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Automated transfer of inactivated virus samples from individual 2ml tubes to 96-well plate

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.bfb2jiqe

Crick COVID-19 Consortium



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ABSTRACT



This protocol is part of the [Crick COVID-19 RT-PCR Testing Pipeline](#) collection.



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Purpose of examination / Clinical relevance

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

Principles of Examination

The assay uses the Hamilton robot to transfer 150 µl of 93 samples of inactivated viral media samples from Barcoded 2 ml tubes (Cat no. 72.694.005) to Barcoded 96 well Nunc plates (Cat no. 260251) maintaining sample identity.

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The authors wish to thank Heather Ringrose for support with the Hamilton liquid handling workstation.

GUIDELINES



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Grade of operator

All qualified members of staff who have been signed off as competent and supervised trainees.

Disposal

If the need arises to dispose of tubes or plates (e.g. due to spillage), all contaminated tubes and reagents used are discarded into plastic disposable jars and then into autoclave waste bins. All sharps are placed in a sharps container prior to incineration.

MATERIALS

NAME	CATALOG #	VENDOR
Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates	260251	Thermo Fisher Scientific
Adhesive PCR Plate Seals	AB0558	Thermo Fisher Scientific

MATERIALS TEXT

Specimen Requirements

Specimens will be inactivated virus derived from throat swab and Bronchoalveolar Lavage Fluid (BALF) in 2 ml screwcap tubes prepared in CL2/3 facility following the [virus inactivation protocol](#) and containing [L6 Inactivation Buffer](#).

Equipment / Consumables

- Hamilton STAR or STARlet robot
- Hamilton rack with blue 2 ml tube inserts (Cat no. SMP_CAR_32_A00)
- Nunc 96-Well Polypropylene DeepWell Storage Plates (Cat no. 260251)
- Hamilton 1000 µl Pipette tips
- Screw cap (coloured) for micro tube (e.g. red - Cat no. 65.716)
- Plate carrier (Cat no. PLT_CAR_L5MD_A00)
- Tip Carrier (Cat no. TIP_CAR_480_A00)
- *optional*: plastic seal (Cat no. AB-0558)

SAFETY WARNINGS

Health and Safety

All practices must be carried out in accordance with the current health and safety policies and procedures. If in any doubt about the aspects of health and safety concerning this procedure, seek advice from the departmental Safety Officer or the health and safety team. Guanidine thiocyanate is harmful if inhaled, swallowed or if it comes into contact with skin and causes severe skin burns and eye damage. SARS-CoV-2 is currently classified as a Hazard Group Three (HG3) agent by Advisory Committee on Dangerous Pathogens (ACDP). As such this work falls within the Control of Substances Hazardous to Health (COSHH) Regulations. The Government has provided guidance for clinical diagnostic laboratories safety, sampling and packaging of specimens associated with COVID-19. For hazards, risks and appropriate control measures identified in the risk assessment relevant to this procedure.

PPE

General personal protective equipment (PPE) Control Measures for laboratory work include the wearing of closed toe footwear, laboratory coat, appropriate disposable gloves (nitrile for general work or specified gloves for chemical work), and safety spectacles should be worn throughout this procedure.

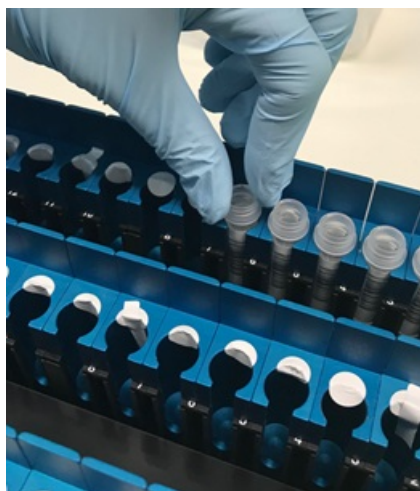
Spillage

The spill kits provided for use in the department can be used for both biohazard and chemical spills. If a spill does occur follow the procedure within the spill kit.

Transfer of inactivated virus to 96 well plates

- 1 Sample tubes will arrive from the CL2/3 facility after [virus inactivation](#) at [Room temperature](#). Working in the reception area (in a separate room), wipe outside of tube with alcohol wipe, invert tube twice, and place in centrifuge. Pulse spin tubes.

- 2 Remove tubes from centrifuge, place in new box (10x10) and transfer to automation lab.
- 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:



The method has been installed on both our Hamilton STAR and STARlet instruments but this protocol only describes the procedure on the STAR.

- 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 93 samples, leave rack 3, spaces 30, 31 and 32 empty for controls to be added later.

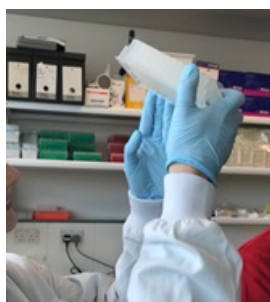


- 5 Place an LPL barcode label on lower edge on the right-hand side of a 96-well Nunc (1.3 ml) plate (Cat no. 260251).



- 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) in track position 49-54 on Hamilton robot.
- 7 Access sample transfer protocol from 'H' 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_A00). 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 **200 µl 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. Visually check volume in plate (this is easiest from below):



- 12 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.
- 13 **Either:** Cover plate and handling very carefully, pass plate immediately to RNA extraction team to be placed on BioMek Fx.
Or: If plate is not to be used immediately, add plastic seal (cat no. AB-0558 located on bench next to Hamilton robot) and store 96-well Nunc plate at **Room temperature** in clear box labelled "to be extracted" (located on bench along from Hamilton robot) in ASF until extraction robot is available.
- 14 Remove 2 ml tube containing residual sample from the Hamilton rack and cap with new coloured lid (located on bench in front of Hamilton and labelled as below).



Archiving

- 15 The app that 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:

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 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 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

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

Archiving box barcode:

Full Name of person carrying out process:

SUBMIT



NOTE: This app is also used for archiving the [RNA samples after extraction](#).

- 16 When this step is carried out in Clarity LIMS, 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:

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

- 17 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):

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:

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 :
- Box Number:

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

Covid Sample Finder

Find a Sample Tube

Enter the sample barcode you're looking for

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
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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
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- 19 Place samples in freezer box labelled with barcode from [go to step #6](#) and store at -80°C .