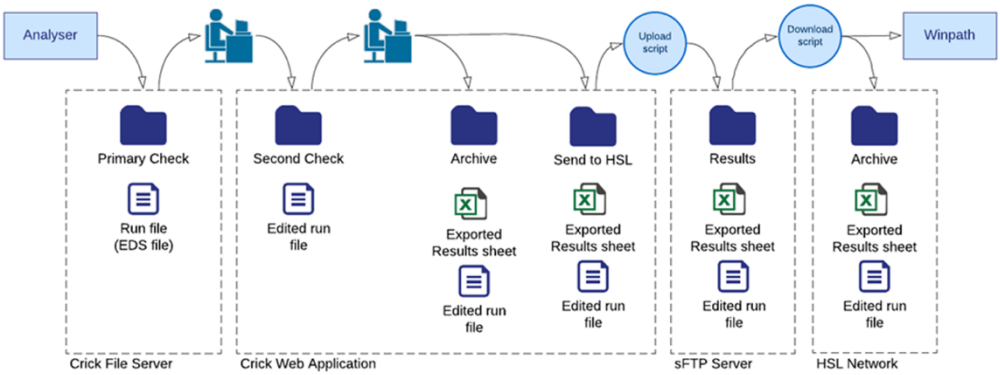
Supplementary Method 8 COVID-19 RT-PCR (BGI kit) results reporting

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

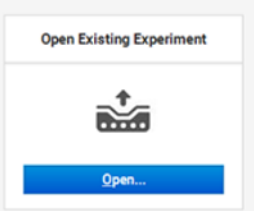
Access to PC Windows PC with QuantStudio software

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.



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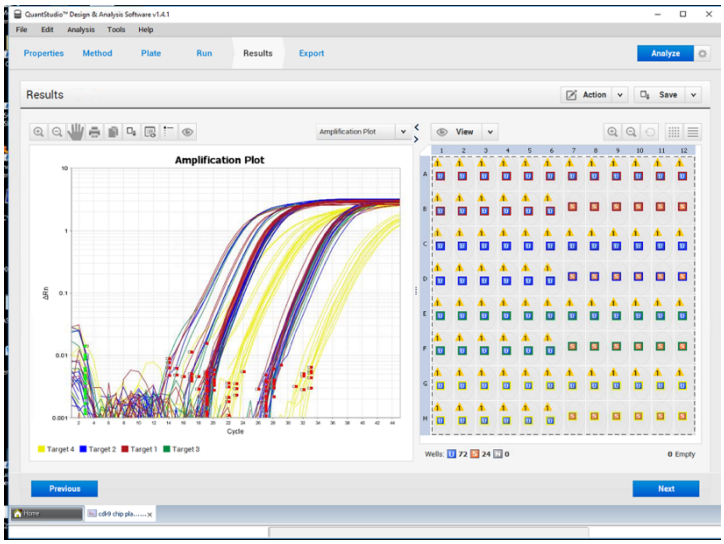

- If the run shows curves but there is widespread failure due to the automatic thresholding, the first reporter discusses this with a second reporter. **See second reporter section below.**

- 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	202(
50U080371	D:3	CFH0PID8	test	2020-03-29	SPL00005	Laura Cubitt	2020-03-29	LPL00505	LC	202(
21U325621	A:2	CFH0PID8	test	2020-	SPL00005	Laura	2020-03-	LPL00505	LC	202(

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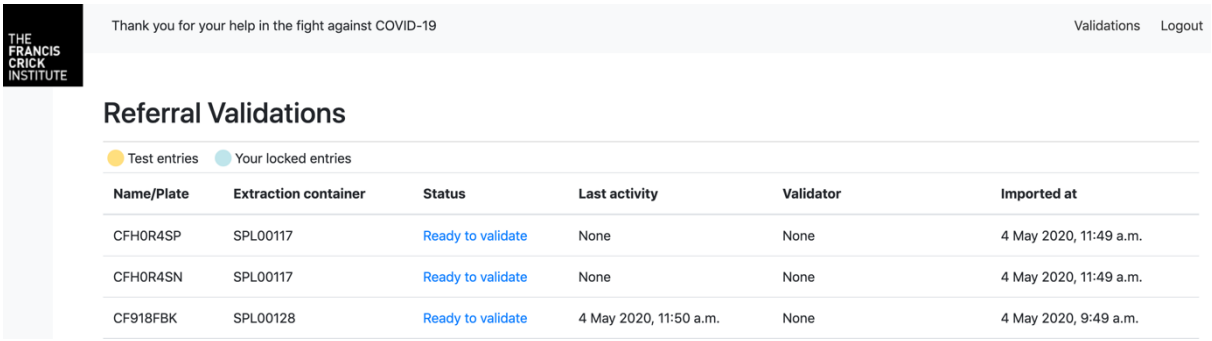
Each run will be run in duplicate and once both runs have passed the first check the First Reporter will save the eds files in a location where they would then be uploaded to the online portal and become available for external clinically registered second reporters to access as shown in the screenshot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

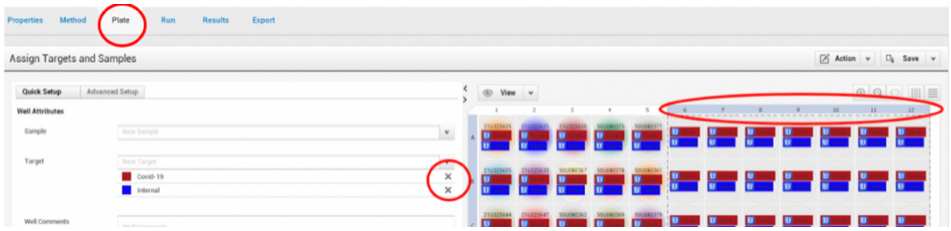


Name/Plate	Extraction container	Status	Last activity	Validator	Imported at
CFH0R4SP	SPL00117	Ready to validate	None	None	4 May 2020, 11:49 a.m.
CFH0R4SN	SPL00117	Ready to validate	None	None	4 May 2020, 11:49 a.m.
CF918FBK	SPL00128	Ready to validate	4 May 2020, 11:50 a.m.	None	4 May 2020, 9:49 a.m.

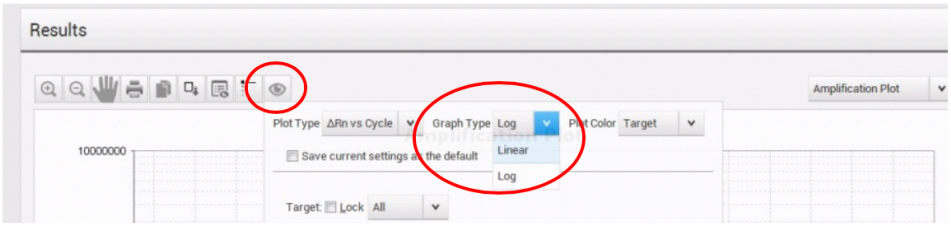
Second Reporter Analysis

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.

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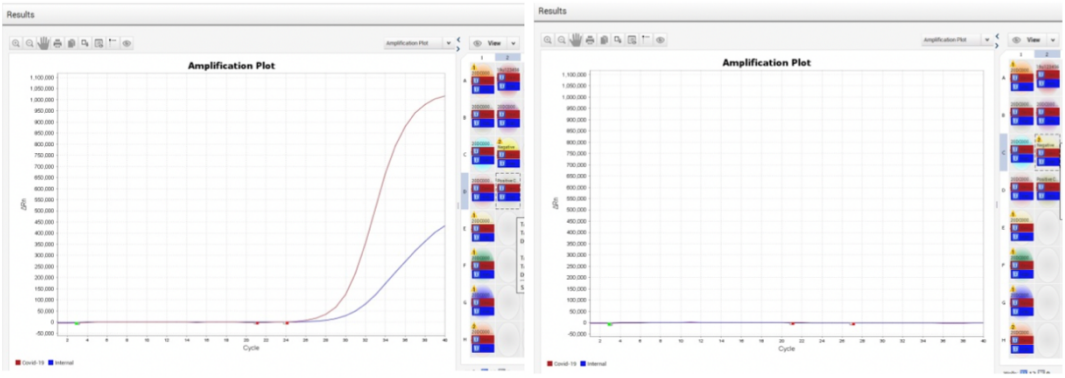


To begin the threshold should be set to automatic in accordance with the BGI kit guidelines and the run checked as a whole on the amplification plot.

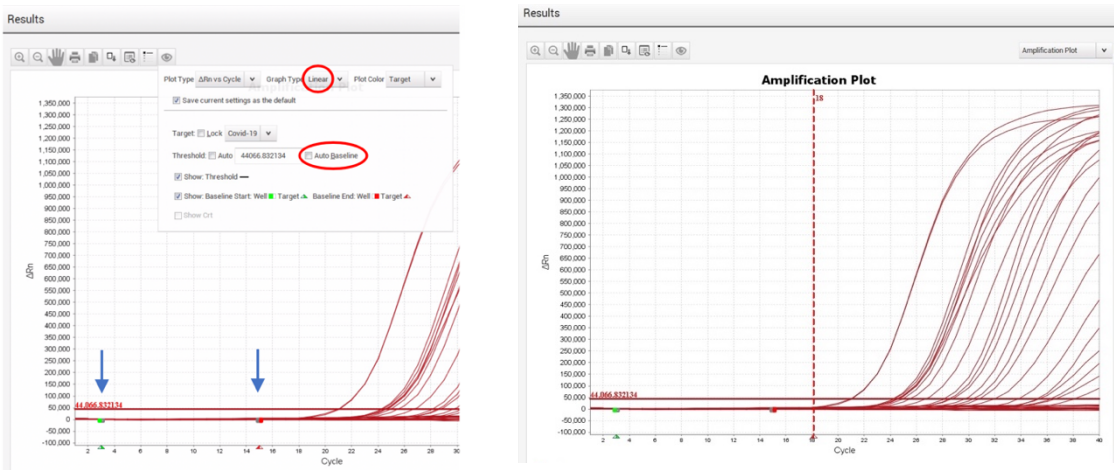
The second reporter should again ensure the following results for the controls:

- 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.



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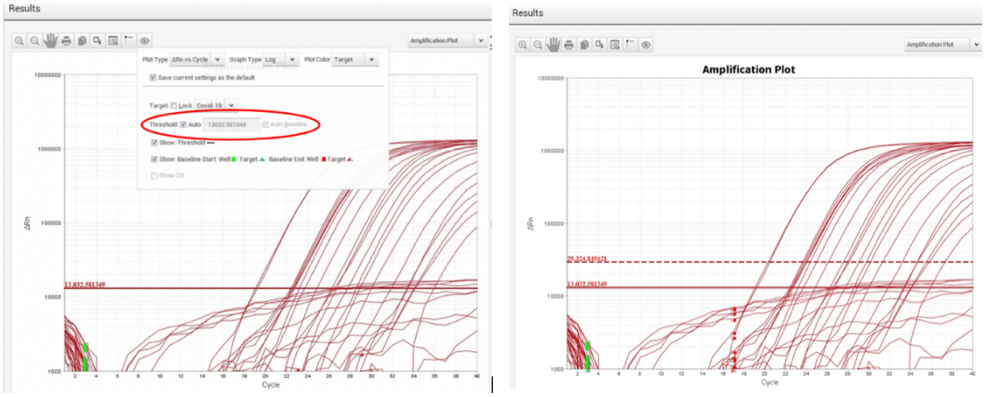
Once the baseline has been adjusted the result analysis can be done using manual thresholding to remove any noise or abnormal curves that could be interfering with the appropriate Ct being given for each sample. In some cases the automatic thresholding will have called all results appropriately and the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

<u>Duplicate run outcomes</u>
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)

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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.

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APPENDIX - *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.