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.
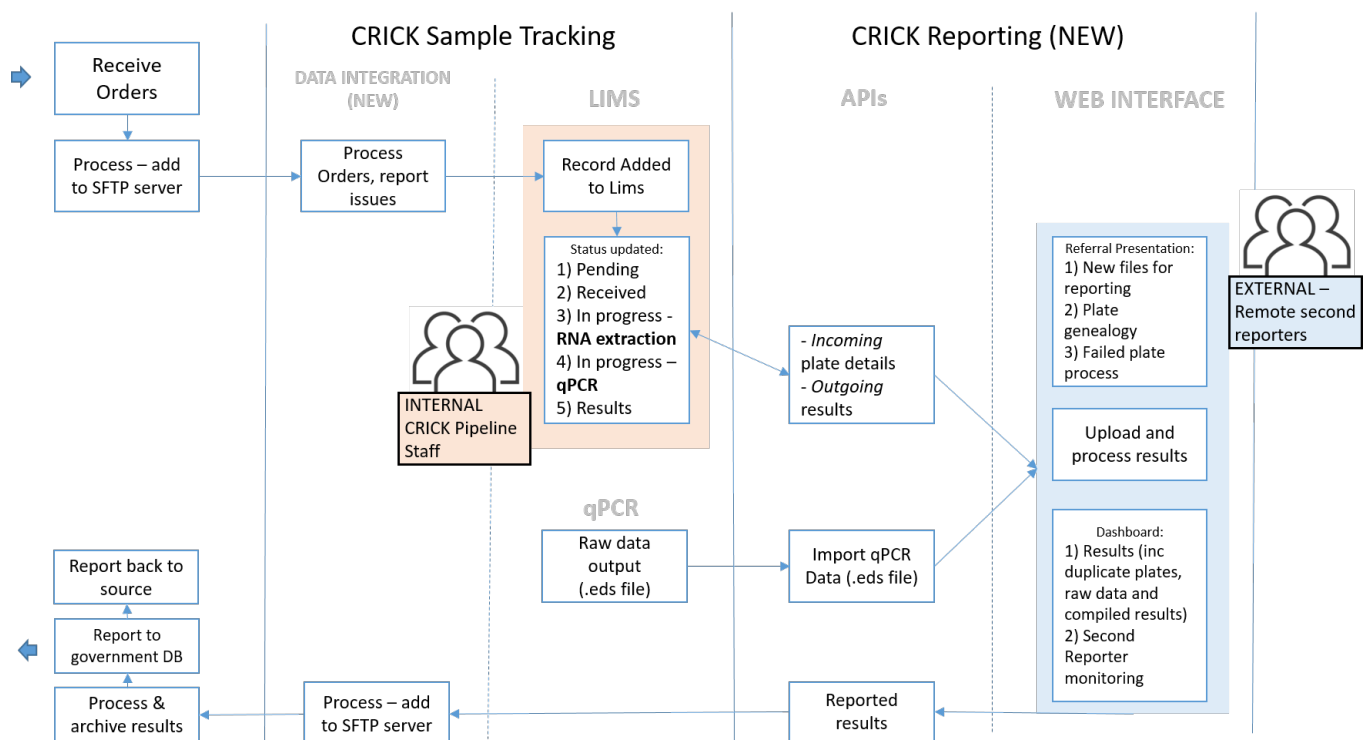
TRACKING SAMPLES AT THE CRICK
A sample tracking dashboard has been developed to allow the tracking of the samples along the pipeline. This is a bespoke piece of software integrated with our internal LIMS system and provides additional functionality to support its use.

asf.thecrick.org   Reception Team   RNA Team   RT-qPCR Team   Results   Trends

search for samples   Search   refresh 4:54

CRICK COVID-19 RNA TEAM STATUS PAGE

Samples Are Ready to Collect

Consolidation

Incoming samples! (this number includes repeats)

🧳 Samples expected: 1 samples scanned in on date: 2020-05-16 hour: 16:00

🧫 Samples expected: 21 samples scanned in on date: 2020-05-19 hour: 09:00

🧫 Samples expected: 4 samples scanned in on date: 2020-05-19 hour:

RNA Extraction

Queued for extraction

RT-qPCR Aliquoting

Ready for RT-qPCR Aliquoting

1. Container name: SPL00281
   🚒 PANIC!!! Urgent samples in this plate
   - Date Scanned: date: 2020-05-19 hour: 17:00
   - Samples in plate: 96

HTS Plate Collection

Plates ready for RT-qPCR

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:

Crick Sample Reception Application

This page should let you use a barcode reader to register the arrival of swab samples into the Crick

1. Whilst using a barcode reader, ensure the below input box is highlighted
2. Scan the barcode to register the sample as received
3. A message will let you know if successful, or if there has been a problem
4. The input box will clear, ready for the next scan
   - A submit button is provided in case the barcode scanner does not automatically submit the form (it should)
   - If a sample is not recognised, it should be put to one side for returning to the Halo lab, as we currently have no way of tracking samples that have not been submitted to our LIMS

Prerequisites
- Sample information has previously been submitted to LIMS with the barcode number becoming the sample name. Samples that have not been submitted to the LIMS cannot proceed through this step

SUCCESS: PROCESSED 50U080343 : GOL743A10

Scan Samples

submit

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

Crick Sample Reception Application v1.005

This page should let you use a barcode reader to register the arrival of swab samples into the Crick

1. Whilst using a barcode reader, ensure the below input box is highlighted

2. Scan the barcode to register the sample as received

3. A message will let you know if successful, or if there has been a problem

4. The input box will clear, ready for the next scan

- A submit button is provided in case the barcode scanner does not automatically submit the form (it should)

- If a sample is not recognised, it should be put to one side for returning to the Halo lab, as we currently have no way of tracking samples that have not been submitted to our LIMS

Prerequisites

- Sample information has previously been submitted to LIMS with the barcode number becoming the sample name. Samples that have not been submitted to the LIMS cannot proceed through this step
- Sample Barcodes only contain uppercase alphabetic characters (A-Z), numbers (0-9), hyphens (-) or underscores (_). Any other characters will be stripped from the barcode during upload

FAILURE: FAKE1 : not found in LIMS

To notify HSL about this unregistered sample, press the Notify button below. Submitting a new barcode is currently disabled to try to ensure this failure is captured

Barcode Length Warning

- Please be extra careful when notifying about this sample (FAKE1). We expect barcodes to have 9 characters, but this one has 5

Notify

Alternatively, if this sample is not to be processed further, click the reset button to reset the form

reset

Scan Samples

| Successful samples scanned (0) | Failed samples scanned (1) FAKE1 |

Mark Urgent Sample:

submit

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"

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.

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.

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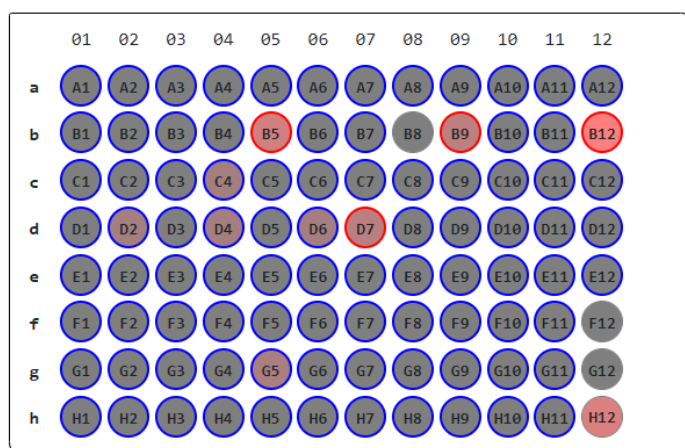
The result file is the Excel output from the QuantStudio. It has 3 tabs:

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.

Supplementary Method 16 COVID-19 Sample Tracking Pipeline
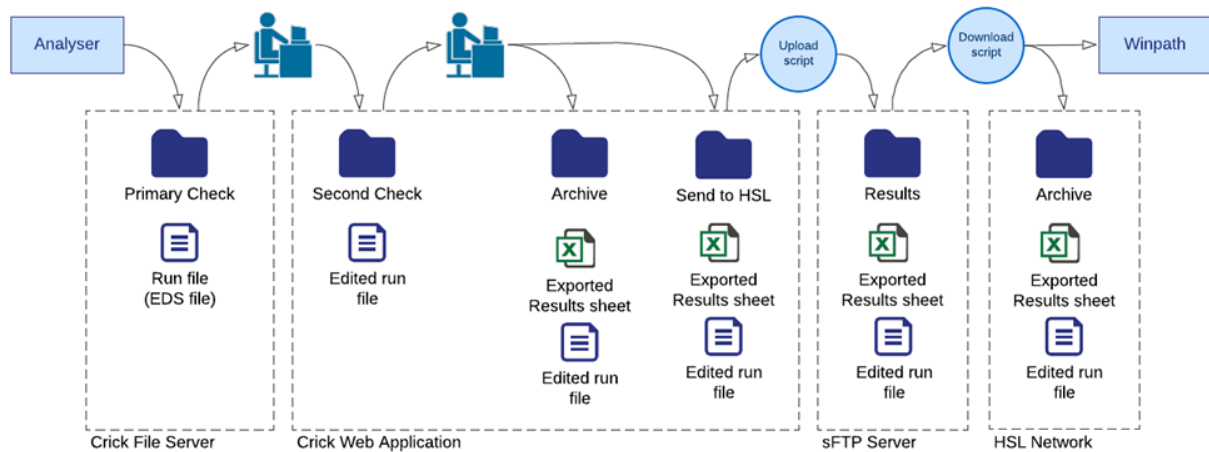
SAMPLE TRACKING FOR RETESTING

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:

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