May 16, 2020

SARS-CoV-2 detection using BGI RT-PCR kit

In 1 collection

Wei-Ting Lu[1], Ming Jiang[1], Robert Goldstone[1], Karen Ambrose[1], Chris Ekin[2], Amy Strange[1], Nnenna Kanu[3], Paul Grant[2], Efthymios Fidanis[1], Jerome Nicod[1], David Moore[3], Michael Howell[1]

[1]The Francis Crick Institute, [2]Health Services Laboratories, [3]University College London

1 *Works for me* dx.doi.org/10.17504/protocols.io.bfc8jizw

Crick COVID-19 Consortium

Jerome Nicod
The Francis Crick Institute

ABSTRACT

This protocol is part of the Crick COVID-19 RT-PCR Testing Pipeline collection.

THIS DOCUMENT MUST BE READ AND UNDERSTOOD BY STAFF USING IT AND DOCUMENTED EVIDENCE PROVIDED THEREOF.

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 COVID19. 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.

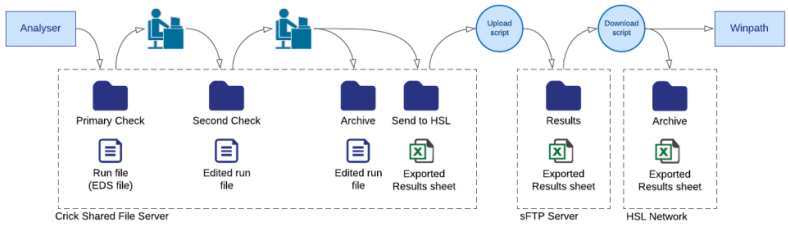
This protocol describes the preparation of the RT-PCR master mix plate, automated addition of RNA template and the reverse-transcriptase quantitative PCR of viral RNA to detect the Orf1ab region of the viral genome, which has high specificity to the 2019-nCoV virus. Furthermore, the protocol describes the analysis of the COVID-19 test results.

Principles of Examination

The kit is a qualitative in vitro nucleic acid amplification assay to detect the new coronavirus 2019-nCoV identified in China in 2019 using Reverse transcription PCR of RNA derived from specimen of throat swabs and Bronchoalveolar Lavage Fluid (BALF) from individuals suspected to have been exposed to the virus.
The kit is based on in vitro RT-PCR with fluorescent probes. Primers and sequence-specific fluorescence probes were designed tailored to the high conservative ORF1ab region of the 2019-nCoV genome. The probes are oligonucleotides with attached fluorophores at the 5' end (FAM as reporter) and at the 3' end (quencher). In addition, a specific primer and probe set designed to human b-actin is included as an internal reference with fluorophores VIC/HEX attached at 5' end as reporter. During the PCR procedure, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye when the probes hybridize to the target DNA. This cleavage results in a fluorescent signal generated by the cleaved reporter dye, which is monitored in real-time by the PCR detection system. Monitoring the fluorescence intensities during Real Time allows the qualitative

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

detection of 2019-nCoV in specimens.
Analysis of the COVID19 test results will allow the qualitative detection of the 2019-nCoV nucleic acid in specimens from patients with suspected 2019n-nCoV. This process will enable the assessment of the infection in clinical and public health practice. The workflow for reporting results from this test is illustrated below:



GUIDELINES

THIS DOCUMENT MUST BE READ AND UNDERSTOOD BY STAFF USING IT AND DOCUMENTED EVIDENCE PROVIDED THEREOF.

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.

The Biomek FX robot carries out the following automated protocol (Appendix):
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

Performance Criteria and Potential sources of variation – Including Interferences and Cross Reactions
Performance Criteria
The sensitivity and specificity calculated from the original validation data were both 100%.
Assay Limitations
- It should be noted that if samples are tested at a dilution then the sensitivity of detection will be reduced accordingly.
- Limitations to the procedure are associated with specimen collection, clinical details, storage, transport and processing.
- The interpretation of results should always include consideration of the clinical picture, epidemiological data and other laboratory results.
- The Results of the test is just for information in clinical practices to assess infection condition of patients combining with clinical presentations and other laboratory markers.
- The incorrect result can be caused by incorrect operations in sample collection, transportation or processing, very low concentration of target virus in the specimens, mutations within the viral genome covered by the kit's primers and/or probe, and unproved external interference factors, such as PCR inhibitor.

Performance Characteristics
The package is intact and liquid contents are clear, transparent and no sediments. All contents are in correct quantity as the package insert listed.

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

Positive control is positive at both FAM and VIC/HEX channel in testing while blank control is negative at both channels.
Limitation of Detection (LOD) of the kit is 100 copies/mL for detecting 2019-nCoV.
The kit was validated by national positive and negative standards.
Cross reactivity
It should be noted that if samples are tested at a dilution then the sensitivity of detection will be reduced accordingly A potential cross-reactivity of the RT-PCR Kit was tested and none of the tested pathogens and human gene have been reactive. The tested pathogens include 54 pathogens, such as human coronavirus includes OC43,229E, HKU1 and NL63(HCoV-OC43, HCoV-229E, HCoV-HKU1, HCoV-NL63) and other pathogens.
Assay Reproducibility
The reproducibility of the assay was validated by manufacturer's precision standards (CV1 and CV 2), LOD standard and negative standard. All samples were tested repeatedly for 20 times, respectively. Coefficient of variance (CV) for Ct values were analyzed to evaluate the variability of inter- and intra-batches, within day and day-to-day operation. The CVs are all less than 5% respective (n=20).
Assay Repeatability

- The repeatability of assay was validated by manufacturer's repeatability standards, LOD standard and negative standard repeatedly for 20 times. Coefficient of variance (CV) for Ct values were analyzed to evaluate the interbatch variability. They are all less than 5%.
- Interference trial shows that performance of the kit is stable with endogenous and exogenous interferin substances such as some anti-microorganism drugs, nasal sprays and nasal drops in specimen. Specimen with elevated level of mucoprotein at a concentration of 60 mg / mL and other substances do not influence the kit performance at virus concentration higher than Limit of Detection.

For details on the import script used in HSL to import the QuantStudio file to Winpath and for more information on the Winpath rules, please refer to the Appendix of the CC006 document added in section 'Description'.

References

- LU Rou-jian, ZHANG Ling-lin, TAN Wen-jie, ZHOU Wei-min, WANG Zhong, PENG Kun, RUAN Li. Development and Comparison of Real-Time and Conventional RT-PCR Assay for Detection of Human Coronavirus NL63 and HKU1[J]. CHINESE JOURNAL OF VIROLOGY, 2008(4).
- NIU P, LU R, LAN J, LIU G, WANG W, TAN W. Development of Novel Multiplex Real-time RT-PCR Assays for Detection of MERS-CoV Infection[J]. CHINESE JOURNAL OF VIROLOGY, 2016(3).
- CHEN Yu-jing. Development of two-panel reactions of real-time PCR for detection of 18 types/subtypes of respiratory viruses [D]. 2015
- Corman V et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan 23; 25(3).

MATERIALS

NAME	CATALOG #	VENDOR
MicroAmp™ Optical Adhesive Film	4311971	Thermo Fisher
MicroAmp™ Adhesive Film Applicator	4333183	Thermo Fisher
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4366932	Thermo Fisher
RNaseZap™ RNase Decontamination Wipes Refill	AM9788	Thermo Fisher
Eppendorf twin.tec® PCR plate 96 LoBind	0030129512	Eppendorf
Adhesive PCR Plate Seals	AB0558	Thermo Fisher Scientific
Biomek Tips P50 Sterile Filtered	A21586	Beckman Coulter

MATERIALS TEXT

Sample Requirements

Extracted viral RNA samples in a 96-well barcoded plate.

Equipment / Consumables

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

Required for preparation of Mastermix plate:

Item (50 tests/kit)	Specification	Quantity	Description
2019-nCoV Reaction Mix	1 ml/vial	1 vial	Composed of reagent for amplification and probes and primers of target gene and internal reference
2019-nCoV Enzyme Mix	80 µl/vial	1 vial	Taq polymerase, Reverse transcriptase and UDG
2019-nCoV Positive control	750 µl/vial	1 vial	Mix solution of pseudo-virus with target virus genes and internal reference
2019-nCoV Blank control	750 µl/vial	1 vial	(Not used in protocol)

Real-time fluorescent RT-PCR kit for detecting 2019-nCoV (BGI, 50 reactions; Cat MFG030010)

- Ranin L-100 manual single pipette
- Ranin L-1000 manual single pipette
- Ranin 20-300 µl LTS multichannel pipette
- Ranin 10-10 0µl or 20-200 µl LTS electronic single channel pipette
- Ranin filtered tips: green box, blue large box, and grey/yellow box
- Eppendorf Tube rack
- 25 ml 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

Required for automatic transfer of RNA with Biomek FX:

Consumables	**Used/run**
Eppendorf 96-well skirted plates LoBind (Eppendorf, cat.no. 0030129512)	1
MicroAmp Fast optical 96-well plate with Barcode (Thermo Fisher, 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
RNaseZap RNase Decontamination Wipes (Thermo Fisher, cat no. AM9788)	1
Equipment	
Beckman Biomek FX workstation	1
Barcode Reader	1

- Alcohol wipes / 70% ethanol

Required for RT-PCR (qPCR):

- Barcode scanner and laptop computer

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

- Quantstudio Real time PCR System
- Underbench centrifuge

Required for Analysis of the test results:
Access to Windows PC with QuantStudio 1.4 software

Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

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. 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.

Preparation of PCR master mix plate

1

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. RT-PCR mastermix is aliquoted into PCR plates in designated hoods in the HTS tissue culture room. All personnel must have full PPE when entering the tissue culture room, which includes clean lab coat, fresh nitrile gloves, and full-face guard. Additionally, the individual pipetting in the hood must wear disposable forearm sleeves.

Take required number of BGI RT-PCR kits from **-20 °C** freezer (2 Kits per 96w plate of RT-PCR reactions, 4 kits per full RNA plate since RT-PCR reactions are run in duplicates) and remove the **2019-nCoV Enzyme mix** to maintain **On ice** , remove the Negative Control to **-20 °C** freezer, and allow the remaining tubes (**2019-nCoV Reaction mix**, **Positive Control**) thaw up at **Room temperature** .

2 **2019-nCoV Reaction mix** has thawed, retrieve **2019-nCoV Enzyme mix** tubes from ice, quick spin down all tubes.

Clean hood and everything that enters hood with DNA ZAP by spraying Solution 1 followed by Solution 2 and then wiping dry with clean paper towels.

Dispense PCR Master mix into plates as follows:

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

- 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 or more: 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 into all 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, 8 aliquots, 1/1 Asp/Dsp Speed

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

Seal the plates with AlumaSeal. Check each well is sealed.

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

Clean up hood by spraying with both DNA ZAP solution 1 followed by DNA ZAP solution 2 and wiping with paper towel.

7 Briefly spin the plate at **1000 rpm 00:01:00** , and store **On ice** or at **4 °C** , together with the thawed positive control tube.

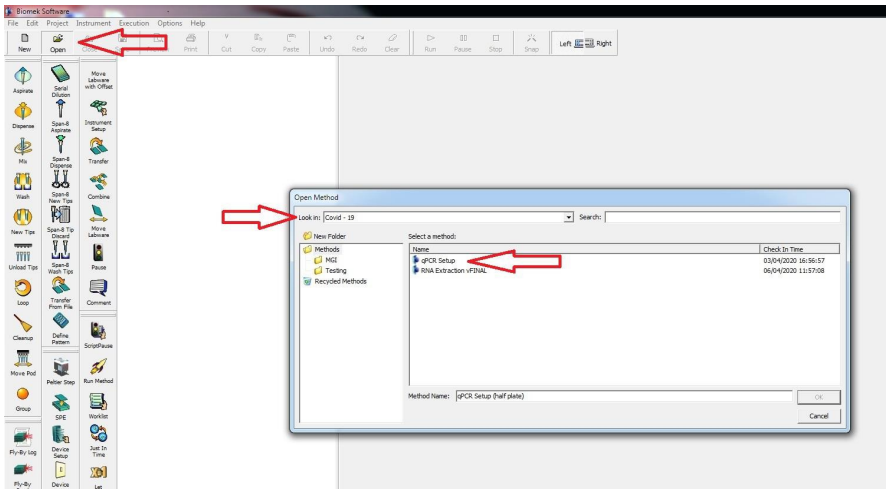
RNA Transfer: Biomek FX Setup

9

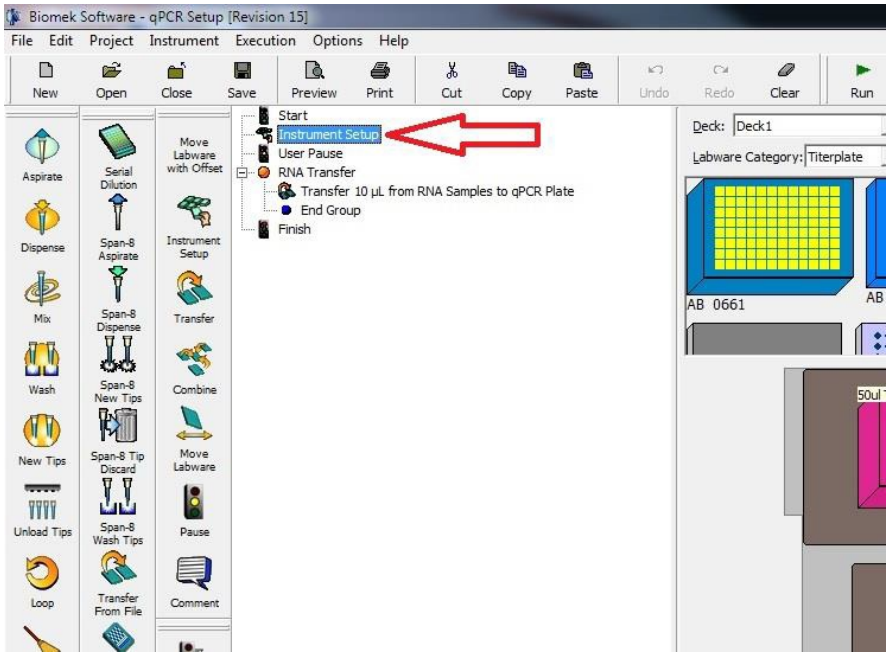
Note: The RNA transfer into PCR plate can also be done manually with a multichannel pipette with filtered tips in a prepared PCR Hood (10 µl of RNA from RNA plate into corresponding wells of the PCR plate, 10 µl of positive control into empty well H12).

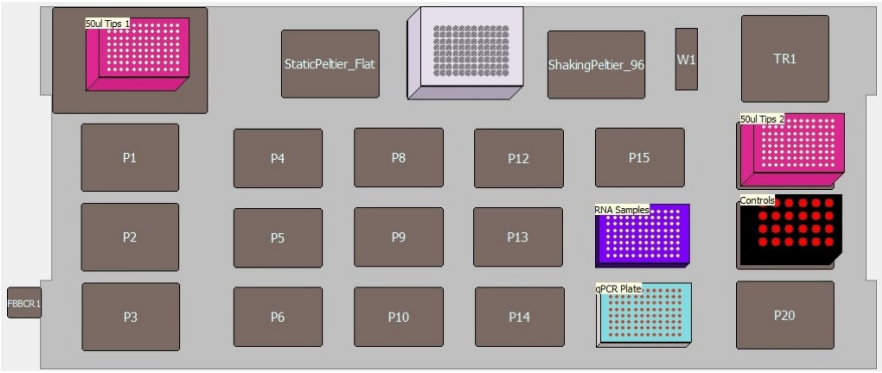
Open the Biomek software, and select the program "**qPCR Setup**" which is located in the "**Covid-19**" project:

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw



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





FX2 deck layout

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

11 Wipe the deck using an **RNaseZap wipe**, followed by an **alcohol wipe** or [M]**70 % ethanol** .

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

13 Take the RNA sample plate (Eppendorf LoBind 96-well skirted plate) with the SPL barcode on the front side, and place in position **P16**.

14 Pulse spin the ABI MicroAmp Optical plate containing the **qPCR reagents mix** in the table top centrifuge if it has been stored at **4 °C** , then stack on a black Greiner CellStar plate. Place on the deck in position **P17**.

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

16 Place a black microfuge tube rack in position **P19**.

17 Place an eppendorf tube (cap open) with **TET buffer** in position **A1** and the **Positive Control** tube (cap off) in position **D1** of the microfuge tube rack.

Position of control tubes on rack

18 Home the instrument by going to “Instrument” and selecting “Home All Axes”.

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

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

20 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.

21 To START the program press the Run button (green triangle) located in the software.

The robot carries out the following automated protocol:

1. Load 50mL tips using pod 1.
2. Aspirate 10mL of RNA samples (10mL/sec).
3. Dispense 10mL to the qPCR plate (10mL/sec).
4. Unload tips.
5. Load one 50mL tip using pod 2.
6. Aspirate 10mL of TET buffer from position A1 of the microfuge rack.
7. Dispense 10mL to the qPCR plate position G12 (this constitutes the 'negative control').
8. Unload tip.
9. Load one 50mL tip using pod 2.
10. Aspirate 10mL of Positive Control from position D1 of the microfuge rack (20mL/sec).
11. Dispense 10mL to the qPCR plate position H12 (30mL/sec).
12. Unload tip.

22 The prompt on the screen will remind you **to remove two tips** from position **G12** and **H12** of the **"50 µl Tips 1"** box**.** Click OK to continue.

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

24 The last prompt will remind you of the microfuge rack setup:
A1: TET buffer in Eppendorf tube
D1: Positive Control

25 Once the program is complete, carefully remove the MicroAmp plate from the deck.

26 Seal with a MicroAmp Optical Adhesive Film.

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

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

28 Repeat steps **go to step #12** to **go to step #27** with a second MicroAmp plate for a total of 2 transfers.

34 Handover both MicroAmp plate to RT-PCR operator or store temporarily at **4 °C** .

29 Seal the RNA sample plate using a Thermo Fisher PCR seal and move to storage at **-80 °C** .

RT-PCR Set Up

35

PCR machines are situated in a separate area of the building to minimise risk of contamination of pre-PCR steps

Take PCR Plate to PCR lab. On designated laptop in PCR lab, open "plate handover" App in Chrome browser.

36 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**.

37 Check seal integrity on PCR plate and spin briefly in underbench centrifuge (**1000 rpm 00:01:00**).
Open the sample drawer of an available QuantStudio 3 PCR machine and place PCR plate.

38 Confirm that the barcode is visible and facing the front of the machine, and **A1** is on top-left side.

39 On the PCR machine-associated laptop, open the "design file" app in Chrome browser.

40 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**.

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

41 Download of text file will happen automatically. Save to “Plate Layout” folder on Desktop with the Barcode as filename.

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

43 Open QuantStudio software.

44 Click on the button to “Open Existing Experiment” and find the **Covid-19 Mater Template.edt** on Desktop.

45 When prompted, click on “Edit” and enter password: **covid192020**.

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

47 Click in field box for **Name** and scan barcode again to replace default, which is “covid 19 master template”.

48 Click in field box for User and enter your Crick user name.

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

50 You should be prompted that current info will be lost and replaced. Hit “yes”.

51 Confirm the following settings by clicking on “Method” on menu near top of window.

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

52 Confirm following settings by clicking on “Plate”.

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

- (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.

53 Click on “Run” window, and hit “Start Run” button.

54 When prompted, save barcode.eds file in “Covid19 Results” folder on Desktop.

55 FINAL CHECKPOINT (stop the run if the following is not true): The PCR run should last **01:28:41** .

56 After the amplification is complete, remove PCR plates from the thermal cycler and discard plate for autoclave and decontamination.

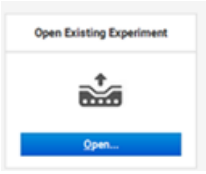
First Reporter QuantStudio 3 Analysis: Initial Data Analysis, Internal Quality Control Procedures and Criteria

57 The run file from the analyser (EDS file) are exported to a shared drive. This folder will be called “Primary Review”.

IMPORTANT: The file must have a unique name that identifies the run and can be linked to the electronic sign-off in Clarity. In this case it will be the unique barcode associated with the patient hospital number.

58 **First reporter/checker** should:

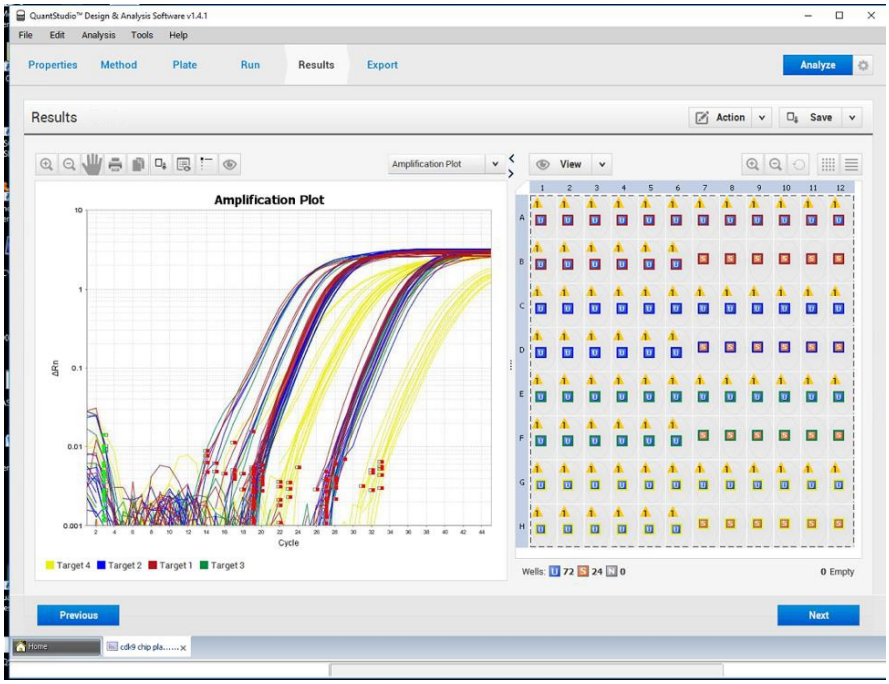
Access the run in Quantstudio 1.4.



59 Ensure threshold is set to automatic.

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

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw



61 If the run shows a lack of typical PCR amplification across the plate (whole plate failure), reject the run and repeat set up.

62 Ensure positive control is ‘positive’ for nCOVID19 and internal control and the negative controls are ‘negative’ for nCOVID19 and internal control:

- Blank control (TET Buffer) and extraction 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 showexponential amplificationwith Ct values not greater than37 and 35 respectively.

Above requirements should be met on the individual plate otherwise the entire plate is invalid. However in borderline cases this may be affected by manual thresholding and can be refered to the second reporter for final decision on plate failure.

63 Click on properties tab and put their name in the “user” free text box.

64 Use the text box to the right of the properties tab to include any additional information that needs to be communicated to the second reporter.

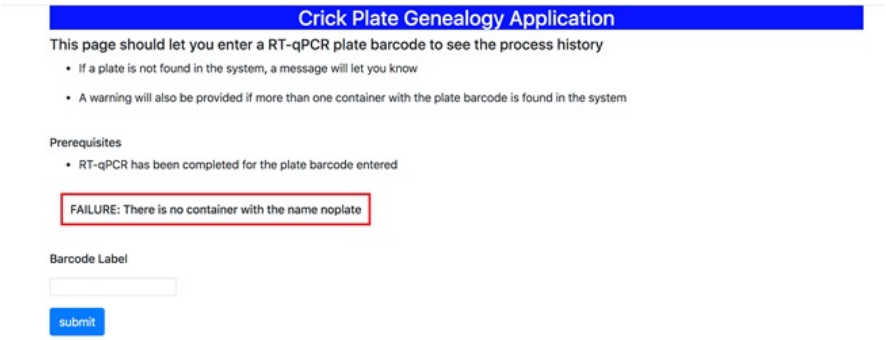
66 In the results tab check all samples using the algorithm below and for cases which should be omitted right click the well and select OMIT.

67 The checked .eds file is then saved to the /report1 folder from where it will be uploaded to the online portal.

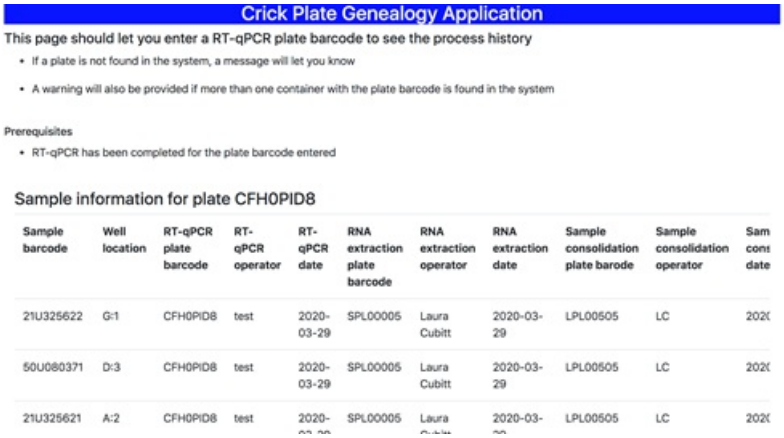
Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

68 The first reporter must extract the plate geneology data from ClarityLims allowing them to documentthe history of a plate so the stages and operators involved. This will support their sign off decision.

A **failed genealogy request** will be illustrated as:



A **successful genealogy request** will be illustrated as:



69 If entire plate has failed and needs to be retested, go back to stored RNA and repeat RT-PCR.

Each run will be run in duplicate and once both runs have been checked and passed the .eds file for one of these runs (“principal run”) is then saved to the /report1 folder from where it will be uploaded to the online portal.

Second Reporter QuantStudio 3 Analysis: Recording, Calculation of Results and Laboratory Clinical Interpretation

70 The second reportor will be able to download the run results for each run and its duplicate through a web app. Following this they will analyse each plate individually and upload the analysed results back onto to the web app and a final outcome for each sample will be determined based on the result from both runs.

Positive and negative results will all be automatically generated in the HSL LIMS system (WinPath) based on Cts and concordance.

71 The second reporter repeats the checking of positive and negative controls as described above (**go to step #62**)

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

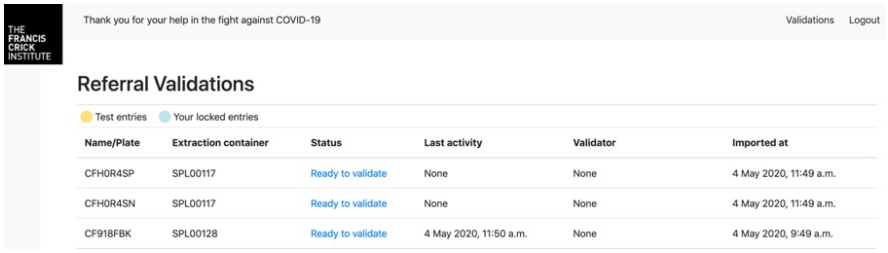
and makes sure of the following:

- The acceptance criteria have been passed
- The overall plate curves suggest appropriate amplification
- There is a complete audit trail for all sample processing steps (At the time of writing this functionality has yet to be completed but will be available in a later edition)

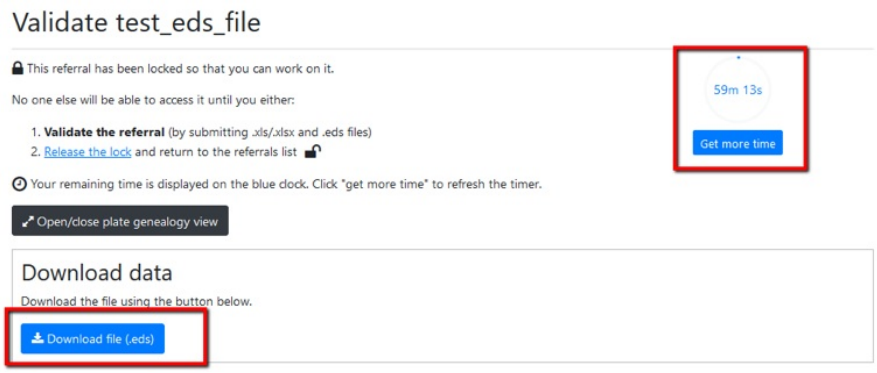
Second Reporter: Import of eds file

72 The results are checked by the second reporter using the online portal.

Any completed plate runs will have the status “Ready to validate” which is a clickable link. The extraction container relates to the plate of RNA that each run originated from, duplicates can be identified as they have the same extraction container ID. The two runs should be opened at the same time thus locking each from interaction by another reporter.



73 When the “Ready to validate” link is clicked the run will become locked to other reporters for an hour. If more time is need, the reporter can click “Get more time” or if they do not continue the validation the can click “Release the lock” to allow other reporters to have access to these runs. To import the eds file the reporter can click on “Download report file”.



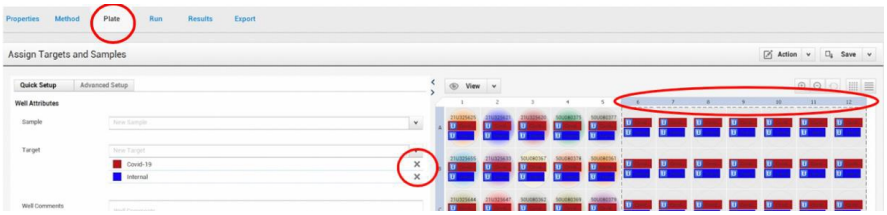
Second Reporter: Using QuantStudio Software

74 The QuantStudio software will open and the password for the locked template is **covid192020 - this must be entered to allow for customisation of the eds file**.

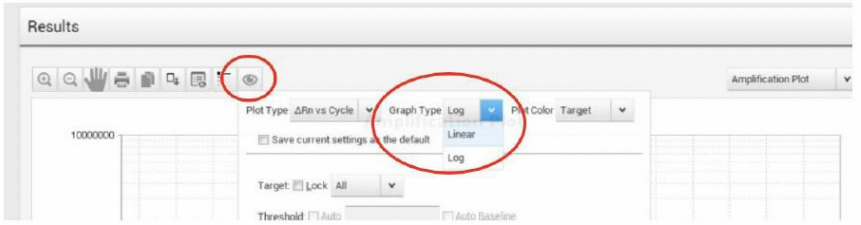
Check the comments box under the properties tab for any comments made by the first reporter.

The eds file automatically opens 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.

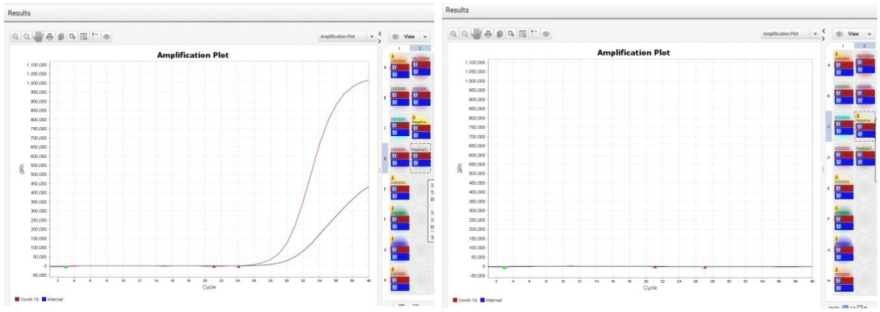
Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw



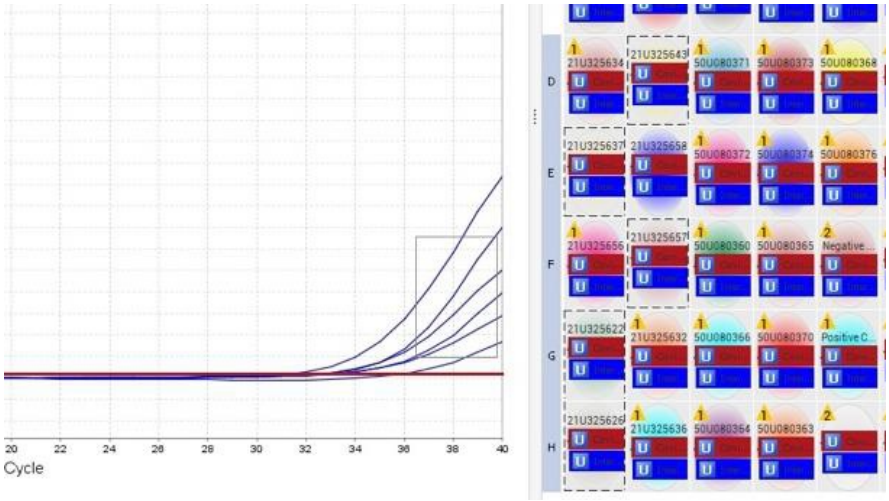
75 **Curve visualistion:** To visualise S curves back on the results page change to a linear graph by clicking the eye symbol and change this under graph type. Then press ANALYSE button, top right.



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.

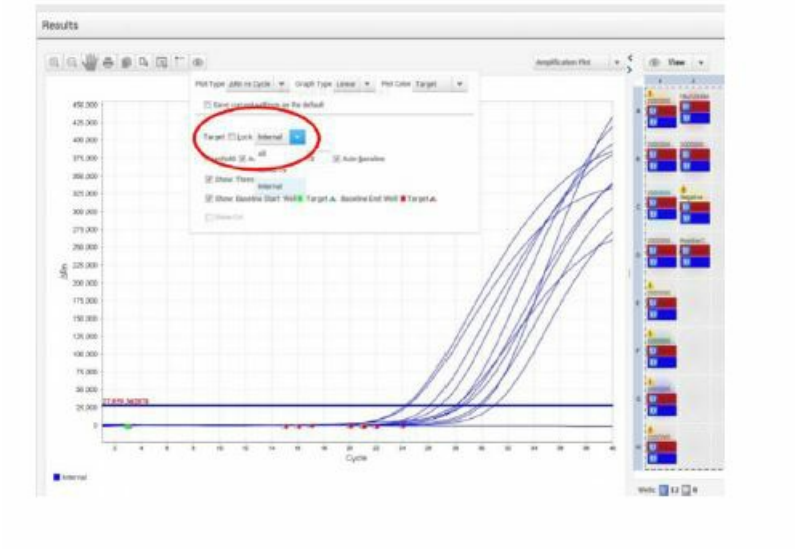


To look closer at any curves or identify which well they are in you can drag the mouse over these to select them and they will become highlighted and the wells they correspond to will be marked with a dotted square box.

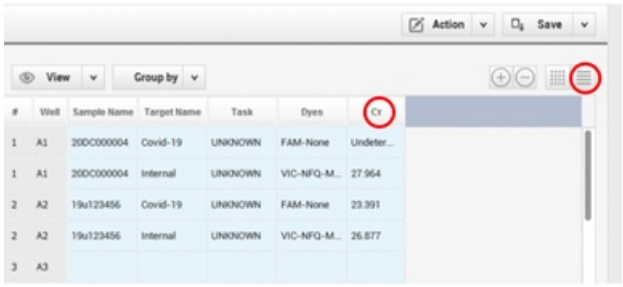


Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

76 **Targets:** To visualise just one target at a time, either covid-19 or internal, this can be done under the plot settings (eye symbol) by changing the target from all to covid-19 or internal.

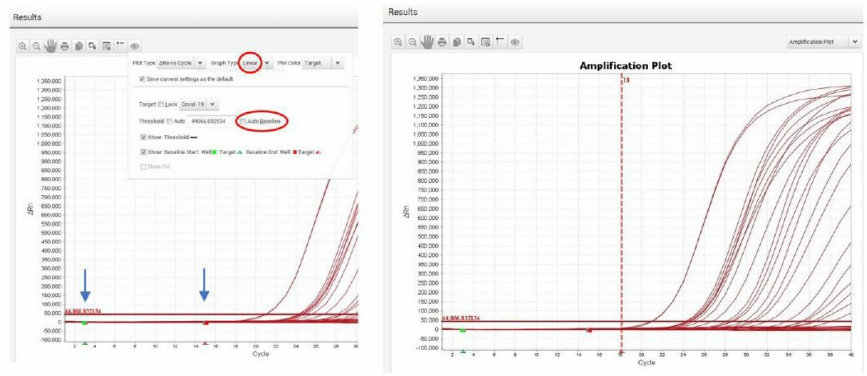


77 **Ct visualisation:** For an easier view of the Ct produced by each curve use the list graph list screen rather than the 96 well screen on the right hand side (button and Ct column circled).



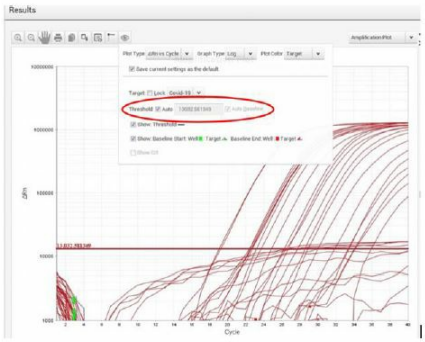
Second Reporter: Threshold

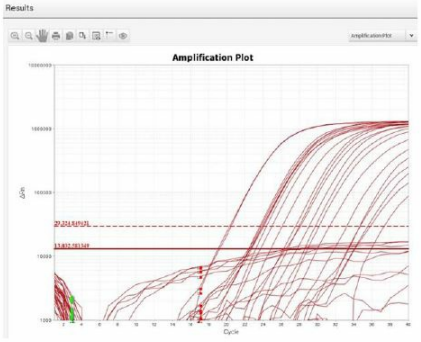
78 **Manual baseline and threshold:**
The baseline will be automatically set between 3 to 15 cycles as according to the BGI kit guidelines however may be manually adjusted to reflect the amplification on the plate. Click on the eye symbol and untick the auto baseline box (circled below left) then drag 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.



Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

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 Ct values will automatically be reanalysed once this has been moved. This is best done in the log scale graph.

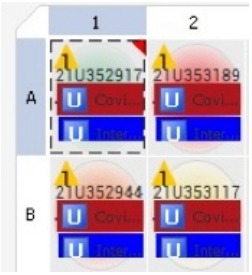


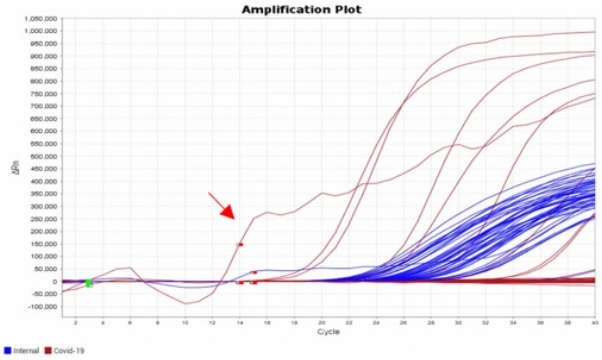


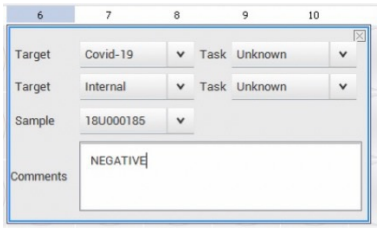
Adding Comments

INVALID

If a sample hasnon exponential amplification (red arrow below) of both or either target with Ct values <37 covid and <35 internal it must be failed.To do so go to the plate screen and double click the well and write “INVALID”.The well to which a comment has been added will show as a red triangle in the top right corner (e.g. see belos, A1).







Second Reporter: Result Anaylsis

79 The second reporter should again check that the controls meet these criteria:

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

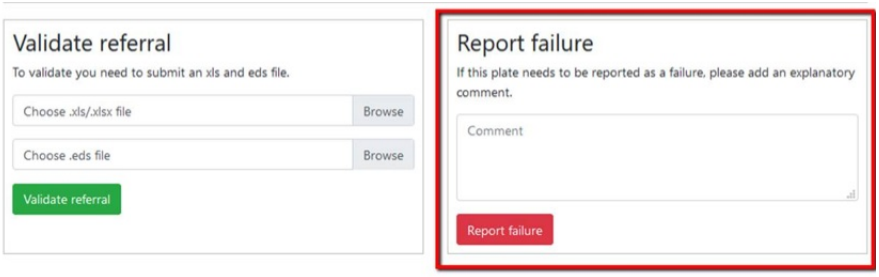
- Positive control:Standard curves at channel FAM (Covid-19) and VIC/HEX (Internal Control) channels showexponential amplificationwith Ct values not greater than 37 and 35 respectively.
- Negative controls: Ct values at FAM and VIC/HEX channels are greater than 37.0 and 35 respectively or no data available. There are two negative controls – one with viral inactivation L6 buffer that has been through the inactivation process (well F12) and one no template control at the RT-PCR stage (well G12).

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

	Covid-19	Internal Control
Positive	Ct <37.0, exponential amplification	Ct <35
Negative	Ct >37.0 or undetermined, Any non exponential amplification	Ct <35
Sample failure	Any Ct, any non exponential amplification	Undetermined or >35
Sample failure (2)	Non exponential amplification of both or either target with Ct values <37 covid and <35 internal	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.

81 **Plate failure:** The plate should be failed if there is signal of <35 for IC or <37 for covid in the negative control. The plate fails if there is no signal for either or both targets for the positive control. If the plate is to be reported as failed reason should be added in the comment box on the online portal and submitted using the “Report Failure” button. This will trigger an email to the PCR and repeat testing will be set up. The plate will be removed from the referrals homepage.



Duplicate Run Outcome

The second reporter will have completed the steps outlined in 6.3 in QuantStudio for both runs and applied the same acceptance criteria for the controls and patient samples outlined in 6.4.

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	Expected result
1	<37	<37	POSITIVE
2	>37/UD	>37/UD	NOT detected
3	35-37	>37/UD	NOT detected

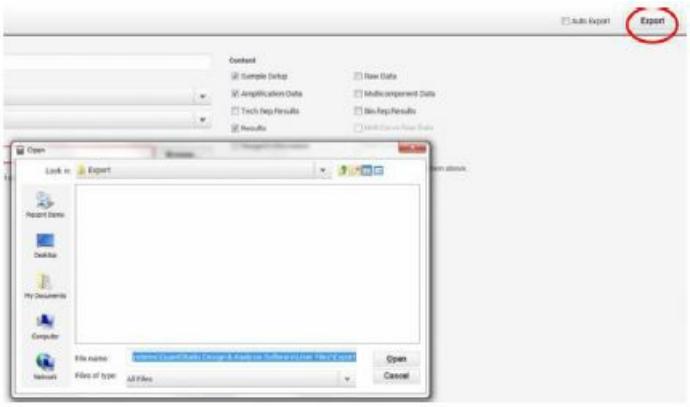
Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

4	>37/UD	35-37	NOT detected
5	<35	>37/UD	Invalid (discordant)
6	>37/UD	<35	Invalid (discordant)

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.

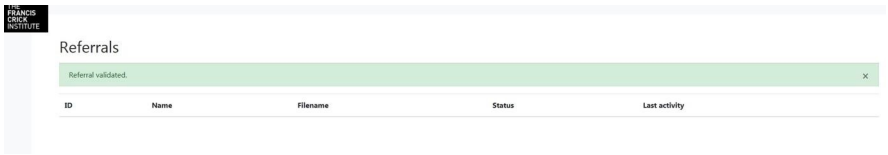
82 **Exporting the xls file from QuantStudio**

Once the results have been analysed and the plate has passed the eds file must be exported to an xls file to upload back onto the results portal. Under the export screen select the browser location to export this file to, ensure this is a .xls file (not .xlsx) and then click export button circled. Once exported the xls form will automatically open, save in a folder on your drive. The modified eds file must also be saved in this folder.



83 **Uploading the xls file to the online portal**

For each run upload the .xls file (not .xlsx) to the portal by clicking browse and uploading the file. Do the same for each with the eds file. Once both files have been uploaded press the “Validate” button. The reporter will then be returned to the Referrals list where any other reports awaiting validation will be available.



Sample Requeue Process

84 If the PCR plate has failed for one run, the RNA from this run will go into a new barcoded PCR plate.

The RNA team will requeue the RNA extraction plate into the aliquot step and scan the new destination plate barcode into the Claritylims system.

This will maintain the link to the original barcoded RNA plate and samples, so that we can also identify the number of times this plate has been repeated.

Result File Structure

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

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

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 51 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.

Example:

	A	B	C	D	E	O	AC
40	Stage/	Stage2, Step2					
41	User N	luw					
42							
43	Well	Well Position	Omit	Sample Name	Target Name	CT	Comments
44	1	A1	FALSE	50U113793	Covid-19	Undetermined	This is a comment on A1
45	1	A1	FALSE	50U113793	Internal	23.219	This is a comment on A1
46	2	A2	FALSE	50U113810	Covid-19	25.308	
47	2	A2	FALSE	50U113810	Internal	19.451	
48	3	A3	TRUE	50U113807	Covid-19	36.615	A3 comment
49	3	A3	TRUE	50U113807	Internal	21.235	A3 comment
50	4	A4	FALSE	50U113791	Covid-19	26.361	
51	4	A4	FALSE	50U113791	Internal	23.133	

Translates to this result in Winpath:

Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw

	Tests	Results
n H	CORONAVIRUS CRICK INSTITUT	
a H	Specimen type	Swab
a H	Report from	The Francis Crick Institute
a H	SARS CoV-2 RNA	NOT detected
a H	Comment	This test is not UKAS accredited, but has been
a H		verified.
t H	- - - - - - - - - - - - - - -	
p H	Date Sent to Crick	Not recorded
a H	Crick CoV-2 CT-Value	0
a H	Crick CoV-2 Internal Control	23.6

- The files should be dropped to the sFTP server RESULTS folder.
- The files should have a unique name.
- If a run is exported with failed run controls by mistake the interface will prevent it being transmitted to Winpath.
- The Winpath rules will look for technical failures and prevent reporting if an invalid result is identified.

86 SFTP Server

The SFTP server is hosted by HSL.

Folder structure:

```
TEST/
        ORDERS/
                *.ORD
        RESULTS/
                *.xlsx
LIVE/
        ORDERS/
                *.ORD
        RESULTS/
                *.xlsx
```


Citation: Wei-Ting Lu, Ming Jiang, Robert Goldstone, Karen Ambrose, Chris Ekin, Amy Strange, Nnenna Kanu, Paul Grant, Efthymios Fidanis, Jerome Nicod, David Moore, Michael Howell (05/16/2020). SARS-CoV-2 detection using BGI RT-PCR kit. https://dx.doi.org/10.17504/protocols.io.bfc8jizw