

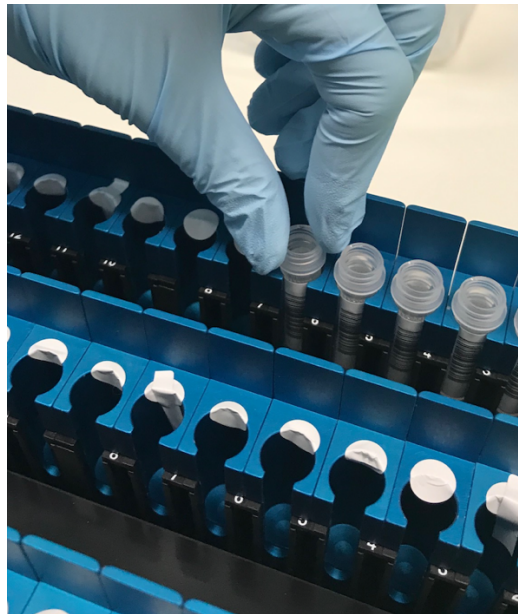
## Supplementary Method 3 Aliquoting Inactivated Virus to 96-DeepWell Plates

### 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  $\mu$ L 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:

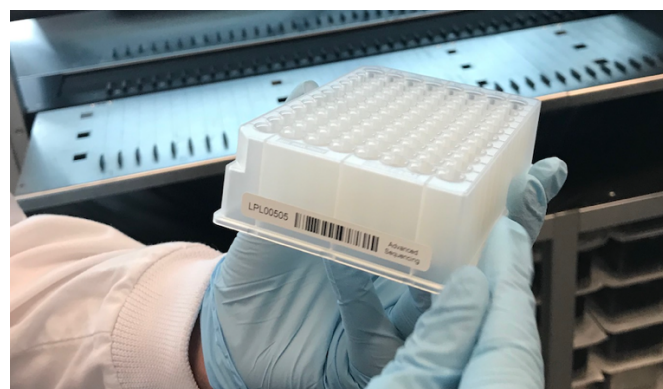


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- 4 Place rack in robot track position 45-47 (racks 1-3 respectively) depending on number of samples to process. Align the barcodes on the tubes to be visible in the inserts window. If processing 94 samples, leave rack 3, spaces 31 and 32 empty for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



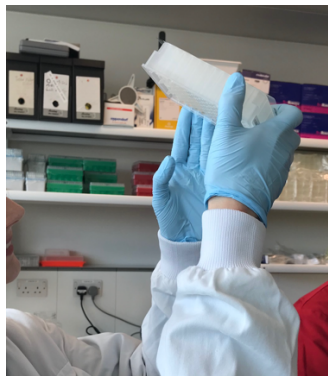
- 5 Place an LPL barcode label on lower edge on the right-hand side of a 96-well Nunc (1.3 mL) plate (Cat no. 260251).



- 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.

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- 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  $\mu$ L 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.

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### 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:

**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

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:

### 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:

<b>Consolidation</b> Incoming samples! (includes repeats)  Samples requested for repeat	<b>RNA Extraction</b> Queued for extraction
<b>Tubes boxes to be Archived</b> RNA plates waiting to be archived / recorded 1. Container name: LPL00505 Date produced: 2020-03-31 12:32:08	<b>Stock RNA Plates to be Archive</b> 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 you the chance to enter the storage information (see below):

Covid-19 sample archiving application	
<b>Sample tube archiving</b> The following tube boxes need archiving:	<b>Stock RNA Plate Archiving</b> The following stock RNA plates need archiving:
1. LPL00505: Select this record : <input type="radio"/> <ul style="list-style-type: none"><li>Date: 2020-03-31 12:32:08</li><li>Work done by: Laura</li></ul>	1. SPL00005: Select this record : <input type="radio"/> <ul style="list-style-type: none"><li>Date: 2020-03-30 20:17:08</li><li>Work done by: Laura Cubitt</li></ul>
Record details below	Record details below
<ul style="list-style-type: none"><li>Freezer: <input type="text"/></li><li>Slot Number: <input type="text"/></li></ul>	<ul style="list-style-type: none"><li>Freezer: <input type="text"/></li><li>Compartment : <input type="text" value="Top"/></li><li>Box Number: <input type="text"/></li></ul>
<input type="button" value="submit"/>	<input type="button" value="submit"/>

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

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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
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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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- 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.

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Notes – errors, variations on SOP etc