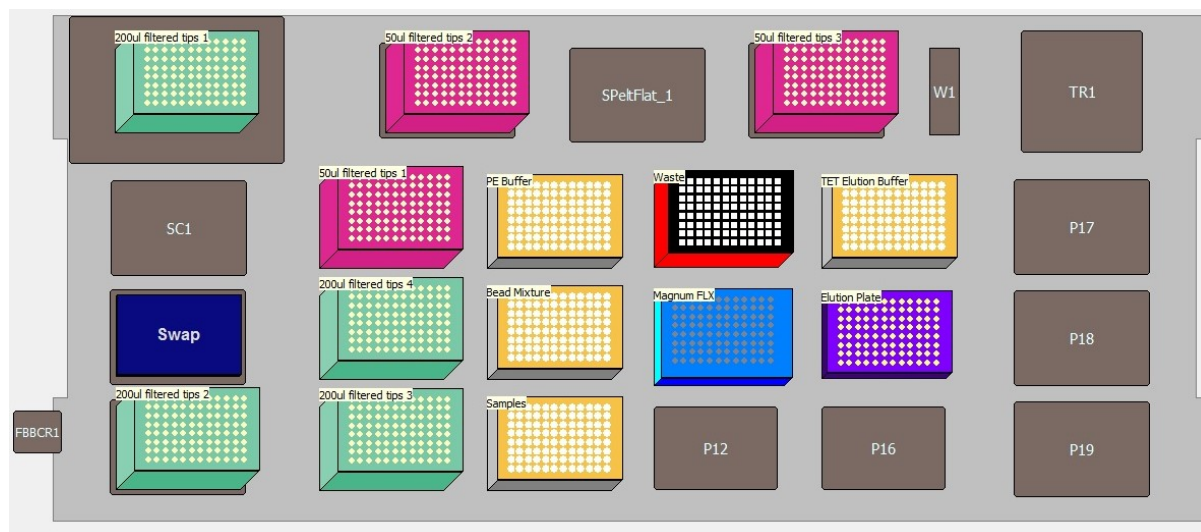
3. Place 4 boxes (lid off) of **250 µL filter tips** (green boxes) as shown above.

4. Place 3 boxes (lid off) of **50 µL filter tips** (pink boxes) as shown above.

5. Place an **Axygen Deep well plate** in position **P10**.

At a clean bench prepare reagents as follows:

Prepare beads for 96 samples**:**

6   Take a 1.5 mL tube of pre-aliquoted silica beads (550 µL) from the fridge and vortex well.

7   Place on the single tube magnet for 1 minute or until supernatant is clear.

8   Remove and discard supernatant (into the tip waste bin).

9   Remove tube from magnet and add **500 µL TET**. Resuspend the beads by vortexing.

10 Pulse spin the tube and place on the magnet for 1 minute or until supernatant is clear.

11 Remove and discard supernatant.

12 Repeat steps 10 to 12 for a total of 3 times.

13 Remove the tube from the magnet, resuspend the beads in **500 µL TET**, vortex and spin down.

Supplementary Method 4 Automated RNA extraction using Biomek FX

Prepare binding buffer/bead mix:

14 In the Falcon tube containing the 50 mL binding buffer (labelled BB, pre-aliquoted) add the beads prepared in 6.

15 Mix well by inverting.

16 Pour into a reservoir and aliquot 400 μL <u>immediately</u> in each well of a 96 deep well Nunc plate, using a multichannel pipette. Place on the deck **P8.**

17 Add PE buffer to a new reservoir and aliquot 600 μL into a new Nunc plate and place plate in position **P7**.

18 Add TET to new reservoir and aliquot 70 μL TET (from batch certified aliquot) into a new Nunc plate and place in position **P14**.

19 Place the sample plate from Supplementary Method 3 (*seal removed*) in position **P9**.

   NB: If samples have been temporarily stored, briefly spin in the plate centrifuge.

20 Label an empty Eppendorf LoBind 96-well skirted plate with a pre-printed SPL barcode (e.g. SPL00000) on the front side, and place in position **P15**.

21 Place the **Alpaqua Magnum FLX** in position **P11.**

The final set up should look like the photo below:
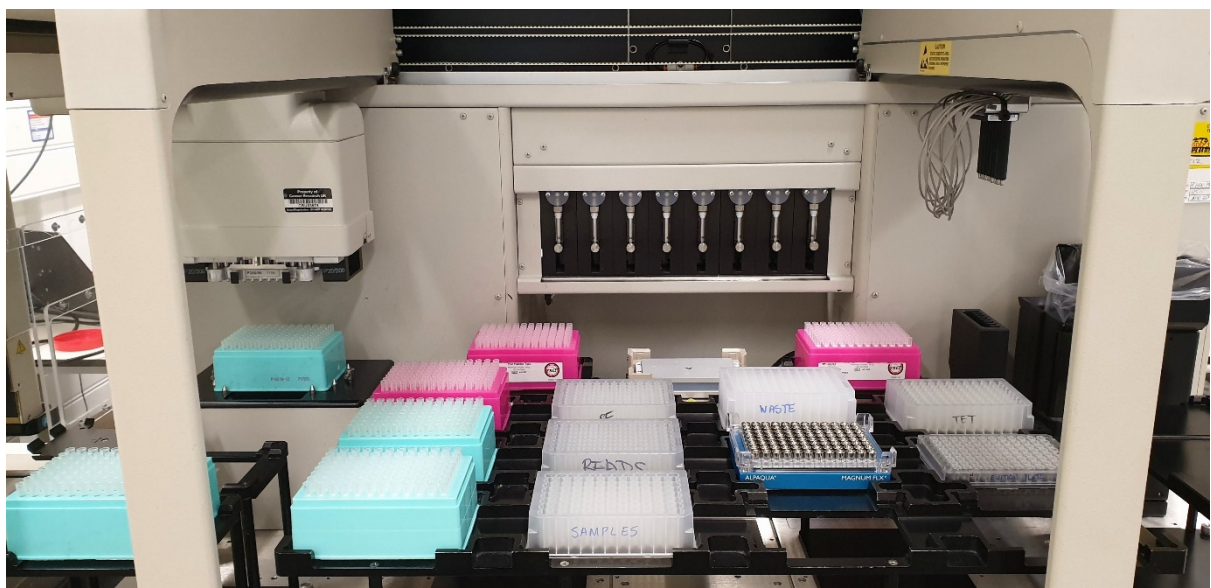
Supplementary Method 4 Automated RNA extraction using Biomek FX

22 Using the laptop associated with the Biomek FX, push the samples through the Extraction step in ClarityLIMS.

23 Make sure you switch ON the Inheco (blue box).

24 To START the program press the green triangle ▶ located in the software.

25 When then program is complete, remove the Eppendorf 96-well skirted plate from the deck, seal with a Thermo Adhesive PCR plate seal and place in the -80℃ until it is used for qPCR.

<u>Archiving</u>

26 Archiving of Inactivated viral samples would have already been performed in Supplementary Method 3. For archiving the stock RNA plates using the app in ClarityLims, a database table is updated with the relevant information

27 This results in an entry appearing on the dashboard to let the team know the samples are ready to be archived:

Consolidation

Incoming samples! (includes repeats)

Samples requested for repeat

Tubes boxes to be Archived

RNA plates waiting to be archived / recorded

1. Container name: LPL00505
   ○ Date produced: 2020-03-31 12:32:08

RNA Extraction

Queued for extraction

Stock RNA Plates to be Archive

RNA plates waiting to be archived / recorded

1. Container name: SPL00005
   ○ Date produced: 2020-03-30 20:17:08

28 An archiving app then displays the boxes and plates that are waiting to be archived, and gives the team the chance to enter the storage information:

Covid-19 sample archiving application

Sample tube archiving

The following tube boxes need archiving:

1. LPL00505: Select this record : ○
   ○ Date: 2020-03-31 12:32:08
   ○ Work done by: Laura

   Record details below

   - Freezer: _____
   - Slot Number: _____

   submit

Stock RNA Plate Archiving

The following stock RNA plates need archiving:

1. SPL00005: Select this record : ○
   ○ Date: 2020-03-30 20:17:08
   ○ Work done by: Laura Cubitt

   Record details below

   - Freezer: _____
   - Compartment : [Top ⬍]
   - Box Number: _____

   submit

Supplementary Method 4 Automated RNA extraction using Biomek FX

29 Once logged, the record disappears from this screen, and the archiving information will appear in the archive search app:

Covid Sample Finder

Find a Sample Tube

Enter the sample barcode you're looking for

[   ]

[find]

Tube Box Archive

Use this table to find the storage location for an archived tube box

Use the search to the left to find out which box holds a particular sample

Box Barcode	Freezer	Slot	Date

Stock RNA Plate Archive

Use this table to find the storage location for an archived stock RNA plate

Use Clarity to find out which plate has a particular sample

Plate Barcode	Freezer	Shelf	Box	Date

APPENDIX

The robot carries out the following automated protocol:

1. The robot transfers 150 µL of the samples to the 400 µL (2.6x Vol) of Binding Buffer (BB) containing the Silica beads and mixes 10 times by pipetting up and down.
2. Move the plate on the Shaker for 5 min
3. Move plate to position P8 and mix 20 times
4. Move plate to Shaker for 2 min
5. Move plate to P8 and incubate for 3 min
6. Move plate to Magnet for 5 min
7. Remove supernatant
8. Move plate from magnet to position P8

   Wash with PE (1)
9. Transfer 180 µL of PE Buffer in each well of the Bead Mixture plate
10. Move plate to the Shaker for 20 sec
11. Move plate to the Magnet for 60 sec
12. Remove supernatant
13. Move plate from the magnet to position P8

   Wash with PE (2)
14. Transfer 180 µL of PE Buffer in each well of the Bead Mixture plate
15. Move plate to the Shaker for 20 sec
16. Move plate to the Magnet for 60 sec
17. Remove supernatant
18. Move plate from the magnet to position P8

   Wash with PE (3)
19. Transfer 180 µL of PE Buffer in each well of the Bead Mixture plate
20. Move plate to the Shaker for 20 sec
21. Move plate to the Magnet for 60 sec
22. Remove supernatant
23. Move plate from the magnet to position P8
24. Wait for 2 min (drying)

Supplementary Method 4 Automated RNA extraction using Biomek FX

Elution

25. Transfer 50 μL of TET Buffer to the Bead Mixture plate and mix 10 times
26. Move plate to the Shaker for 5 min
27. Move plate to the magnet for 60 sec
28. Transfer 40 μL from the Bead Mixture plate to the Eppendorf twin.tec plate

Supplementary Method 4 Automated RNA extraction using Biomek FX

Checklist Automated RNA extraction on Biomek FX

Name(s) of operator(s)	
Sample box barcode	LPL
RNA plate barcode	SPL
Bead Lot No.	
qPCR plate barcode	

Biomek FX operation

Operator name:_____ **Date/time:**_____

- ❑ Wipe down the bench.
- ❑ Initialise the FX by homing all axes.
- ❑ Open the RNA extraction program on the FX computer.
- ❑ Load the Biomek FX deck with 4x 250 µl and 3x 50 µl pipette tip boxes and a waste plate.
- ❑ Wash aliquot of beads 3x in TET, add to Binding buffer and dispense 400µl into plate. Load onto deck.
- ❑ Dispense 600µl Buffer PE into plate. Load onto deck.
- ❑ Dispense 60µl remaining TET into plate. Fill the negative control well with 60µl water. Load onto deck.
- ❑ Load LPL sample plate onto deck.
- ❑ Affix an SPL barcode to an Eppendorf Twin-tec skirted plate. Load onto deck.
- ❑ Verify that all items have been loaded onto the deck correctly. Second person sign-off:_____
- ❑ Complete the RNA extraction step in Clarity. Scan the SPL barcode in.
- ❑ Run the RNA extraction program.
- ❑ After program completion, visually check volumes in the RNA plate.
- ❑ Wait for qPCR team member to arrive with qPCR plate.
- ❑ Open the program 'qPCR setup' on the FX computer.
- ❑ Load the RNA plate and qPCR plate. Ensure the qPCR plate is placed on an adapter plate.
- ❑ Load pink pipette tip box. Remove the tip from position H12.

Supplementary Method 4 Automated RNA extraction using Biomek FX

- ❑ Run the transfer program.
- ❑ Add positive control manually in H12.
- ❑ Seal the qPCR plate, hand to qPCR team member.
- ❑ Seal the RNA plate and place inside small -80°C freezer, ready to archive box.
- ❑ Staple this sheet to the corresponding Page 1 with sample reception and Hamilton steps. Place the sheets in the binder.
- ❑ Wipe down the bench.

Archiving

Note: wait a reasonable amount of time before archiving in case the qPCR is unsuccessful and needs repeating.

- ❑ Take sample box to CovFreezer 1, place in next available slot in the rack and record location details in the COVID-19 Landing page.
- ❑ Take RNA plates to CovFreezer 2, place in next available box and record location details in the COVID-19 Landing page.

Supplementary Method 4 Automated RNA extraction using Biomek FX

Notes – errors, variations on SOP etc

Supplementary Method 5 Manual Preparation of RT-PCR master mix plate

Equipment / Consumables

- Rainin L-100 manual single pipette

- Rainin L-1000 manual single pipette

- Rainin 20-300µl LTS multichannel pipette

- Rainin 10-100µl or 20-200µl LTS electronic single channel pipette

- Rainin filtered tips: green box, blue large box, and grey/yellow box

- Eppendorf Tube rack

- 25ml individually wrapped reservoirs

- PCR plate (MicroAmp™ Fast Optical 96-well reaction plate with barcode, Cat. no. 4346906)

- MicroAmp™ Optical Adhesive Film (Cat. no. 4311971)

- Seal roller

- PCR chiller plate

- Vortex

Reagents

Real-time fluorescent RT-PCR kit for detecting 2019-nCo (BGI, 50 reactions; Cat MFG030010)*			
Item（50 tests/kit)	Specification	Quantity	Description
2019-nCoV Reaction Mix	1mL /vial	1 vial	Composed of reagent for amplification and probes and primers of target gene and internal reference
2019-nCoV Enzyme Mix	80µL /vial	1 vial	Taq polymerase, Reverse transcriptase and UDG
2019-nCoV Positive control	750µL/vial	1 vial	Mix solution of pseudo-virus with target virus genes and internal reference
2019-nCoV Blank control	750µL/vial	1 vial	(Not used in protocol)

Supplementary Method 5 Manual Preparation of RT-PCR master mix plate

<u>**Procedure**</u>

In RNA extraction lab:

1. Prepare sealed and barcoded empty PCR plates and store in lab. Barcodes of one PCR plate will be scanned and linked to a barcoded RNA plate when the RNA is eluted.

2. **The coupled empty PCR plate and RNA plate will be taped together and handed over if RT-PCR staff are retrieving straight away or otherwise stored at -80ºC.**

3. Take both linked plates to PCR lab. Place RNA plate on ice. Take PCR plate only to RT-PCR mix aliquoting bench.

Manual Preparation of PCR master mix in 1 x 96 well MicroAmp™ plate

NOTE: Reagents are stored at -20ºC. Take out all the kit contents and thaw them thoroughly at ambient temperature. Vortex and centrifuge briefly. The enzyme mix should be kept on ice at all times

4. When email indicating 'RNA plate is ready' is received, take required number of kits from the -20°C freezer (2 Kits per 96w plate of RT-PCR reactions) and remove the *2019-nCoV Enzyme mix* and Negative Control tubes from them to maintain in -20ºC freezer while the remaining tubes (*2019-nCoV Reaction mix*, Positive Control) thaw at RT.

5. When *2019-nCoV Reaction mix* has thawed, retrieve *2019-nCoV Enzyme mix* tubes from -20°C, quick spin down all tubes.

6. Using an L-100 pipette with filtered tip from green tip box, add 80µl of *2019-nCoV Enzyme mix* (entire tube) to one *2019-nCoV Reaction mix* tube to make final PCR Master mix. Mix well by vortex and spin down.

7. Dispense PCR Master mix into plates as follows:

   • For half plate, leave PCR mix in tube and use an electronic single channel pipette fitted with a filter tip to draw up 100 or 200µl and repeatedly dispense 20µl into individual wells of the 96 well PCR plate.

   • For Full Plate: Using an L-1000 pipette with filtered tip from Blue tip box, pipette all the Master Mix from individual tubes into a 25ml individually wrapped reservoir. Dispense 20ul of Master mix from step into the first 7 wells of a barcoded MicroAmp™ Fast optical 96-well plate, using an LTS 20-300µl multichannel pipette.

Pipette settings: From Main menu, select Multi-Disp setting with Aliquot volume 20 µl, 7 aliquots, 1/1 Asp/Dsp Speed

Supplementary Method 5 Manual Preparation of RT-PCR master mix plate

8 Eject any remaining PCR master mix left in tips back into reservoir.

9 Use 2 tips to take up PCR Master mix and dispense into wells of last row, 2 wells at a time keeping the same pipette setting.

10 Seal the plates with MicroAmp™ Optical Adhesive Film. Check each well is sealed.

11 Briefly **spin** the plate at 1000rpm, 1min, and store on ice or at 4°C, together with the thawed positive control tube.

12 Transfer remaining PCR master mix back into a Reaction Mix tube, label with date, and put in box in fridge under the bench

13 Clean up bench by spraying with 70% ethanol or distal and wiping with paper towel.

Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX

Equipment and Consumables

Consumables	Used/run
Eppendorf 96-well skirted plates LoBind (Eppendorf, Cat.no. 0030129512)	1
MicroAmp™ Fast optical 96-well plate with Barcode (ThermoFisher, Cat no. 4366932)	1
MicroAmp™ Optical Adhesive Film (Thermo Fisher, Cat no. 4311971)	1
MicroAmp™ Adhesive Film Applicator (Thermo Fisher, Cat no. 4333183)	1
Adhesive PCR Plate Seals (Thermo Fisher, Cat no. AB0558)	1
Biomek FX 50 µL tips, Filtered, Sterile (Beckman Coulter, Cat no. A21586)	1
RNAse ZAP wipes	1
Equipment	
Beckman Biomek FX workstation	1
Barcode Reader	1

Reagents

Extracted viral RNA samples (prepared using **Supplementary Method 4**) in a 96-well barcoded plate.

ABI MicroAmp™ Fast Optical PCR plate containing RT-PCR Master Mix for detecting SARS-CoV-2 (prepared using **Supplementary Methods 5)**.

Positive control
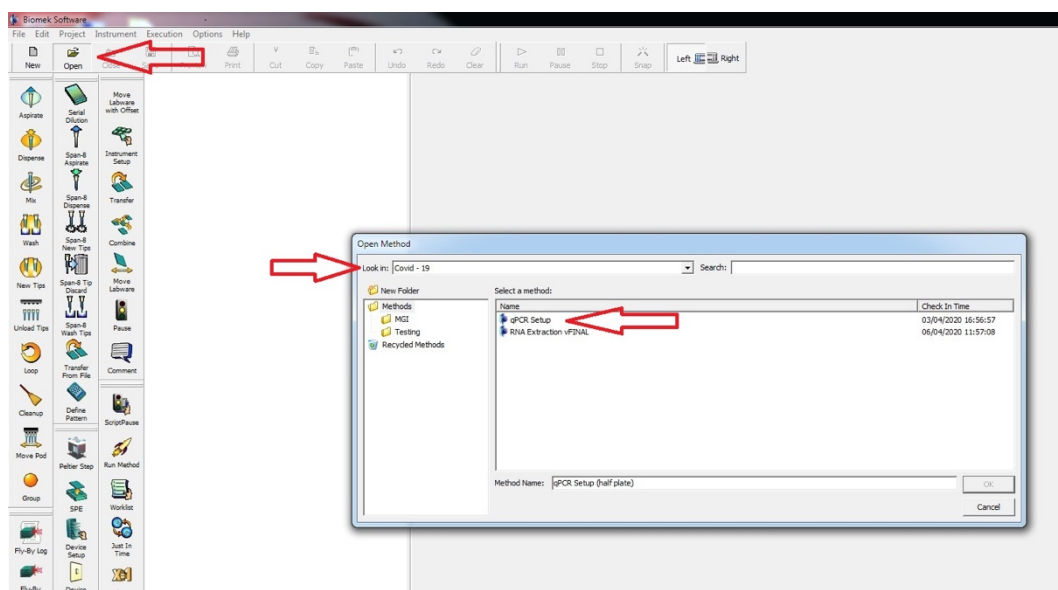2019-nCoV Positive control - included in the BGI kit

Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX
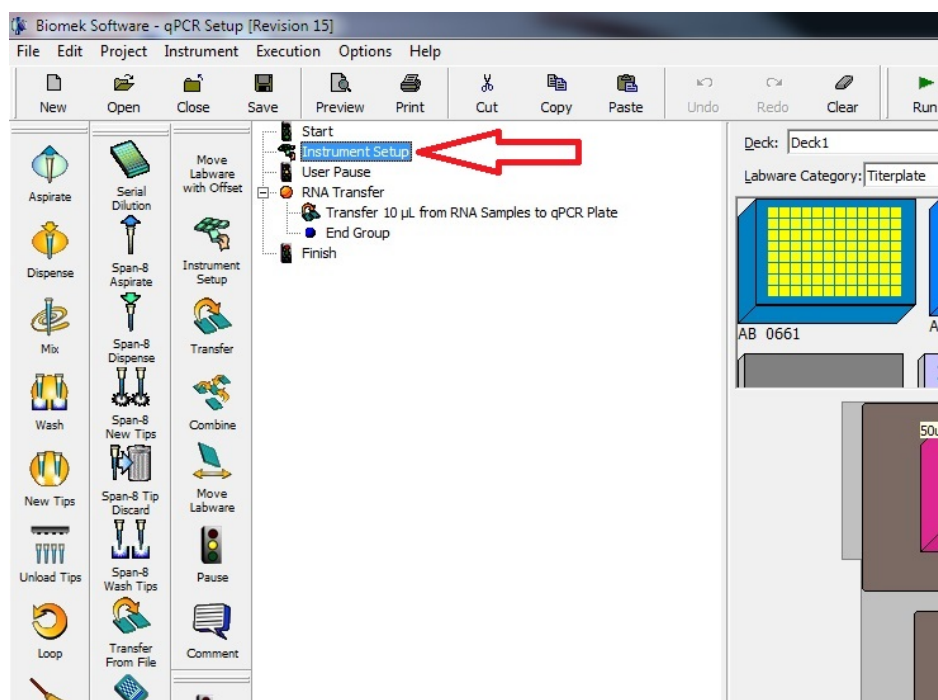
Procedure

Biomek FX Setup

1. Open the Biomek software, and select the designated program (see Appendix) for "**qPCR Setup**" from the relevant location:
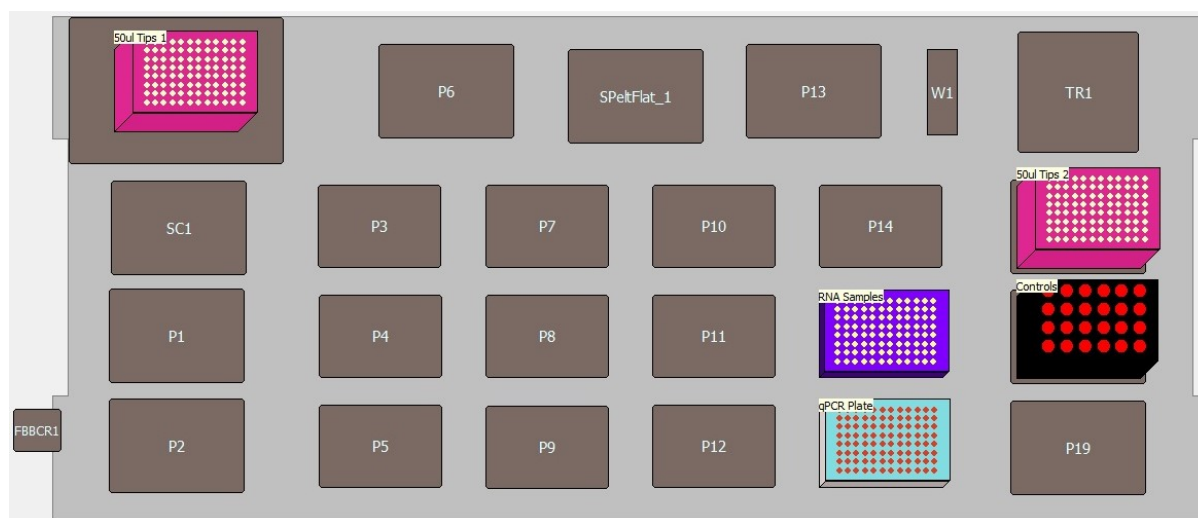

2. Click on "**Instrument Setup**" as shown below to view the deck layout:

Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX

FX1 DECK LAYOUT

3. Wipe the deck using an RNAse ZAP wipe, followed by an alcohol wipe or 70% ethanol.

4. Place 2 boxes (lid off) of **50 μL filter tips** as shown above.

5. Take the **RNA sample plate** (Eppendorf LoBind 96-well skirted plate), remove the seal, and place in position P15 with the SPL barcode on the front side.

6. If the ABI MicroAmp™ Optical plate containing the **qPCR reagents mix** has been stored at 4°C, spin at 1000 rpm for 1 min in the table top centrifuge.

7. Then stack on a black Greiner CellStar plate. Place on the deck in position P16.

8. Make sure you **scan the barcode** of the qPCR plate on the Clarity LIMS. This will link the barcode of the qPCR plate with the RNA samples.

9. Place a black microfuge tube rack in position P18.

10. Place a 2ml screwcap tube (cap off) with TET buffer (or H2O) in position A1 and the Positive Control tube (cap off) in position D1 of the above rack.

11. Home the instrument by going to "Instrument" and selecting "Home All Axes".

12. Make sure to purge the Span-8 syringes until no bubbles are seen in the tubing.

13. Check the level of the Biomek FX Span-8 water container. Re-fill with Milli-Q water if the level is too low. Empty the waste bottles in the sink when they start getting full.

Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX

14. To START the program, press the Run button (green triangle ▶ Run) located in the software.

15. The prompt on the screen will remind you to remove two tips from positions G12 and H12 of the "50 µL Tips 1" tipbox. Click OK to continue.

16. The next prompt will remind you that the MicroAmp™ plate should be stacked on top of a black Greiner plate.

17. The last prompt will remind you of the microfuge rack setup:
    A1: TET buffer (or H2O) in 2ml tube
    D1: Positive Control

18. When the program is complete, seal the RNA sample plate using a Thermo Fisher PCR seal and place at -80°C freezer for temporary storage.

19. Carefully remove the MicroAmp™ plate from the deck.

20. Seal the plate with an Optical Adhesive Film (located on the bench next to the Biomek).

21. Press the seal down to ensure a good seal on each well using the MicroAmp™ Adhesive Film Applicator.

22. Spin the MicroAmp™ plate at 1000rpm for 1 min using the table top plate centrifuge.

Archiving

23. Once the RT-PCR is completed successfully (see **Supplementary Method 7**), the RNA plate can be moved to an archive freezer.

24. Archiving information must be recorded in the COVID-19 web app.

Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX

Appendix

The robot carries out the following automated protocol:

1. Load 50 μL tips using pod 1.

2. Aspirate 10 μL of RNA samples (10 μL/sec).

3. Dispense 10 μL to the qPCR plate (10 μL/sec).

4. Unload tips.

5. Load one 50 μL tip using pod 2.

6. Aspirate 10 μL of TET buffer from position A1 of the microfuge rack.

7. Dispense 10 μL to the qPCR plate position G12 (this constitutes the 'negative control').

8. Unload tip.

9. Load one 50 μL tip using pod 2.

10. Aspirate 10 μL of Positive Control from position D1 of the microfuge rack (20 μL/sec).

11. Dispense 10 μL to the qPCR plate position H12 (30 μL/sec).

12. Unload tip.

Supplementary Method 7 RT-PCR for SARS-CoV-2 using the BGI kit

Equipment / Consumables

- Barcode Scanner and laptop computer

- QuantStudio Real time PCR System

- Benchtop centrifuge

RT-PCR set up procedure

1. Take RT-PCR Plate to PCR lab. Ensure that the PCR plate is correctly assigned to the PCR stage of the process in a LIMS system. If using ClarityLims to confirm receipt of plate, click in empty field box in app, then scan the barcode with a handheld barcode scanner to enter PCR plate name. Hit **SUBMIT**.

2. Check seal integrity on PCR plate and spin briefly in centrifuge (1000rpm, 1min)

3. Open the sample drawer of an available Quant 3 PCR machine and place PCR Plate in the machine.
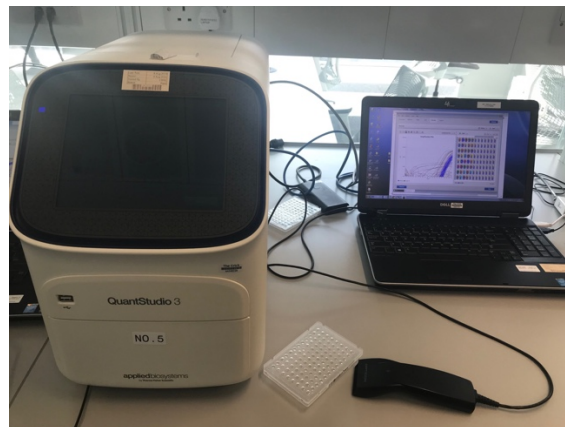
4 Confirm that the barcode is visible and facing the front of the machine, and A1 is on top-left side

5 On the PCR machine-associated laptop, ensure that the correct annotation for the specific PCR plate is downloaded from a LIMS system. If using ClarityLims, click in empty field box in app and scan the barcode on the PCR plate with handheld barcode scanner to enter PCR plate name. Hit **SUBMIT.**

6 Downloading of text file will happen automatically. Save to designated Plate Layout folder on Desktop with the Barcode as filename.

7 **At this point, a second operator must confirm plate orientation.**

Supplementary Method 7 RT-PCR for SARS-CoV-2 using the BGI kit

8 Open QuantStudio software.

9 Click on the button to "Open Existing Experiment" and find the designated **Covid-19 Master Template.edt** on Desktop

10 When prompted, click on '**Edit**' and enter designated password.

11 Click in field box for **Barcode** and use handheld scanner to scan plate barcode again to populate the field and replace default.

12 Click in field box for **Name** and scan barcode again to replace default.

13 Click in field box for **User** and enter your designated user name.

14 Under 'File' menu click on 'Import Plate Setup' and browse to identify the text file with same barcode on Desktop\Plate Layout. Hit '**Apply**'.

15 You should be prompted that current info will be lost and replaced. Hit '**yes**'.

16 Confirm the following settings by clicking on 'Method' on menu near top of window

Step	Cycle	Temperature	Duration	Fluorescence measure
1	1 cycle	50℃	20 min	N
2	1 cycle	95℃	10 min	N
3	40 cycles	95℃	15 sec	N
		60℃	30 sec	Y

17 Confirm following settings by clicking on 'Plate'
- **(Advanced Setup Tab) Target1: FAM, Quencher: None for Covid-19**
- **(Advanced Setup Tab) Target2: VIC, Quencher: None for internal control**
- **(Quick Setup Tab) Passive Reference Dye: None**
  in 'Plate' window in Quick Setup Tab that plate is populated with sample barcodes.

18 Click on 'Run' window, and hit 'Start Run' button

19. When prompted, save barcode.eds file in the designated folder on Desktop.

20. **FINAL CHECKPOINT (stop the run if the following is not true): The PCR run should last 88min 41sec.**

21   After the amplification is complete, remove PCR plates from the thermal cycler and discard plate for autoclave and decontamination in line with laboratory procedures.

Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting

In the CCC pipeline, samples have been submitted to an accredited reporting laboratory with testing being completed in a research laboratory. The test results have been analysed by both a research scientist (First reporter) and an individual with BMS, CS or FRCPath clinical registration (Second reporter) and reported via the accredited reporting laboratory.
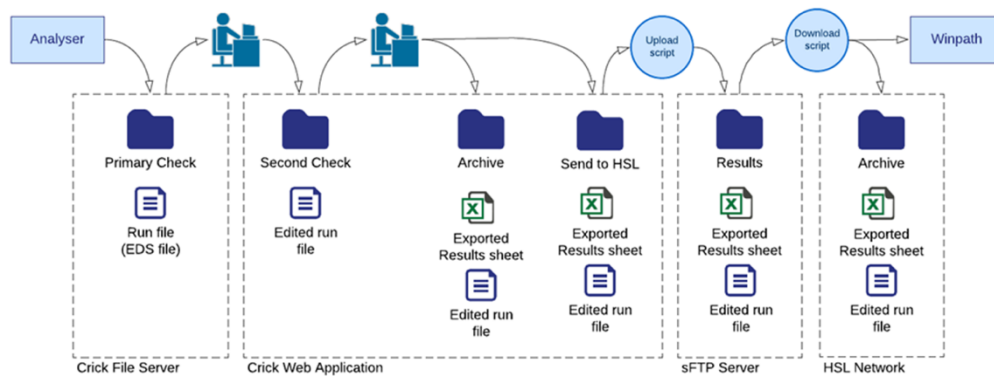
Equipment
Access to PC Windows PC with QuantStudio software

First Reporter Analysis

The first checker would export the run result (EDS file) to a shared drive into a primary review folder. The file would have a unique name that identifies the run and could be linked to the sample auditing in the research laboratory internal LIMS.

The first checker would then:

- access the run in Quantstudio 1.4

- Ensure the threshold is set to automatic

- Check the run as a whole on the amplification plot.

Open Existing Experiment
Open...
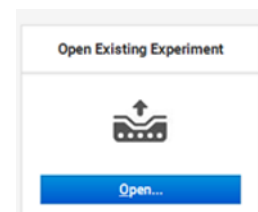
Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting
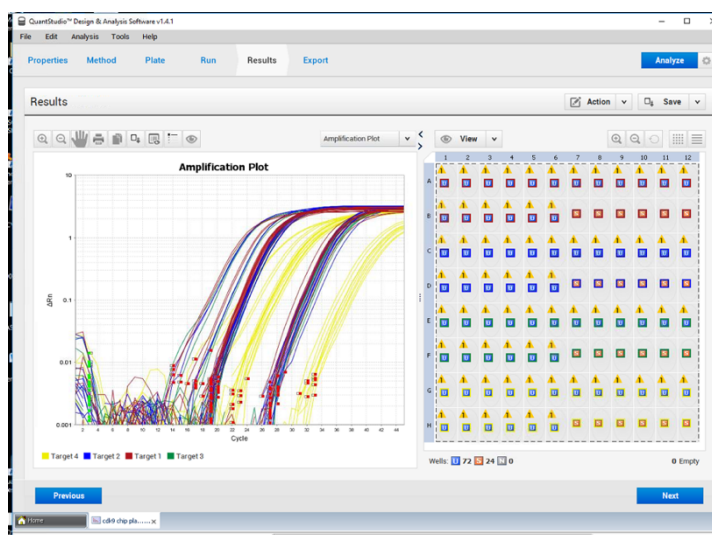

- If the run shows curves but there is widespread failure due to the automatic thresholding, the first reporter discusses this with a second reporter. **See second reporter section below.**

- The primary reporter will ensure:
  - Blank control: Ct values at FAM and VIC/HEX channels are greater than 37 and 35 respectively or undetermined.
  - Positive control: Standard curves at channel FAM and VIC/HEX channels are show exponential amplification with Ct values not greater than 37 and 35 respectively.

- Above requirements should be met on the individual plate otherwise the entire plate is invalid. If entire plate has failed and needs to be retested, go back to stored RNA and repeat RT-PCR (See Supplementary Method 5A).

- The first reporter should then click on properties tab and put their name in the 'user' free text box for sample audit purposes. The text box to the right of the properties tab can be used to include any additional information that needs to be communicated to the second reporter. The exported eds is then saved on an internal drive to be uploaded to the online portal for the second checker.

- The first reporter will also extract plate genealogy data from the research laboratory internal LIMS system allowing them to document the history of a plate so the stages and operators involved. This will support their sign off decision.

Example of sample audit trail:

Sample information for plate CFH0PID8

Sample barcode	Well location	RT-qPCR plate barcode	RT-qPCR operator	RT-qPCR date	RNA extraction plate barcode	RNA extraction operator	RNA extraction date	Sample consolidation plate barode	Sample consolidation operator	Sam cons date
21U325622	G:1	CFH0PID8	test	2020-03-29	SPL00005	Laura Cubitt	2020-03-29	LPL00505	LC	2020
50U080371	D:3	CFH0PID8	test	2020-03-29	SPL00005	Laura Cubitt	2020-03-29	LPL00505	LC	2020
21U325621	A:2	CFH0PID8	test	2020-03-29	SPL00005	Laura Cubitt	2020-03-29	LPL00505	LC	2020

Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting

Each run will be run in duplicate and once both runs have passed the first check the First Reporter will save the eds files in a location where they would then be uploaded to the online portal and become available for external clinically registered second reporters to access as shown in the screenshot below.

Second Reporter Analysis

The second reporter will be able to download the run results for each run and its duplicate (identified by the same extraction container as shown in screenshot above) through a web app. Once two results for a sample have been uploaded there is computer logic in place to generate a result. To ensure that this happens in a reasonable time duplicate runs should be looked at by the same reporter and uploaded at the same time.

Once the second reporter has downloaded the eds file of each run from the online portal they would enter the password to allow for customisation of the eds file. The eds file will open to the results page in log format. If the plate to be analysed is only half full, to remove any of the wells that are not to be analysed go to the plate screen and select these wells and deselect the targets.
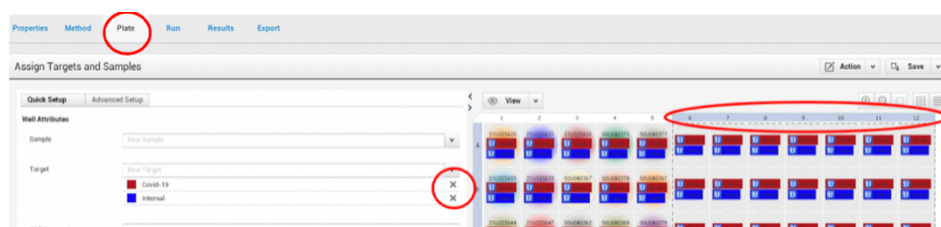
To visualise S curves back on the results page change to a linear graph by clicking the eye symbol and change this under graph type.
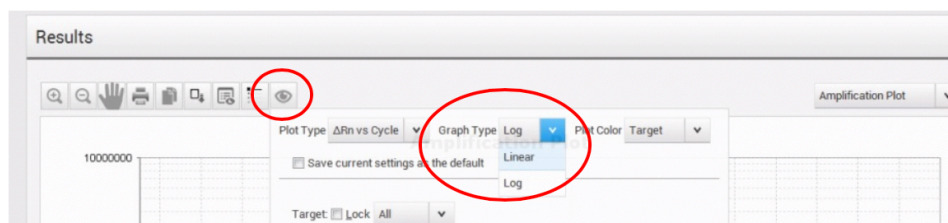
COVID-19 fluoresces in the FAM channel and is shown in red. The internal control fluoresces in the VIC channel and is shown in blue. To visualise the curves for individual samples click on their well, e.g. the positive control (left) and negative control (right) shown below.

Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting
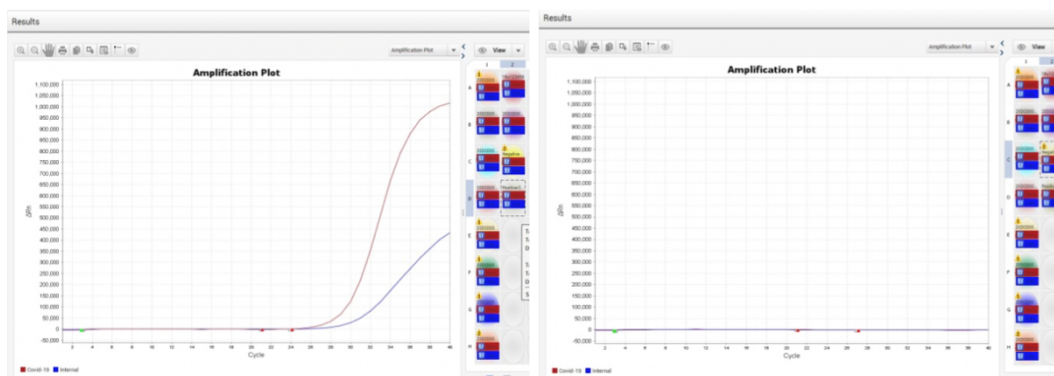
To begin the threshold should be set to automatic in accordance with the BGI kit guidelines and the run checked as a whole on the amplification plot.

The second reporter should again ensure the following results for the controls:
- Positive control: Standard curves at channel FAM (Covid-19) and VIC/HEX (Internal Control) channels show exponential amplification with Ct values not greater than 37 and 35 respectively.
- Negative control: Ct values at FAM and VIC/HEX channels are greater than 37.0 and 35 respectively or no data available.

The plate should be failed if there is signal of <35 for IC and <37 for COVID-19 in the negative control. If only one of these criteria are met then the plate is not automatically failed but the whole plate should be reviewed with a high index of suspicion for overall failure, based on plate wide increase in high Ct signals. The plate fails if there is no signal for both targets for the positive control. If the plate has failed the second reporter would report the plate as failed on the online portal after which repeat testing of the plate would be set up (See Supplementary Method 5A).

Manual baseline and threshold
If the run shows widespread failure due to automatic thresholding, the baseline and threshold may be manually adjusted. The baseline will be automatically set between 3 to 15 cycles as according to the BGI kit guidelines. To manually adjust click on the eye symbol and untick the auto baseline box (circled below left) then drag the baseline end to just before the first true amplification on the plate. The Ct values will automatically be reanalysed once this has been moved. This is best done in the linear graph.
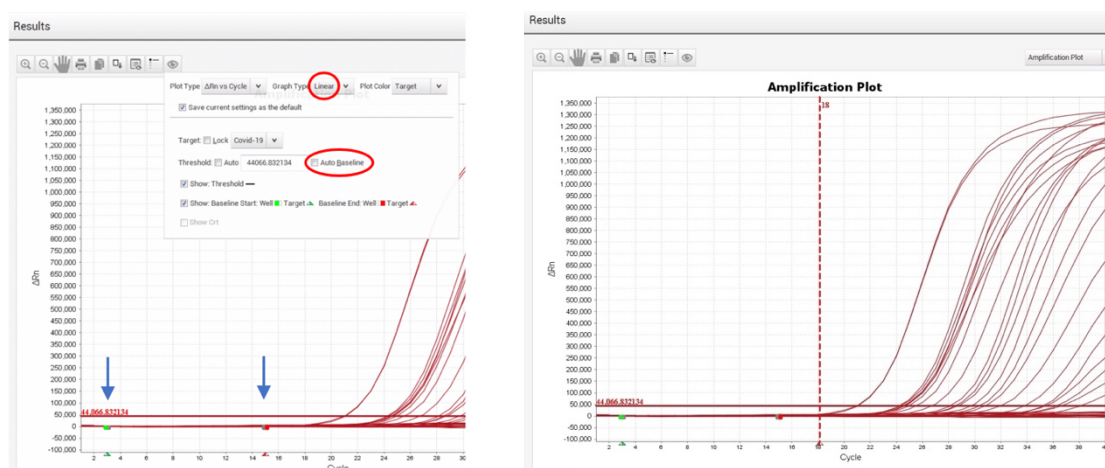
Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting

Once the baseline has been adjusted the result analysis can be done using manual thresholding to remove any noise or abnormal curves that could be interfering with the appropriate Ct being given for each sample. In some cases the automatic thresholding will have called all results appropriately and the threshold can be manually set to the same level.

Click the eye symbol and untick the auto threshold box, (circled below left). The threshold line will then become draggable by mouse (below right) and can be raised to a level above any "noise" on the plate. The minimum value to which the covid-19 threshold should be set is 30,000. The Ct values will automatically be reanalysed once this has been moved. This is best done in the log scale graph.
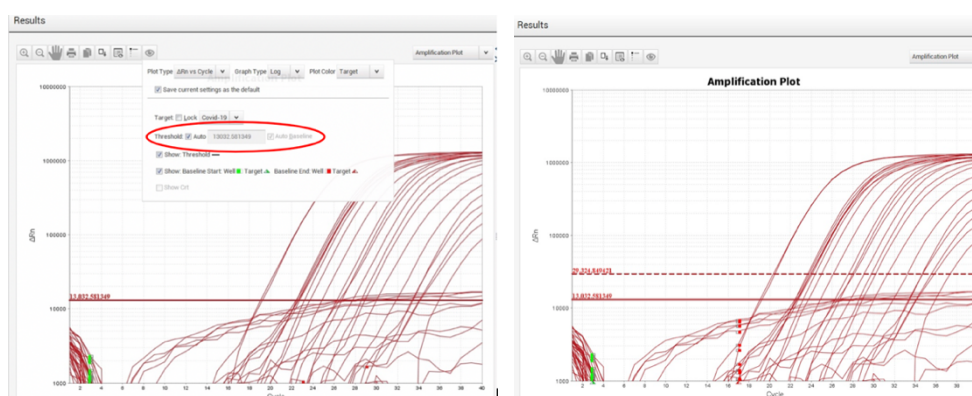
Assessment of clinical specimen test results

The three following results are the possible outcomes for each sample:

	COVID-19	Internal Control
Positive	Ct <37.0, S-shaped curve	Any Ct or undetermined
Negative	Ct >37.0 or undetermined Any non S-shaped curve	Ct <35
Sample failure	Any Ct, any non exponential amplification	Undetermined or >35
	Non exponential amplification of both or either target with Ct values <37 covid and <35 internal	

Positive and negative results are generated automatically in the LIMS based on the Ct values in the uploaded xls and released immediately. Sample failure results are generated automatically in the LIMS by an "Invalid" comment in the xls results and also released immediately.

Duplicate run outcomes
The automated outcome will be calculated by the logic in the online system that will take into account the Ct value and any well comments. Once the results for both runs have been uploaded the following outcomes will be possible:

No	Covid CT Value 1	Covid CT Value 2	Result Outcome
1	<37	<37	POSITIVE
2	>37/UD	>37/UD	NOT detected
3	35-37	>37/UD	NOT detected
4	>37/UD	35-37	NOT detected
5	<35	>37/UD	Invalid (discordant)
6	>37/UD	<35	Invalid (discordant)

Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting

Any samples with the comment invalid in either one or both of the runs will be called invalid. Any samples that have an internal control failure in one or both runs will be failed.

The second reporter would then upload the eds and xls files to the online portal which releases results to the clinically accredited laboratory's LIMS. The second reporter would then be returned to the Referrals list on the online portal where any other reports awaiting validation would be available.

Result file structure

The result file is the Excel output from the QuantStudio.

It has 3 tabs:

Sheet #	Sheet Name	Description
1	Sample Setup	Information about the specimens contained in each well, and the run setup.
2	Amplification Data	Raw data of the well curve X/Y coordinates.
3	**Results**	The main sheet containing the well numbers, sample numbers and result values (CT values). This will be used for import to Winpath.

The results tab has a table starting on row 43 with the results. The key columns for import are:

Column position	Column header	Description
D	Sample Name	The lab number associated with the sample.
E	Target Name	The assay processed. This needs to be mapped to the result code in Winpath as part of the interface.
I	CT	The quantitative result. This will either be a number or 'Undetermined'. This will interface to an internal line in Winpath. Winpath rules will then generate the final result.
AC	Comment	Used for manual overrides. This will be "INVALID" for sample failure.

EXAMPLE of the resulting LIMS:

		Tests	Results	Test Code
n	H	CORONAVIRUS CRICK INSTITUT		CCO1
a	H	Specimen type	Swab	CCO2
a	H	Report from	The Francis Crick	CCO3
a	H	SARS CoV-2 RNA	Indeterminate	CCOV
a	H	Comment	This test is not U	CCOC
a	H		verified.	
t	H	- - - - - - - - - - - - - - -	|	CCO4
p	H	Date Sent to Crick	30/03/20	CCSE
a	H	Crick CoV-2 CT-Value	0	CCCT
a	H	Crick CoV-2 Internal Control	23.2	CCIC
a	H	Crick CoV-2 Well Omitted	False	CCOO
a	H	Crick CoV-2 Comment	This is	CCMT
n	H	Note		CCO5
t	H	=====================		CCO6

Other notes:
- The files should have a unique name.
- If a run is exported with failed run controls by mistake the interface should prevent it being transmitted to the reporting laboratory LIMS.
- The reporting laboratory LIMS rules will look for technical failures and prevent reporting if an invalid result is identified.

Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting

<u>APPENDIX -</u> *Web application software is available on request*

REQUIREMENTS

The key requirement for this software was to enable a wide pool of *remote working* clinicians to fulfil the 'second reporting' requirements for the testing pipeline. The application needed all the usual basic requirements for an access controlled application – user authentication, role based privileges and encryption – as well producing a workflow for 'Second Reporting' of Covid-19 test results as quickly as possible.

WEB APPLICATION

The best way of providing this was through the development of a secure and robust externally facing web application. The Crick instance is hosted locally on VM infrastructure (which is already fully backed-up and maintained), however the local development environment is containerised in Docker providing all the configurations required to implement either locally or in the cloud. It should be deployed with encryption (and forced HTTPS) and provided with an SSL cert. The Crick have set-up automated deployment using Jenkins, and code is version controlled using Git. It has also been important throughout - despite the speed of development – to continuously share knowledge of the software within a small group of developers and in doing so remove human dependencies.

The application has been developed in Python using the Django framework with an Apache web server, the supporting database is MySQL.

FUNCTIONALITY

The fasted most efficient way for us to make the First Reporter QA results available to the Second Reporter was via utilising the same locally installed and openly available (for MS Windows) version of the Quant Studio software used by the First Reporter on the bench to analyse the RCP output. These files once QA approved are saved in a secure location where they can be picked up by the web application and presented as a referral requiring analysis and approval. The presentation of the results is via a simple web interface listing results by plate and timestamp and with a process of automatically locking the 'under review' file to prevent the possibility of more than one Second Reporter accessing the results at the same time. Once the Second Reporter has completed their work, the results and the edited Quant Studio files are uploaded back into the website, the Plate's status is updated, results are persisted in the DB and the results files are moved into a second secure location for downstream processing and reporting back.

REUSABILITY

To use this core part of the functionality, all that is needed are a secure 'location one' (where QA'd First Reporter approved files are placed), a secured 'location two' where the approved Second Reporter results files are placed, an SMTP account for password reset and email notifications and the open source based web application software which is available on request.

EXTENDED FUNCTIONALITY

In order to make the whole pipeline as streamlined as possible, the Crick instance of this application includes various bespoke integration points and additional functionality, for example allowing results data to flow back to the main LIMS sample tracking dashboard and to pick up from internal systems plate genealogy to be presented to Second Reporters.

Supplementary Method 9 Preparation of Binding Buffer (BB)

Reagents

- Guanidine Hydrochloride (GuHCl)

- MilliQ water

- Isopropanol

- 3M sodium acetate, pH 5.2

- Tween 20

Equipment

- Weighing scales

- 2L glass beaker

- 3L glass beaker

- 5 L glass beakers

- 2 L graduated cylinder

- Heater/Stirrer

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination. This buffer is made in a fumehood

How to make 2 L + 80 mL Binding buffer BB

1. Weigh out **955.2 g** GuHCl and add to a 2 L beaker.
Initials:
2. Add **500 mL** freshly drawn milliQ. Stir and heat to 60°C to dissolve. Make up to 1200 mL in a 2 L graduated cylinder.
Initials:
3. Add Isopropanol up to 2 L (**800 mL**).
Initials:
4. Add **80 mL** 3M sodium acetate, pH 5.2.
Initials:
5. Add **1mL** Tween-20. Stir well to mix.
Initials:
6. Vacuum filter into sterile bottles.
7. Label with batch and date.
Initials:
Store Binding Buffer BB buffer in a flammables cupboard at room temperature (+15°C – +25°C).

Supplementary Method 9 Preparation of Binding Buffer (BB)

How to make 4L + 160 mL Binding buffer BB

1. Add **1000 mL** freshly drawn milliQ water which has been heated to 60°C to a 5L beaker.

Initials:

2. Weigh out **1.911 Kg GuHCl** in aliquots using a 3 L beaker and carefully add to 5 L beaker Stir and heat to 60 deg C to dissolve A sterile pipette may be required to stir. Once dissolved, make up to 2400 mL with freshly drawn milliQ water using a 2L graduated cylinder and transferring buffer to second 5L beaker.

Initials:

3. Add Isopropanol up to 4 L (**1600 mL**) using a 2L graduated cylinder.

 Initials:

4. Add **160 mL** 3M sodium acetate, pH 5.2.

Initials:

5. Add **2 mL** Tween-20. Stir well to mix.

Initials:

6. Vacuum filter into sterile bottles.
7. Label with batch and date.

Initials:

Store Binding Buffer BB buffer in a flammables cupboard at room temperature (+15°C – +25°C).

How to make 5L + 200 mL Binding buffer BB

1. Add **1250 mL** freshly drawn milliQ water which has been heated to 60°C to a 5L beaker.

Initials:

2. Weigh out **2.388 Kg GuHCl** in aliquots using a 3 L beaker and carefully add to 5 L beaker Stir and heat to 60 deg C to dissolve A sterile pipette may be required to stir. Once dissolved, make up to 3000 mL with freshly drawn milliQ water using a 2L graduated cylinder and transferring buffer to second 5L beaker.

Initials:

3. Add Isopropanol up to 5 L (**2000 mL**) using a 2L graduated cylinder.

 Initials:

4. Add **200 mL** 3M sodium acetate, pH 5.2.

Initials:

5. Add **2.5 mL** Tween-20. Stir well to mix.

Initials:

6. Vacuum filter into sterile bottles.
7. Label with batch and date.

Initials:

Store Binding Buffer BB buffer in a flammables cupboard at room temperature (+15°C – +25°C).

Supplementary Method 10 Preparation of TET Buffer

<u>**Reagents**</u>

- RNAse free -Sigma 270733

- 0.5 M EDTA, pH 8.0

- 1 M Tris-HCl, pH 8.0

- Tween 20

<u>**Equipment**</u>

- 2L single use cell culture bottle

- 5L single use cell culture bottle

- Vacuum filter

- sterile bottles

<u>**Procedure**</u>

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination. This buffer is made in a tissue culture hood to reduce the possibility of contamination by RNAses.

How to make 2 L TET

1. Add **1800 mL** Water (RNAse free -Sigma 270733) to a 2L single use cell culture bottle.
Initials:
2. Add **4 mL** 0.5 M EDTA, pH 8.0.
Initials:
3. Add **20 mL** 1 M Tris-HCl, pH 8.0
Initials:
4. Add **1 mL** Tween-20
Initials:
5. Make up to 2000 mL Water (RNAse free -Sigma 270733). Swirl very well to ensure that Tween-20 is well mixed in.
Initials:
6. Vacuum filter into sterile bottles.
7. Label with batch number and date.
Initials:
Store TET buffer solution at room temperature (+15ºC – +25ºC).

Supplementary Method 10 Preparation of TET Buffer

How to make 5L TET

1. Add **4500 mL** Water (RNAse free -Sigma 270733) to a 5L single use cell culture bottle.

Initials:

2. Add **10 mL** 0.5 M EDTA, pH 8.0.

Initials:

3. Add **50 mL** 1 M Tris-HCl, pH 8.0

Initials:

4. Add **2.5 mL** Tween-20

Initials:

5. Make up to 5000 mL Water (RNAse free -Sigma 270733). Swirl very well to ensure that Tween-20 is well mixed in.

Initials:

6. Vacuum filter into sterile bottles.

7. Label with batch number and date.

Initials:

Store TET buffer solution at room temperature (+15°C – +25°C).

Supplementary Method 11 - Preparation of 5M L6 guanidinium thiocyanate virus inactivation buffer

Reagents

- Guanidinium thiocyanate (GuSCN)

- 0.1M Tris HCl (see **Supplementary Method 12**)

- 0.2M EDTA pH 8.0 (see **Supplementary Methods 13 and 14**)

- Triton X-100

Equipment

- 1L/ 2L/ 3L/ 5L beaker

- Vacuum filter

- 2ml Screw cap tubes (Sarstedt)

- Heater/Stirrer

- Sterile bottles

- Weighing scales

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination.

Upon contact with acids, GuSCN can produce a toxic gas (HCN). As a precaution, this buffer is prepared in a fume hood.

How to make 1L L6 5M Guanidinium thiocyanate Inactivation buffer

1. Weigh out **600 g** GuSCN and add to a 1 L beaker
Initials:
2. Measure out **500 mL** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to beaker.
Initials:
3. Measure out **110 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) pH 8.0 and add to beaker
Initials:

4. Add **13 mL** Triton X-100 to beaker and stir well and heat to 60°C if required.
5. Vacuum filter into sterile bottles.
6. Label with batch and date.
Initials
Store L6 inactivation buffer in dark at solution at room temperature (+15°C – +25°C).

Supplementary Method 11 - Preparation of 5M L6 guanidinium thiocyanate virus inactivation buffer

How to make 2L L6 5M Guanidinium thiocyanate Inactivation buffer

1. Weigh out **1200 g** GuSCN and add to a 2 L beaker

Initials:

2. Measure out **1000 mL** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to beaker.

Initials:

3. Measure out **220 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) and add to beaker

Initials:

4. Add **26 mL** Triton X-100 to beaker and heat to 60°C if required.
5. Vacuum filter into sterile bottles.
6. Label with batch and date.

Initials

Store L6 inactivation buffer in dark at solution at room temperature (+15°C – +25°C).

How to make 4L L6 5M Guanidinium thiocyanate Inactivation buffer (Note this method makes about 4.3 L)

1. Measure out **1.6 L** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to 5L beaker.

Initials:

2. Weigh out **2.4 Kg GuSCN** in aliquots using a 3L beaker and carefully add to 5L beaker whilst stirring and heating to 60 deg C. Rinse 3L beaker with **0.4 L** 0.1 M Tris HCl. Stir manually with sterile 10 ml pipette if required.

Initials:

3. Measure out **440 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) and add to beaker.

Initials:

4. Add **52 mL** Triton X-100 to beaker. Swirl/stir well to mix.

Initials:

5. Vacuum filter into sterile bottles.
6. Label with batch and date.

Initials

Store L6 inactivation buffer in dark at solution at room temperature (+15°C – +25°C).

Supplementary Method 11 - Preparation of 5M L6 guanidinium thiocyanate virus inactivation buffer

How to make 5L L6 5M Guanidine thiocyanate Inactivation buffer (Note this method makes about 5.4 L)

1. Measure out **2.0 L** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to 5L beaker out

Initials:

2. Weigh out **3 Kg GuSCN** in aliquots using a 3L beaker and carefully add to 5L beaker whilst stirring and heating to 60 deg C. Rinse 3L beaker with **0.5L** 0.1 M Tris HCl. Stir manually with sterile 10 ml pipette if required.

Initials:

3. Transfer to 5 L single use tissue culture bottle. Measure out **550 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) and add to bottle

Initials:

4. Add **65 mL** Triton X-100 to bottle. Swirl/stir well to mix.

Initials:

5. Vacuum filter into sterile bottles.
6. Label with batch and date.

Initials

Store L6 inactivation buffer in dark at solution at room temperature (+15°C – +25°C).

Following preparation of L6 5M Guanidine thiocyanate Inactivation buffer:

1. Within a tissue culture hood cupboard, aliquot 1 ml of L6 5M guanidine thiocyanate buffer into 2ml tubes and replace lids (**see Appendix**)

2. Label with batch and date and store in dark at RT

3. After the aliquoting is complete, change gloves.

4. Place tubes in a box clearly marked 5M guanidine thiocyanate L6 Lysis buffer.

5. Clean the hood, dispose of waste and switch off tissue culture hood.

Note:5M Guanidine thiocyanate is stable at room temperature for at least three weeks. (Boom et al, J Clin Microbio 1990. 28, 3, 485-503. Commercial stocks have shelf life of 18 months)

Supplementary Method 11 - Preparation of 5M L6 guanidinium thiocyanate virus inactivation buffer

Appendix:

Racking tubes prior to aliquoting 5M L6 guanidinium thiocyanate virus inactivation buffer

Equipment

- 2ml screw cap tubes Sarstedt 72.694.005 or similar

- Racks (96 position)

- Empty 200 μl Tip Boxes

Procedure

NOTE: Masks should be worn when performing this procedure due to the possibility of asymptomatic COVID-19 infection causing contamination.

Lab coat, gloves, safety glasses and face mask (or face shield) must be worn. A new lab coat should be used each day. Please put your name on your lab coat. Safety glasses should not be shared but be labelled and kept for each person.

Please observe social distancing of 2 m and avoid other areas of the lab where making of buffers could be in process.

1) Make sure work area is clean and clear.

2) Unscrew lid. Place lid in box and tube in rack. Continue until 93 tubes are in rack with two spaces left.

3) Once complete, wrap filled rack in foil and place in transparent really useful box.

4) Continue as required then make sure area is left clean and tidy. Empty tube bags should go in green recycling bin. Leave cardboard boxes for disposal in consolidation space. Lab coats should be placed in the laundry bags, masks in yellow burn bin and gloves in yellow tiger stripe bin.

Supplementary Method 12 Preparation of 0.1M Tris HCl pH 6.4

Reagents

- Tris HCl

- MilliQ water

- 5M NaOH (**see Supplementary Method 13**)

Equipment

- 1L beaker

- 5L beaker

- 1L or 2L graduated cylinder

- Vacuum filter

- pH meter

- Sterile bottles

- Weighing scales

- Magnetic flea

- Magnetic stirrer

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination.

Buffer is not hazardous so can be made on open bench.

How to make 1L 0.1M Tris HCl pH 6.4

1. Weigh out **15.76g** Tris HCl and add to a 1L beaker.
Initials:
2. Measure out **800 mL** milliQ water and add to the beaker.
Initials:
3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution.
4. Add a newly calibrated pH meter into the solution to observe the pH.
   (Reading………..)
5. Tune pH with 5M NaOH. (Batch Number ……………...) Final pH is …………..
Initials:
6. Transfer to a 1L graduated cylinder and make up to 1000 mL using milliQ water.
   Swirl.
Initials:
7. Vacuum filter into sterile bottles.

Supplementary Method 12 Preparation of 0.1M Tris HCl pH 6.4

8. Label with batch number and date.
Initials:

Store 1L 0.1 M Tris HCl, pH 6.4 solution at room temperature (+15°C – +25°C).

How to make 5L 0.1M Tris HCl pH 6.4

1. Weigh out **78.8g** Tris HCl and add to a 5L beaker.
Initials:
2. Measure out **4L** milliQ water and add to the beaker.
Initials:
3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution.
4. Add a newly calibrated pH meter into the solution to observe the pH. (Reading………...)
5. Tune pH with 5M NaOH. (Batch Number ……………...) Final pH is …………..
Initials:
6. Make up to 5L using milliQ water by transferring to a second 5L beaker via a 2L graduated cylinder. Swirl.
Initials:
7. Vacuum filter into sterile bottles.
8. Label with batch number and date.
Initials:

Store 0.1 M Tris HCl, pH 6.4 solution at room temperature (+15°C – +25°C).

Supplementary Method 13 – Preparation of 5M NaOH

Reagents

- NaOH

- MilliQ water

Equipment

- 500mL beaker

- 500 mL graduated cylinder

- Sterile bottles

- Weighing scales

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination. This buffer is made in a fume hood.

How to make 300mL 5M NaOH

1. Weigh out 60 g NaOH. Add to 500 mL beaker.
Initials:
2. Measure out 250 mL milliQ water and add to beaker.
Initials:
3. Transfer to a 500 mL graduated cylinder. Make up to 300 mL using milliQ water, if necessary.
Initials:
4. Transfer to a 500 mL sterile Duran bottle. Label with batch and date.
Initials:

Store solution at room temperature (+15°C – +25°C).

Supplementary Method 14 - Preparation of 0.2M EDTA pH 8.0

Reagents

- EDTA disodium salt, dihydrate

- MilliQ water

- 5M NaOH (**see Supplementary Method 13**)

- Sodium hydroxide (NaOH) pellets

Equipment

- 1L beaker

- 2L beaker

- 1L measuring cylinder

- 2L measuring cylinder

- Vacuum filter

- pH meter

- Sterile bottles

- Stirrer

- Weighing scales

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination. This buffer is made in a fume hood.

How to make 1L of 0.2M EDTA pH 8.0

1. Weigh out **74.45 g** EDTA disodium salt, dihydrate and add to a 1 L beaker.
Initials:
2. Measure out **800 mL** milliQ water and add to the beaker.
Initials:
3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution. The EDTA salt will not go into solution until the pH reaches 8.0.
4. Add a newly calibrated pH meter into the solution to observe the pH.
5. To dissolve the salt, add sodium hydroxide (NaOH) pellets to the solution. Add a few pellets at a time and wait until the pellets have fully dissolved before adding more. It may take around **8** g of NaOH pellets before the pH is at 8.0. (Reading………)

Supplementary Method 14 - Preparation of 0.2M EDTA pH 8.0

    6. Fine tune pH with 5M NaOH. (Batch Number …………………)
Initials:
    7. Once fully dissolved (this will take some time so be patient), transfer to 1L cylinder and top up the solution to 1L using milliQ water, if necessary.
Initials:
    8. Vacuum filter into sterile bottles.
    9. Label with batch and date.
Initials:

Store 0.2M EDTA pH 8.0 solution at room temperature (+15°C – +25°C).

How to make 2L of 0.2 M EDTA pH 8.0

    1. Weigh out **148.89 g** EDTA disodium salt, dihydrate and add to a 2 L beaker.
Initials:
    2. Measure out **1600 mL** milliQ water and add to the beaker.
Initials:
    3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution. The EDTA salt will not go into solution until the pH reaches 8.0.
    4. Add a newly calibrated pH meter into the solution to observe the pH.
    5. To dissolve the salt, add sodium hydroxide (NaOH) pellets to the solution. Add a few pellets at a time and wait until the pellets have fully dissolved before adding more. It may take around **16** g of NaOH pellets before the pH is at 8.0. (Reading………)
    6. Fine tune pH with 5M NaOH. (Batch Number ………………….)
Initials:
    7. Once fully dissolved (this will take some time so be patient), transfer to 2L cylinder and top up the solution to 2 L using milliQ water, if necessary.
Initials:
    8. Vacuum filter into sterile bottles.
    9. Label with batch and date.
Initials:

Store 0.2M EDTA pH 8.0 solution at room temperature (+15°C – +25°C)

How to make 5L of 0.2 M EDTA pH 8.0

    1. Weigh out **372.23 g** EDTA disodium salt, dihydrate and add to a 5 L beaker.
Initials:
    2. Measure out **4000 mL** milliQ water and add to the beaker.
Initials:
    3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution. The EDTA salt will not go into solution until the pH reaches 8.0.
    4. Add a newly calibrated pH meter into the solution to observe the pH.
    5. To dissolve the salt, add sodium hydroxide (NaOH) pellets to the solution. Add a few pellets at a time and wait until the pellets have fully dissolved before adding more. It may take around **40** g of NaOH pellets before the pH is at 8.0. (Reading………)
    6. Fine tune pH with 5M NaOH. (Batch Number ………………….)
Initials:
    7. Once fully dissolved (this will take some time so be patient), transfer to 5 L cylinder and top up the solution to 5 L using milliQ water, if necessary.

Supplementary Method 14 - Preparation of 0.2M EDTA pH 8.0

Initials:
8. Vacuum filter into sterile bottles.
9. Label with batch and date.

Initials:

Store 0.2M EDTA pH 8.0 solution at room temperature (+15°C – +25°C).

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

	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

i   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.

i   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 <u>must be worn at all times</u>.
- ➢ **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.

Set-up

4

6

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

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

> **!** **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
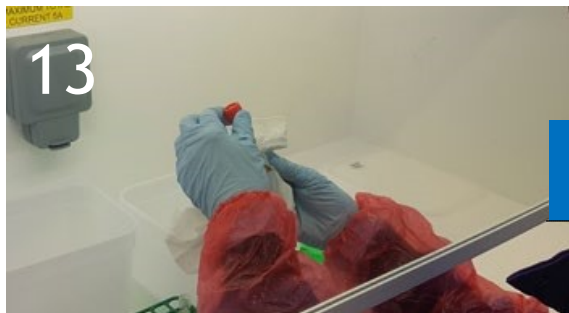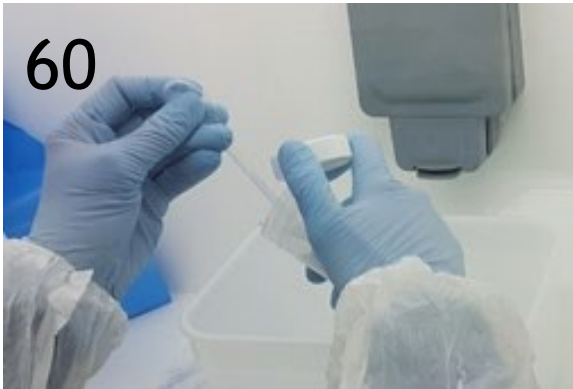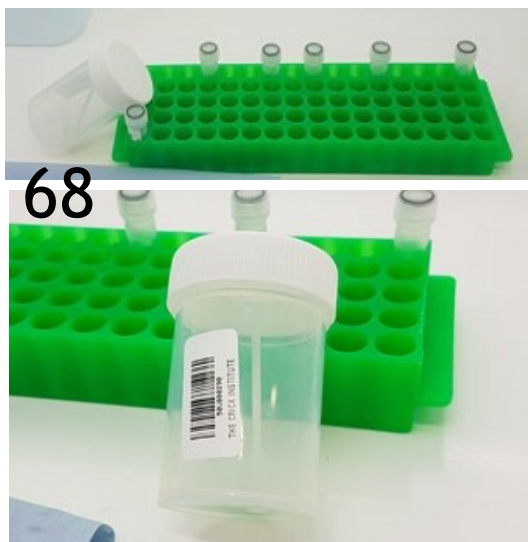
> **!** **The ASF robot will only work if the labels are orientated vertically and <u>as straight as possible</u>!**

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.

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

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

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.
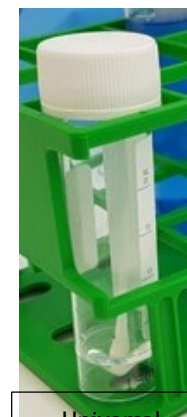
Universal Conical

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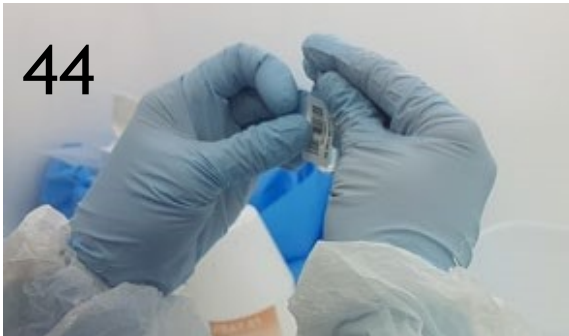
> **!** **If barcodes do not align, DO NOT PROCESS. Spray bag out at end of work to be removed and rescanned at scanning station**

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 <u>as straight as possible</u>!**

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

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

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

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**

> 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. <u>Check the barcodes on the swab container and opened inactivation tube match</u>

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
<u>Removal of</u> <u>racks of inactivated samples from the MBSC</u> section below

3

4

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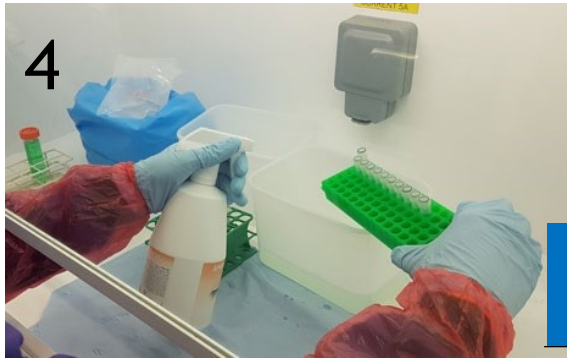
! To prevent fatigue and operator error, work only in pre-arranged 1-hour shifts. If you are in the middle of a batch, stop and hand over to the next person.
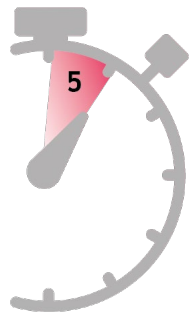
! Robot processing in RNA extraction lab

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.

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).

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

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.

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.

Supplementary Method 16 COVID-19 Sample Tracking Pipeline

TRACKING SAMPLES AT HOSPITAL SITES
1) Swabs taken from patients

2) Details logged onto TABLO OR EPIC LIMS

3) Specimens taken to HSL

TRACKING SAMPLES AT HSL AND ELECTRONIC FILE SUBMISSION TO THE CRICK
A. **IF BARCODE IS PRESENT ON THE SWAB TUBE AT HSL** - sample will be logged directly onto WINPATH and entered into the pipeline:

1. **(AT HSL)** Samples will be booked at the specimen reception at the hospital rapid response lab. Each request will be assigned a unique lab number in the format YYUnnnnnn (i.e. 20U123456). The samples will have a Code128 barcode attached. (Barcode format: 2 numbers, 1 letter, 6 numbers – 9 characters).
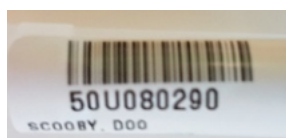
2. Winpath will automatically generate the ORDER FILE when a specimen is booked. A script will upload the order file in csv format to the sFTP server at the HSL end, providing one file per sample.
3. The samples will be batched in the HSL specimen reception. The staff will use the Winpath worklisting to find all unsent samples.
4. The samples will be sent in transport bags via a courier and the transport bags are registered with Tubetracker for delivery. Delivery manifests will not be generated with the order, as all the information required is on the sample label, and in the electronic order csv files.
5. **(AT THE CRICK)** The Crick Institute system will pick up the csv files and import them to an orders database ready for receipt. The order file is transferred to the Crick via the secure FTP site. The order files are processed by a script and the samples are then pre-registered within the existing LIMS system used by the Advanced Sequencing Facility, ClarityLIMS, a product of Genologics, pending entry into the ClarityLIMS workflow, after the sample is physically scanned in at the sample reception step. The order file structure Is outlined next.

ORDER FILE STRUCTURE

Column No	Description	Example	Notes
1	Lab Number	19U123456	9 character alphanumeric
2	Hospital Number	H123456	
3	Request date	20200319	In the format YYYYMMDD. Date the sample was booked in Winpath.
4	Sample date	20200319	In the format YYYYMMDD. Date the sample was collected from the patient.
5	Urgent	Y	Single character. Options Y or N.
6	Comment	Feeling unwell	Freetext clinical details from the hospital system.
7	Tests requested	CCOV	Repeating field with all the tests requested (ie. "CCOV","SRHU",…)

CSV file contents example:
"0019U999907","H123456","20200319","20200319","N","TEST","SRHU"

Other notes:
- The order file will be submitted to the sFTP server with a unique GUID file name (i.e. b602d581-164c-44b0-95fc-95897dac8e35.ORD).
- The file extension will be ".ORD" (order file)
- All fields will be enclosed in quotes, as shown in the CSV file contents example provided previously.

B. **IF NO BARCODE IS PRESENT ON THE SWAB TUBE AT HSL** – barcode to be added by HSL and then logged into WINPATH and the sample will then enter into the pipeline as outlined above.

NOTE - samples will only come to the CRICK if they require COVID19 tests only.

LOGICAL ARCHITECTURAL VIEW OF THE SAMPLE TRACKING PIPELINE

The following diagram is a high level logical architectural view of the sample tracking and management pipeline. This provides a logical overview of the key steps involved in the pipeline, which will be described in greater detail throughout the remainder of this document.
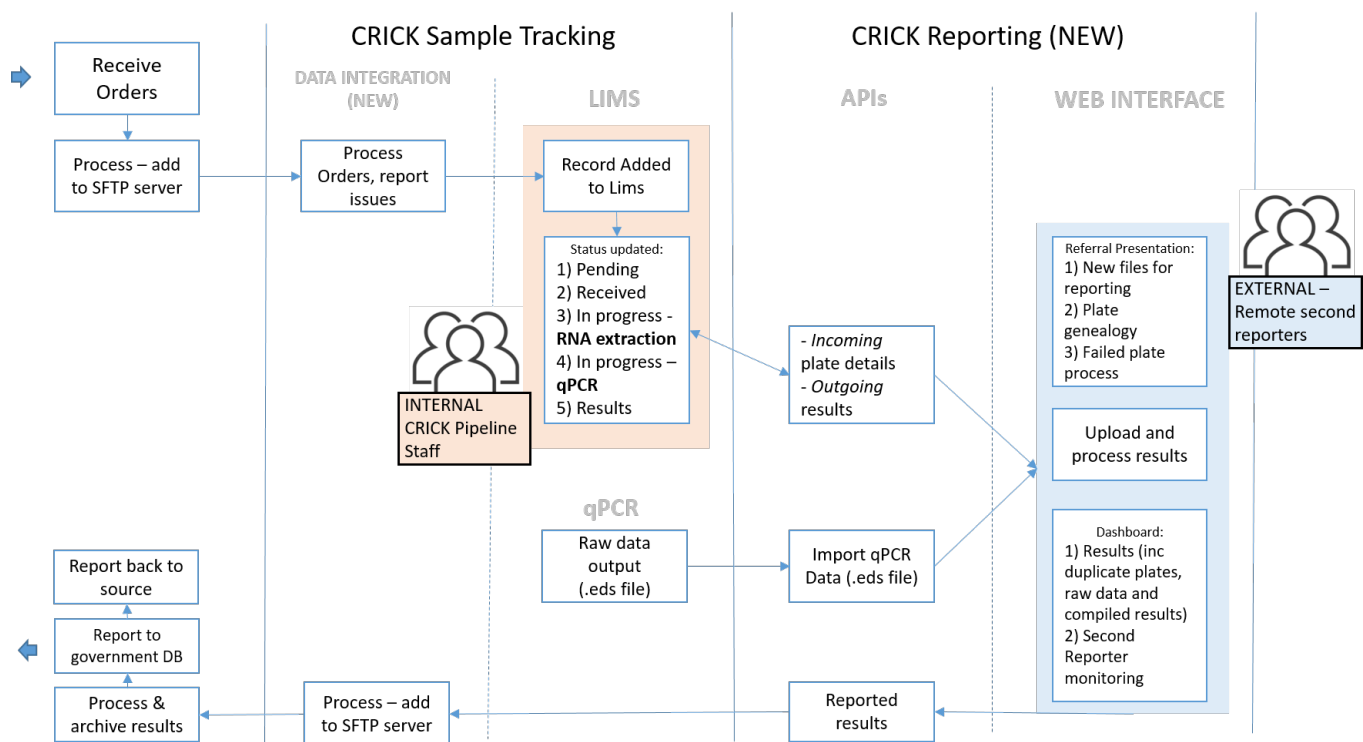
TRACKING SAMPLES AT THE CRICK
A sample tracking dashboard has been developed to allow the tracking of the samples along the pipeline. This is a bespoke piece of software integrated with our internal LIMS system and provides additional functionality to support its use.

The sample will be scanned by the barcode scanning operator into the web based sample reception application at Crick sample reception. Scanning a sample causes the sample to be entered into a predefined workflow in ClarityLIMS (COVID19 RNA EXTRACTION) where the sample queues in the first step of that workflow (sample consolidation).

If the barcode has been scanned successfully, the following notification should be received:

If the barcode scanning has failed, the following notification should be received:

Once the sample has been successfully scanned, the sample will proceed to the next stage.

An electronic "**.rec**" (received) file is created and submitted via the sftp site to confirm the receipt of the sample. The sample ID is electronically processed at HSL and the sample entry updated in Winpath to acknowledge Crick receipt of the sample. Complete the tracking feedback for the sample receipt process.

The barcode is rescanned for each sample to print 3 additional barcode labels and these are then stapled to the outside of the sample bag. Samples then proceed to the next step, viral inactivation.

POTENTIAL RISK: During this manual step, the operator could mis label the bags with the wrong barcode causing a potential sample swap.

RISK MITIGATION: This step will be performed in a conveyor belt manner by four individuals to ensure each operator double checks the bag barcodes and labels

NEAR MISS REPORTING: If during this step any of the operators observe incorrect barcodes being printed or stapled to bags, this needs to be reported on the COVID19 clinical incident page, where a series of questions must be filled in by the reporter.

SAMPLE FAILURE OR REJECTION
If a sample tube has **leaked** its contents or is an **incorrect sample** (urine/stool), the sample must be rejected and the status reported back to HSL to decide the follow up action.

The sample should be scanned into the sample reception application with the barcode as above, print 3 labels, then the barcode is scanned into the sample rejection application to register this sample as **rejected** with a rejection reasons from a drop down menu of,
- "leak",
- "lost",
- "LabError" and,
- "other"

Supplementary Method 16 COVID-19 Sample Tracking Pipeline

This will update ClarityLIMS to register the sample for **disposal** in the CL3 facility or for **return to the HSL**. Faulty samples and incorrect sample forms are completed by the sample registration team.

An electronic "**.rej**" (rejected) file is created and submitted via the sftp site with the sample id and the rejection reason to be electronically processed at the HSL and the sample entry updated in Winpath with the relevant reason to be returned to the Trust that requested the original test

SAMPLES NOT PRE-REGISTERED WITH THE CRICK LIMS

1. There have been instances where a sample had been delivered to the Crick but not pre-registered in the system. Samples scanned into the sample reception software will fail at this stage.
2. An email will be sent to HSL for notification.
3. An electronic csv formatted order file, "**.ORD**", is sent from HSL to register the sample. Following this, a notification is sent to the sample reception team to inform them that the sample can be scanned in and processed in the normal way.

PRIORITY SAMPLES

1. Priority samples can be flagged in the system.
2. Priority samples will be in the same batch and labelled by HSL before they are delivered to the Crick.
3. There is a manual checkbox (highlighted with the yellow arrow) available in the sample reception application which will be checked by the sample reception operator when a priority sample is scanned in.
4. Plates with priority samples are highlighted downstream, in the pipeline for prioritisation.

Supplementary Method 16 COVID-19 Sample Tracking Pipeline

TRACKING SAMPLES IN CL3 CABINET AT THE CRICK

1. Use one barcode sticker to label a 2ml inactivation tube prefilled with 5M guanidine thiocyanate

2. Transfer swab to the barcoded 2ml tube and dispose of specimen bottle in CL3

POTENTIAL RISK: During this manual step, the operator could put the swab in a wrong 2ml tube

RISK MITIGATION: Before leaving the CL3 cabinet, user will cross check the barcode on the swab vial and 2ml tube

RISK MITIGATION: Each user will only inactivate 1 sample at a time

NEAR MISS REPORTING: If during this step any of the operators observe that the wrong barcode label is labelled to an inactivation tube, this needs to be reported on the COVID19 clinical incident page where a series of questions must be filled in by the reporter.

Take 2ml tubes to RNA extraction laboratory and put in a fridge

SAMPLE TRACKING IN RNA Extraction

A. **Tracking inactivated samples during aliquoting on Hamilton**

1. On Hamilton, 200ul of 93 inactivated samples are transferred to a deep 96-well plate. This is the CONSOLIDATION PLATE (See Supplementary Method 3 –aliquoting inactivated virus to 96 well plates)

2. Place a barcode label on right hand side of the CONSOLIDATION PLATE and place on Hamilton Robot.

3. Scan barcode to print a duplicate label (which will be used to label a box for the residual 0.8ml of these samples into the Archive application.

4. As Robot transfers 200 ul of lysate to the 96 well plate, it scans the tube barcodes and produces a .csv file that describes the well locations of the transferred samples in the consolidation plate. This .csv file can be uploaded to the consolidation app hosted. Submitting this .csv to this app will complete the consolidation step in Clarity, automating the assignment of samples to well locations in the electronic record

Consolidate Sample Tubes to 96 Well Plate Format v1.1

For consolidation of inactivated sample tubes to 96 well plate format

This page allows you to upload a the .csv file produced by the Hamilton robot following the sample consolidation step

The Hamilton will scan the sample tubes and allocate each sample to a well of a 96 well plate

Uploading the .csv file here will complete the 'T Consolidation' step in Clarity, and move the new 96 well plate into the queue for RNA extraction

To log the sample tubes as archived, highlight the archiving barcode input box. Using a barcode scanner, scan the barcode on the archiving box

When the SUBMIT button is pressed, this will associate the samples with the archiving box, and carry out the step in Clarity

Prerequisites

- For this app to operate, each of the sample tubes must be currently queued in the T Consolidation step

- IMPORTANT: If the Hamilton has errored and samples have been missed from the plate. If you have transferred these manually you can edit the csv file after selecting it, and change the error flag (which should be set to 1 for samples that have been missed) to 0 before proceeding with the upload

- The archiving box barcode must be available to be scanned in

Please select the .csv file using the button below, and then click submit to send it to Clarity

[Choose File] No file chosen

Archiving box barcode:

[_____]

Full Name of person carrying out process:

[_____]

[Check csv]

Selected csv will be displayed here- it can be edited if incorrect (but be careful!)

NOTE: Initial sample barcode and patient hospital no. are automatically linked to the plate well position on ClarityLIMS.

NOTE: This scanning also tracks the arrival of samples to the RNA extraction step in the pipeline.

POTENTIAL RISK: It is possible that the operator places the barcode label on the left hand side of the 96 well plate and all samples will be mis-tracked

POTENTIAL RISK: It is possible the plate is misoriented in the Hamilton robot and all samples will be mis-tracked

RISK MITIGATION: When the 96-well plate is placed on the Hamilton robot, a second operator double checks/signs off that this has been done in the correct orientation (A1- upper LHS, H12, lower RHS). This can be signed off on the aliquoting section of the backing sheet.

RISK MITIGATION: The Hamilton robot starts the transfer by scanning the barcode label on the 96 well plate. If the plate is not on the right hand side, an error will develop and the transfer will be paused.

NEAR MISS REPORTING: If during this step any of the operators observe that the deep well plate is placed on the Hamilton robot in the wrong orientation this needs to be reported on the COVID19 clinical incident page, where a series of questions must be filled in by the reporter.

Remove the 2ml inactivation tube with residual lysate from the Hamilton rack and place in the ARCHIVE box. Scan the barcode label on the freezer box and transfer the inactivation tubes (containing residual 0.8ml lysate) to log the sample onto the Archive application and place box at -80°C.

B. Tracking sample RNA and RT-PCR reactions

1. RNA extraction is performed and the plate is barcoded and scanned onto ClarityLIMS. From the sample consolidation plate to the extracted RNA plate, the samples should be transferred 1 to 1 which will minimise risk of misassignment.

2. Extracted RNA is aliquoted into a plate for RT-PCR. The source plate (RNA plate) and destination plate (aliquot plate) should be scanned into Clarity using a barcode scanner to prevent errors. Samples should be moved in a 1 to 1 format between the source and destination plate.

3. Scan a new 96-well plate containing RT-PCR master mix (5 ul RNA is transferred to RT-PCR plate.

4. Scan the RNA plate with the residual RNA to log the plate onto the Archive application for storage at -80°C.

5. Scan RT-PCR plate using scanner attached to QUANT STUDIO 3 to name plate being run.

6. Download the platemap for QUANT STUDIO 3 by scanning the plate barcode into the design file creator app. This fetches the sample / well location associations form ClarityLIMS for importing into the PCR machine.

Create a .csv design file for Covid-19 RT-qPCR

- This page allows you to create a.csv for a RT-qPCR aliquot container
- Scanning a plate barcode below will create the .csv design file for this plate, which will download in the browser
- To begin, highlight the barcode input box below. Using a barcode scanner, scan the barcode on the RT-qPCR aliquot plate and click submit when you are ready
- Please note that this app takes about 3 minutes to run, so please be patient

Enter information

RT-qPCR aliquot plate barcode:

Name of person carrying out process:

SUBMIT

POTENTIAL RISK: It is possible the plate is misoriented in the QUANT STUDIO 3 and all samples will be mis-tracked

RISK MITIGATION: When the 96-well plate is placed in the QUANT STUDIO 3, a second operator double checks that this has been done in the correct orientation (A1- upper LHS, H12, lower RHS). This can be signed off on the plate orientation section of the backing sheet.

RISK MITIGATION: The locations of the positive and negative controls in the plate provide an asymmetry allowing any plate misorientation to be spotted by the first reporter. The first reporter can sign off that the controls reported correctly. This can be signed off on the controls section of the backing sheet.

NEAR MISS REPORTING: If during this step any of the operators observe that the RT-PCR plate is loaded in the QuantStudion3 in the incorrect orientation, this needs to be reported on the COVID19 clinical incident page, where a series of questions must be filled in by the reporter.
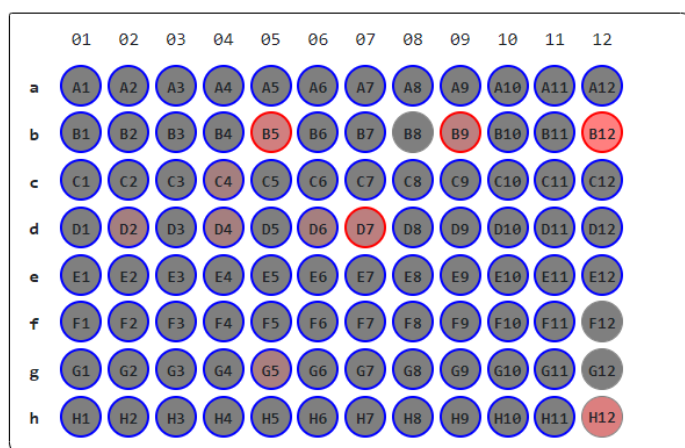
SAMPLE TRACKING AT REPORTING

The plate reporting process is as follows.
1. The PCR run completes and reporter 1 will assess the PCR run on site at the CRICK at the bench.
2. The run is reviewed, QC and analysed using the QuantStudio Software installed on the instrument laptop.
3. Results are submitted via an .eds file output file, which is saved to the reporter1 folder on scientific storage repository.
4. Reporter2 will access this .eds file remotely via an externally facing web portal and analyse the results using locally installed QuantStudio software. (Along with the raw data .eds file, the web portal also provides plate genealogy for reference)

5. They will add any further edits and save the .eds file then export an .xls file and upload both to the web portal.
6. Where plates are being run in duplicate, steps 1-5 are repeated.
7. The reporting application will extract and log results (compiling a combined result if needed) and move the files to the reporter2 folder on scientific storage repository.
8. Both .eds and .xls results files are transferred to the sftp server and picked up by HSL for further processing and results extraction. The results are then uploaded to Winpath and returned to the patient.
9. An API for plate results is provided to the LIMS system to allow results to be available via the sample tracking dashboard, as shown below.

The result file is the Excel output from the QuantStudio. It has 3 tabs:

Sheet #	Sheet Name	Description
1	Sample Setup	Information about the specimens contained in each well, and the run setup.
2	Amplification Data	Raw data of the well curve X/Y coordinates.
3	**Results**	The main sheet containing the well numbers, sample numbers and result values (CT values). This will be used for import to Winpath.

The key columns for import are:

Column position	Column header	Description
D	Sample Name	The lab number associated with the sample.
E	Target Name	The assay processed. This needs to be mapped to the result code in Winpath as part of the interface.
I	CT	The quantitative result. This will either be a number or 'Undetermined'. This will interface to an internal line in Winpath. Winpath rules will then generate the final result.
AC	Comments	A free-text comment that can be applied in QuantStudio to a single well.

The diagram below illustrates the reporting process.
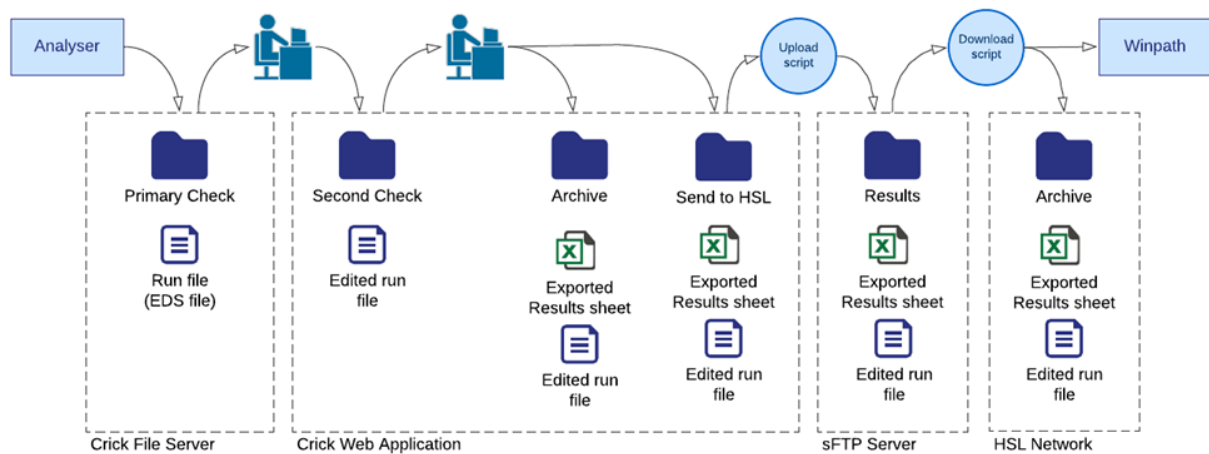
SAMPLE TRACKING FOR RETESTING

Post Rt-PCR, check quality controls –

- If entire plate fails QC, go back to the remaining 25 ul RNA and repeat RT PCR

- If plate passes, continue to first and second reporting. ClarityLIMS will automatically import the following data: Sample barcode and hospital no.

The Crick will only perform a retest on an entire plate. Single failed samples will not be retested.

SAMPLE TRACKING FOR ARCHIVING

- For the archiving of viral inactivation tubes, the app that the lab uses to perform the consolidation step will ask the user to enter the barcode that they will attach to the box that will be used to archive the samples:

- For the stock RNA plates, when the step is carried out in Clarity a database table is updated with the information. This results in an entry appearing on the dashboard to let the team know the samples are ready to be archived:

Supplementary Method 16 COVID-19 Sample Tracking Pipeline

Consolidation

Incoming samples! (includes repeats)

Samples requested for repeat

Tubes boxes to be Archived

RNA plates waiting to be archived / recorded

1. Container name: LPL00505
   - Date produced: 2020-03-31 12:32:08

RNA Extraction

Queued for extraction

Stock RNA Plates to be Archive

RNA plates waiting to be archived / recorded

1. Container name: SPL00005
   - Date produced: 2020-03-30 20:17:08

- An archiving app then displays the boxes and plates that are waiting to be archived, and gives the team the chance to enter the storage information:

Covid-19 sample archiving application

Sample tube archiving

The following tube boxes need archiving:

1. LPL00505: Select this record : ◯
   - Date: 2020-03-31 12:32:08
   - Work done by: Laura

   Record details below

   - Freezer: [____]
   - Slot Number: [____]

   [submit]

Stock RNA Plate Archiving

The following stock RNA plates need archiving:

1. SPL00005: Select this record : ◯
   - Date: 2020-03-30 20:17:08
   - Work done by: Laura Cubitt

   Record details below

   - Freezer: [____]
   - Compartment : [Top ▾]
   - Box Number: [____]

   [submit]

- Once logged, the record disappears from this screen, and the archiving information will appear in the archive search app:

Covid Sample Finder

Find a Sample Tube

Enter the sample barcode you're looking for

[____]

[find]

Tube Box Archive

Use this table to find the storage location for an archived tube box

Use the search to the left to find out which box holds a particular sample

Box Barcode	Freezer	Slot	Date

Stock RNA Plate Archive

Use this table to find the storage location for an archived stock RNA plate

Use Clarity to find out which plate has a particular sample

Plate Barcode	Freezer	Shelf	Box	Date