Introduction

The 2019-20 coronavirus outbreak is an ongoing public health emergency of international concern involving COVID. It is caused by the SARS-CoV-2, first identified in Wuhan, Hubei, China. SARS-CoV-2 is closely related to the original SARS-CoV. It is thought to have a zoonotic origin. Genetic analysis has revealed that the coronavirus genetically clusters with the genus Betacoronavirus, in lineage B or the subgenus Sarbecovirus together with the two bat-derived strains. It is 96% identical at the whole genome level to other bat coronavirus samples (BatCov RaTG13). In February 2020, Chinese researchers found that there is only one amino acid difference in certain genome sequences between the viruses found in pangolins and those from human patients, implying that pangolins may have been an intermediate host.

The BGI 2019-nCoV PCR assay is used with the MGI extraction system as a real-time RT PCR test able to detect the COVID gene (ORF1ab) and human beta actin gene.

Collection and Transport & Storage

Nasopharyngeal or throat swabs collected in viral transport media

- Store collected specimens at 4^{o}C and process specimens as soon as possible.
- If delay of more than one day is expected, aliquot specimens and store at $\leq$-20^{o}C.

Materials, Equipments and Facilities

Clean Room: Biosafety Cabinet (MIBCT3), freezer (MIFTG)

- Specimen Preparation area: Biosafety Cabinet (MIBCT7 or MIBCT8)
- BIO-RAD CFX96 ^{TM} Real-Time System

- BIO-RAD Hard-Shell PCR Plates 96-Well WHT/CLR
- BIO-RAD Microseal ®'B' seal Seals
- 96-Well Loading Block (pre-cooled to -20°C)
- Variable volume Rainin pipettes: 1 to 20 µL, 10 to 200 µL, 100 to 1000 µL
- Variable volume multichannel Rainin pipettes: 1 to 20 µL, 10 to 200 µL, 100 to 1200 µL
- Reagents: MGI Easy DNA/RNA, MGI Easy Magnetic Beads Virus DNA/RNA Extraction Kit, BGI Real time fluorescent RT-PCR kit for detecting 2019-nCoV,
- Positive and negative external Controls

Specimen Processing

Minimum volume for testing is 180 µL.

Heat fix 300 µL sample aliquots at 65°C for 30 minutes to inactivate virus.

NOTE: *For dry swabs, refer the details to COVID 19 Pre-Analytic Processing Procedure from Microbiology Internal Manual*

Procedure

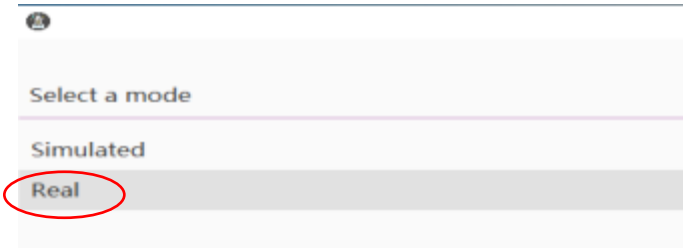
General Precautions

- There must be separate PCR work areas:
  Clean room
  Specimen preparation room
- Powder-free Gloves should only be in use in PCR areas.
- Change gloves frequently and keep tubes closed whenever possible.
- Prepare Working 1% sodium hypochloride daily.
- Specimen Preparation Supplies and equipment must be dedicated to Specimen Prep Area and not used for other activities and never used in Clean Room.
- Change lab coats and gloves between work areas.
- Use only Aerosol Resistant Tips (ART)
- Use only sterile RNase-free, DNAse-free microtubes
- Thaw components completely at room temperature.
- PCR work areas (Clean Room and Specimen Preparation Area) bench tops and equipment after each shift.

Extraction Set up

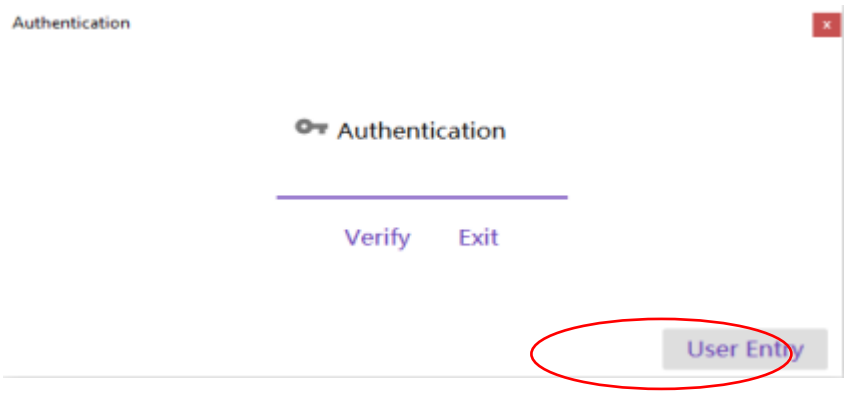
1. Turn on the computer. Turn on the MGI platform.
2. Double click the **MGI SP 960 icon**.

3. Select **Real** in the 'Select the mode' window.

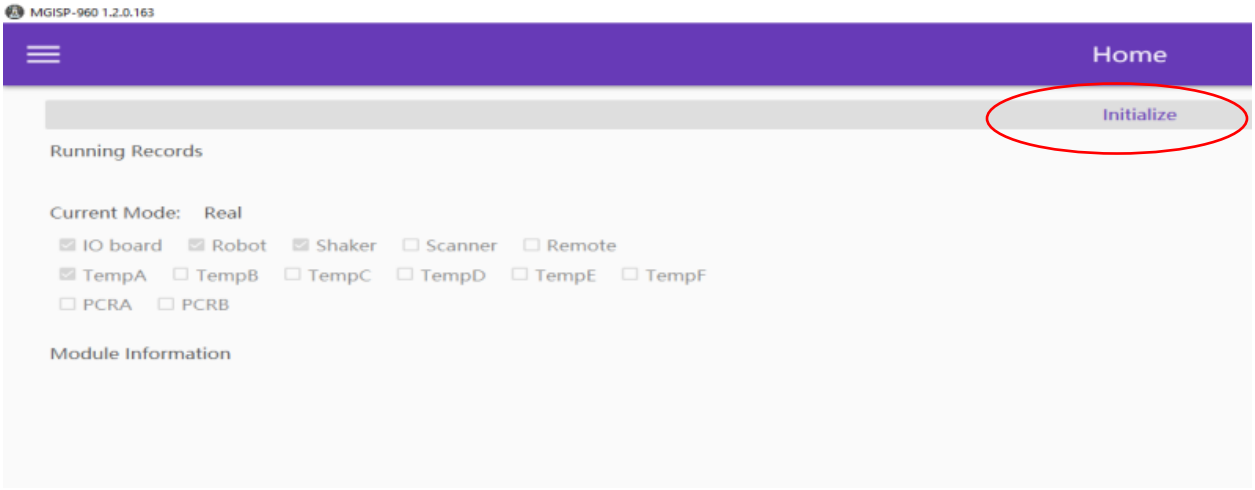


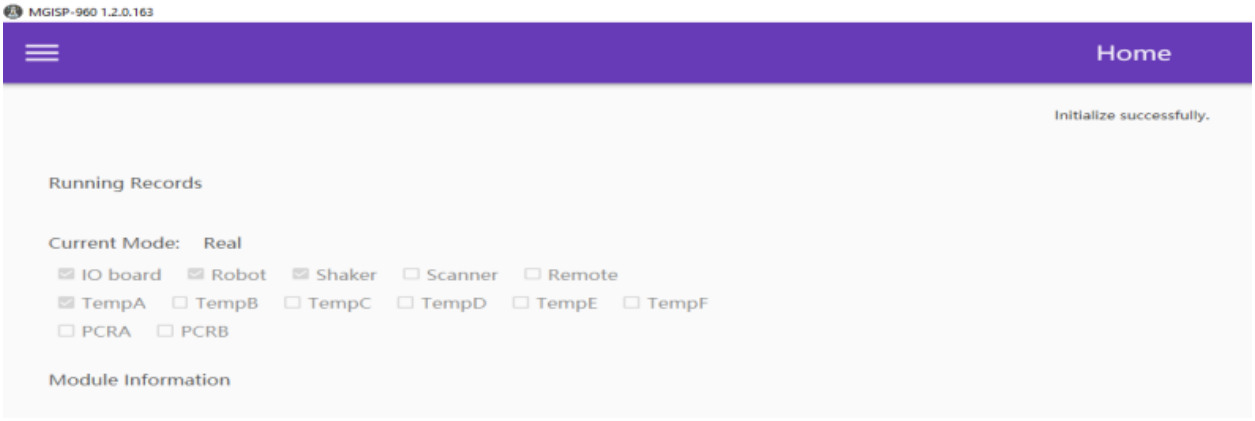
4. Click **Create** button.

5. Authentication window pops up. Click **User Entry** button.

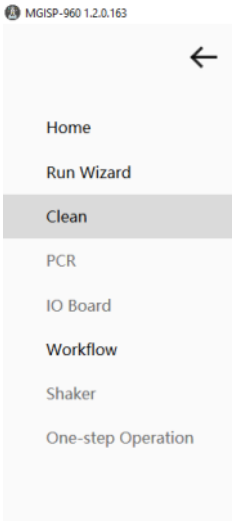


6. MGI SP960 is in the Initializing process.

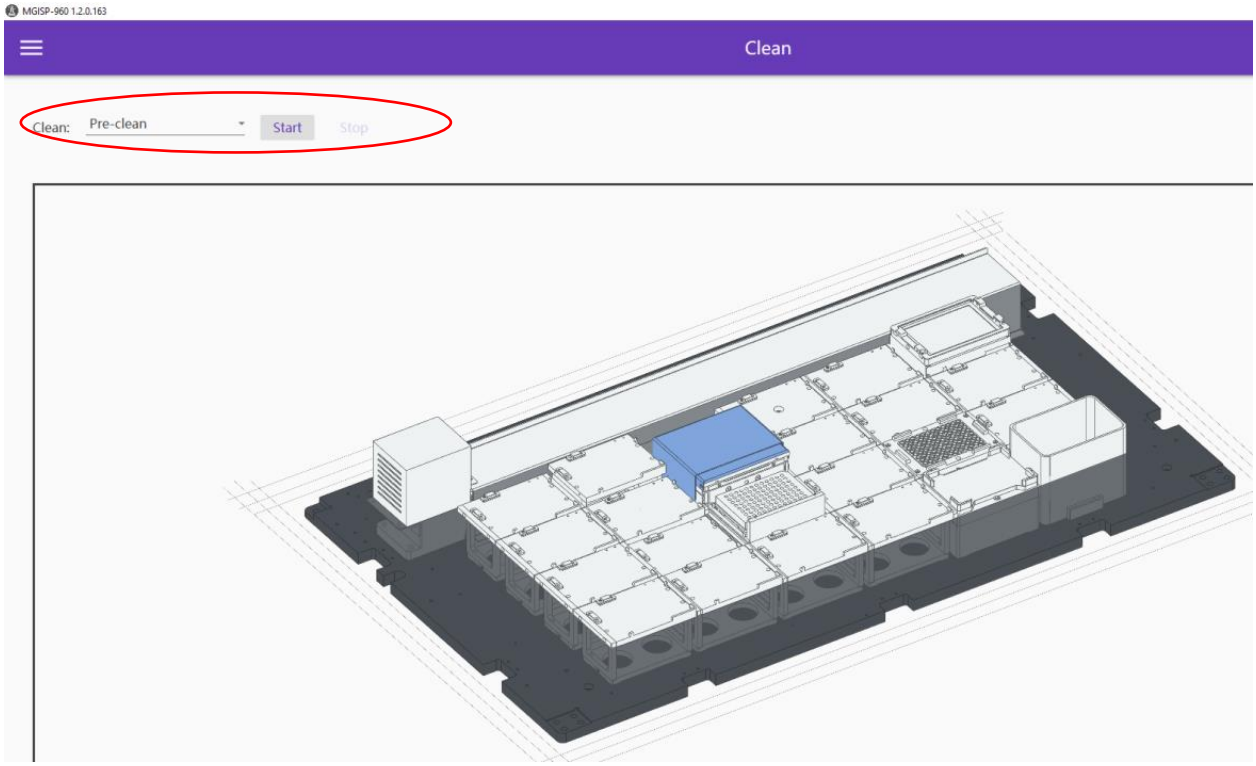




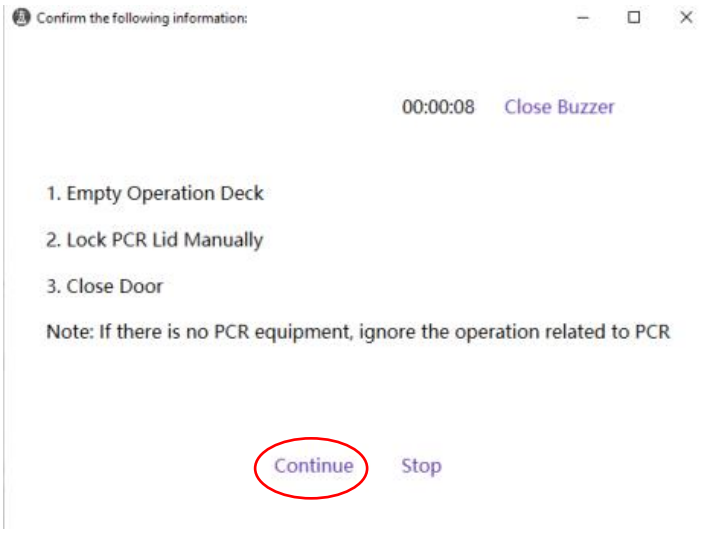
8. Click **Home** bar to go back the main menu
   Select **Clean**.



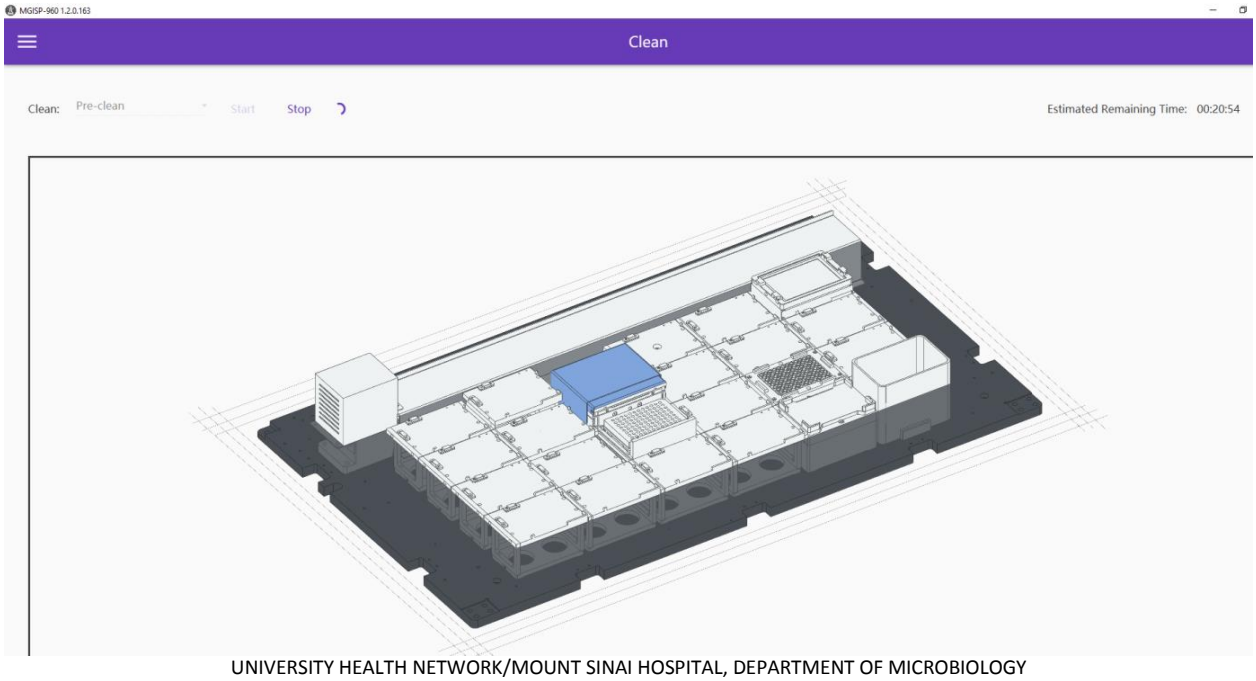
9. Select **Pre Clean**.
   Click **Start** button.

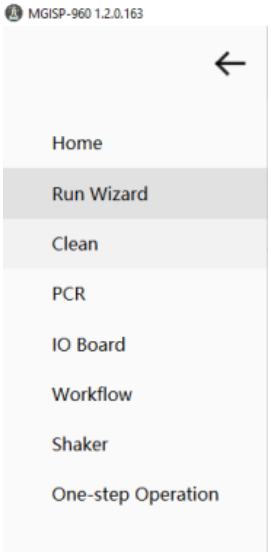


10. Confirm the operation listed in the confirmation window
    Click **Continue** button.

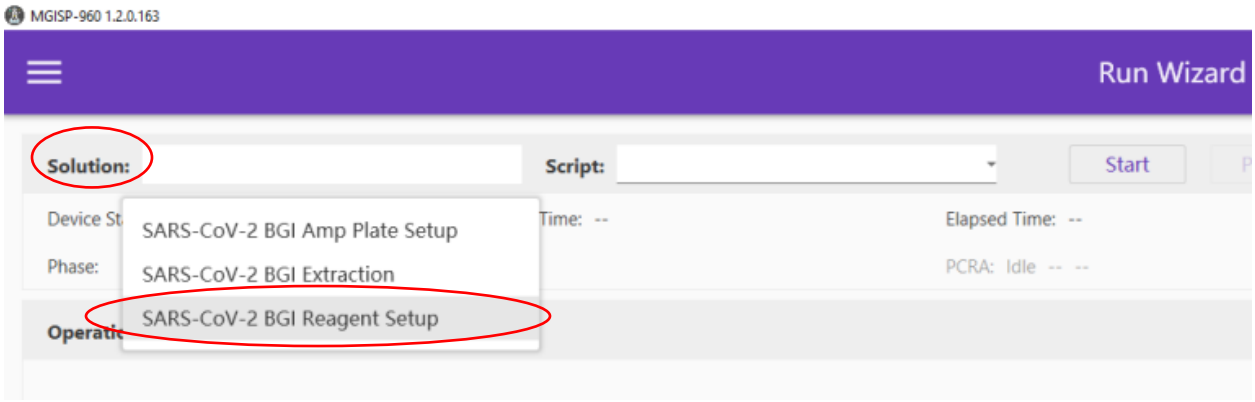


11. MGI SP 960 will start the clean process. It takes 20 minutes to finish.

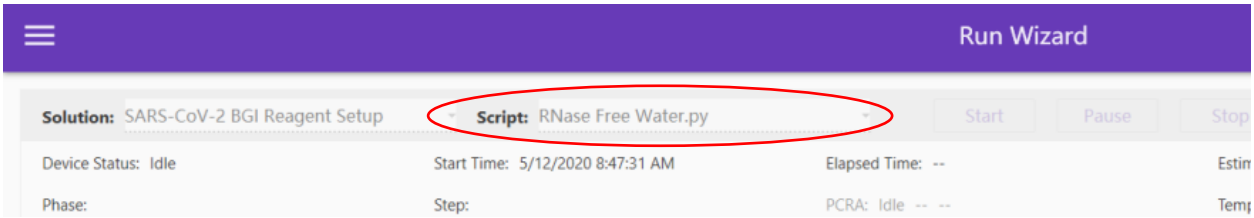




3. Choose **SARS CoV2 Reagent Setup** in the Solution drop-down menu



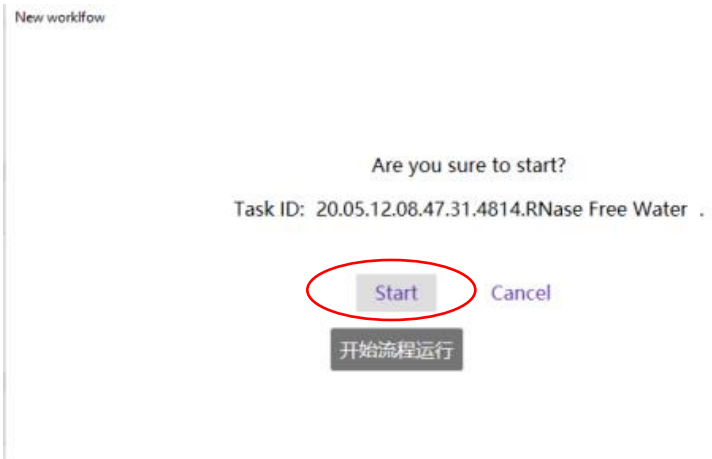
4. Choose **RNase Free Water.py** from **Script** drop-down menu to dispense water.



5. Load tips, deep well plates and the tip lid according to the position on the screen.
6. Measure and transfer 45ml RNase Free Water into the tip lid at position 2.

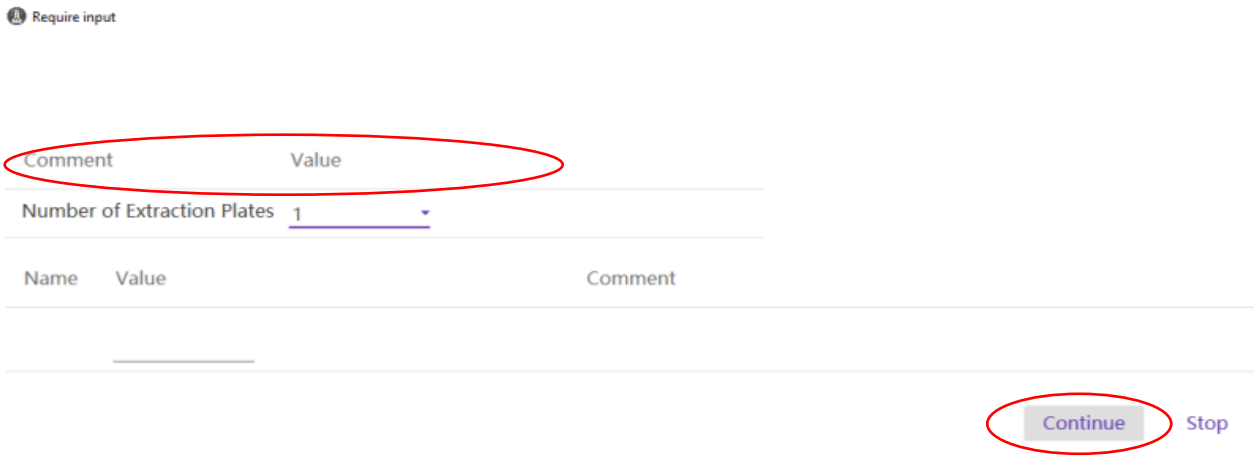
Note: Make sure not to spill onto the surface of the instrument.

7. Press **Start** button.

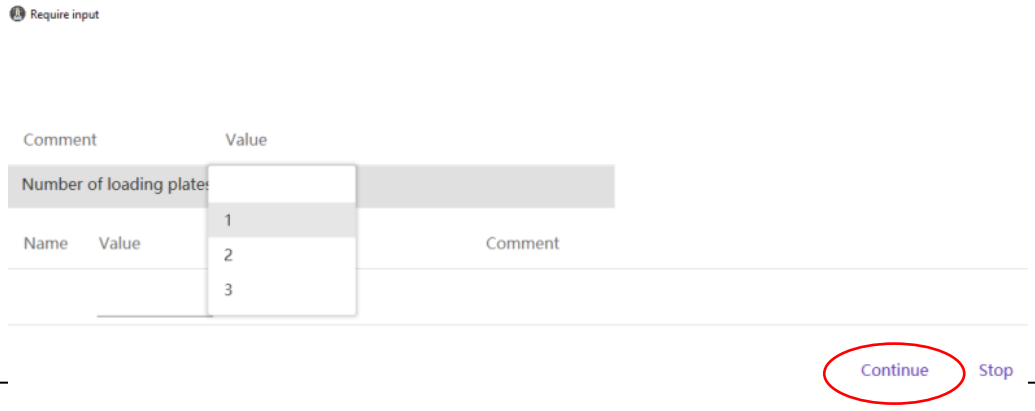


8. Choose number of extraction plate (s) according to the table below
   Click **Continue** button

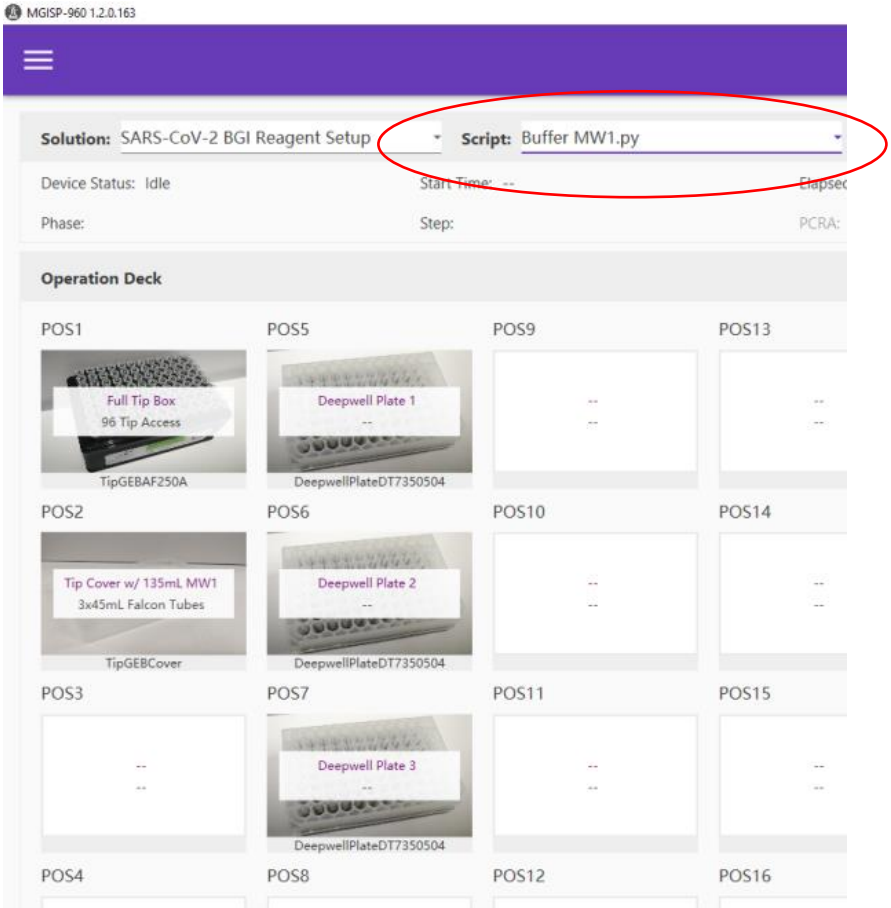
Number of the Extraction Plate(s)	Total number of samples (patient samples + controls)
1	≤96
2	>96 and ≤192



Click **Continue** button

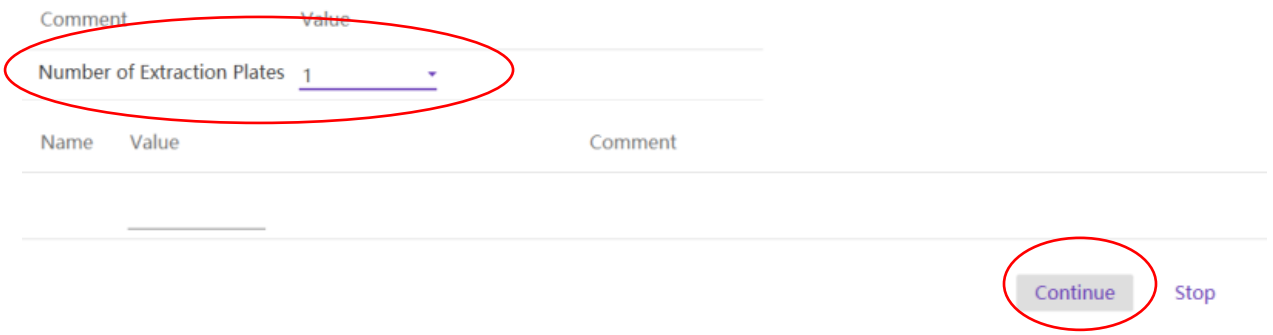


10. After RNase Free Water is dispensed, take out the unused water and pour it back into the original container.
11. Seal the deep well plates with RNase Free Water and leave them on the bench at room temperature.
12. Dispense the next reagent-MW1.
    Choose **Buffer MW1.py** from **Script** drop-down menu to dispense MW1



13. Choose number of extraction plate (s) according to the table below
    Click **Continue** button

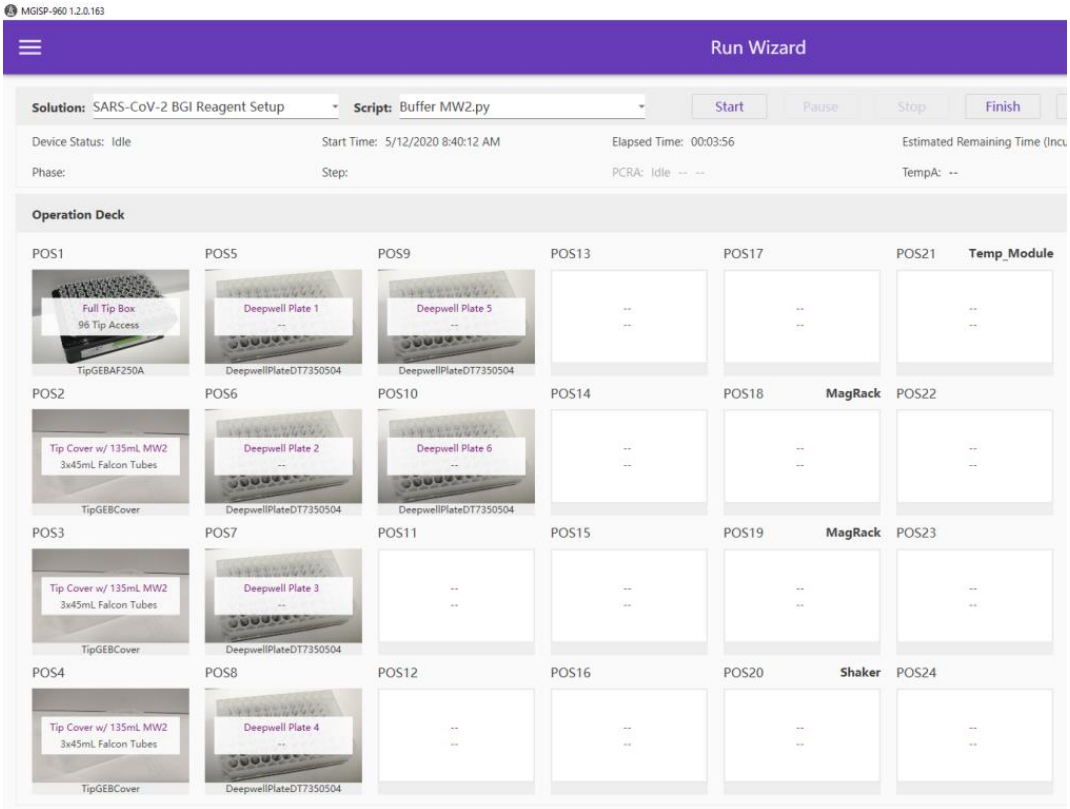
Number of the Extraction Plate(s)	Total number of samples (patient samples + controls)
1	≤96
2	>96 and ≤192



14. Choose number of loading plate(s) (from 1 to 3) for MW1.
    Click **Continue** button

15. Load tips, deep well plates and the tip lid according to the position on the screen.
16. Measure and transfer enough volume of MW1 according to the screen into the tip lid.
    Note: Make sure not to spill on the surface of the instrument.

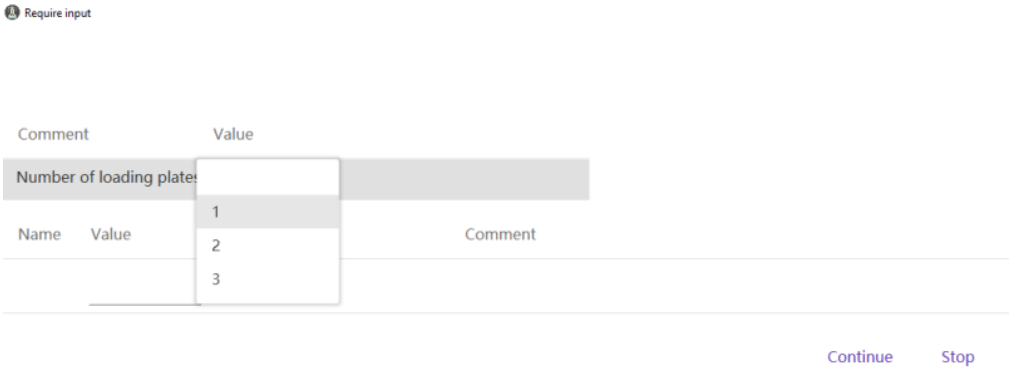
17. Label the deep well plates with MW1
18. Press **Start** button
19. After MW1 is dispensed, seal the deep well plates and leave them on the counter at Room temperature.
20. Pour the unused MW1 reagent back into the original container.
21. Dispense the next reagent MW2.



22. Choose number of extraction plate (s) according to the table below
    Click **Continue** button

Number of the Extraction Plate(s)	**Total number of samples (patient samples + controls)**
1	≤96
2	>96 and ≤192

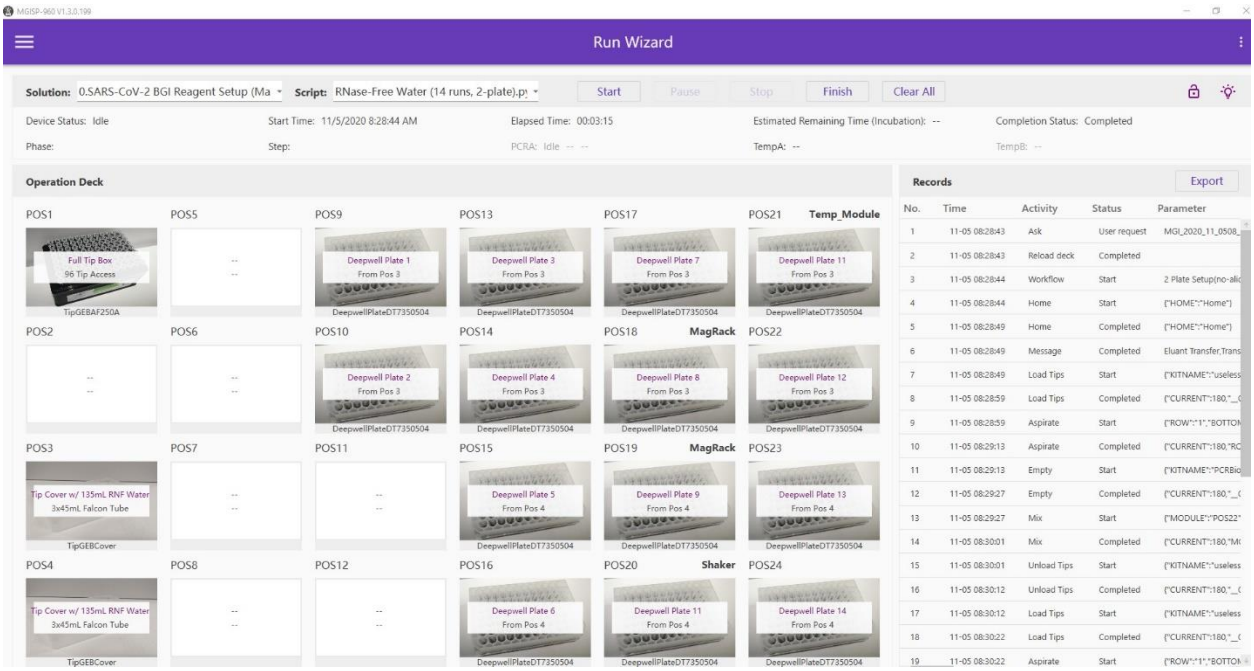
23. Select the number of loading plate.



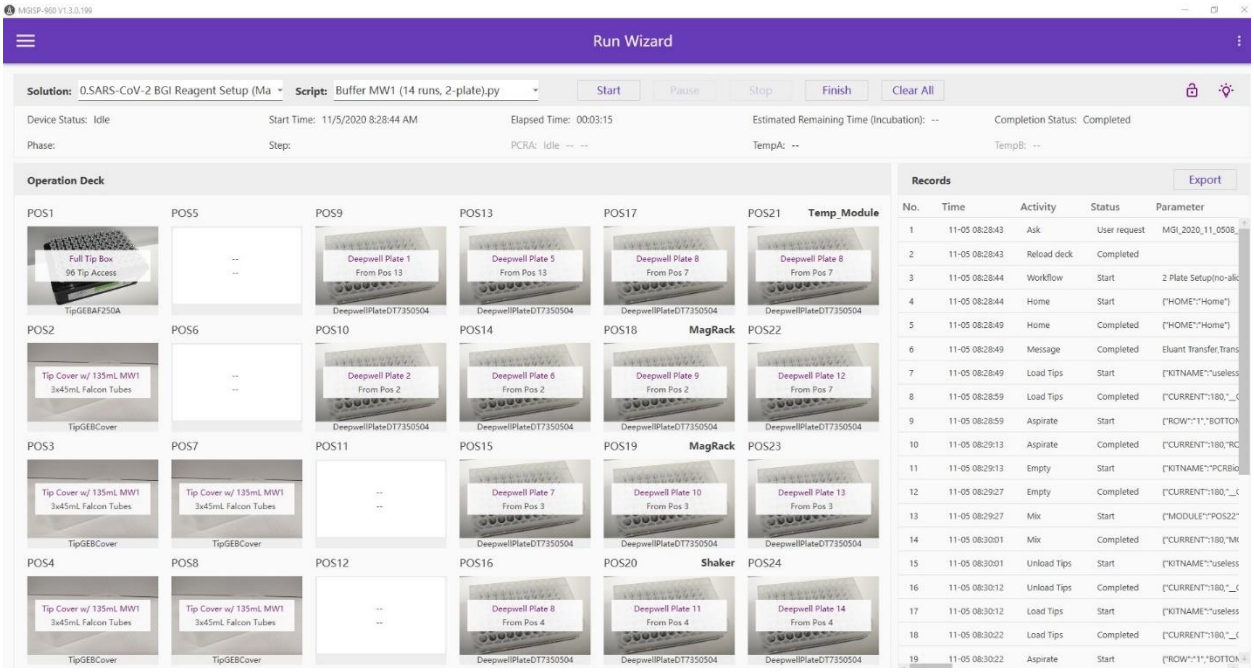
24. For extraction of 96 samples choose 1, for 2 sets of 96 samples choose 2.
25. Dispense the next reagent MW2 buffer.
    Set up tips, plates and tip lids according to the position on the screen. Label the deep well plates as MW2.
26. Measure the volume of the reagent according to the screen into the tip lid. Make sure not to spill on the surface of the instrument.
27. Press **Start**.
28. After the dispense seal the deep well plates and leave them on the counter at Room temperature.
29. Pour the unused reagent back into the original container.

Dispense reagents (massive procedure)

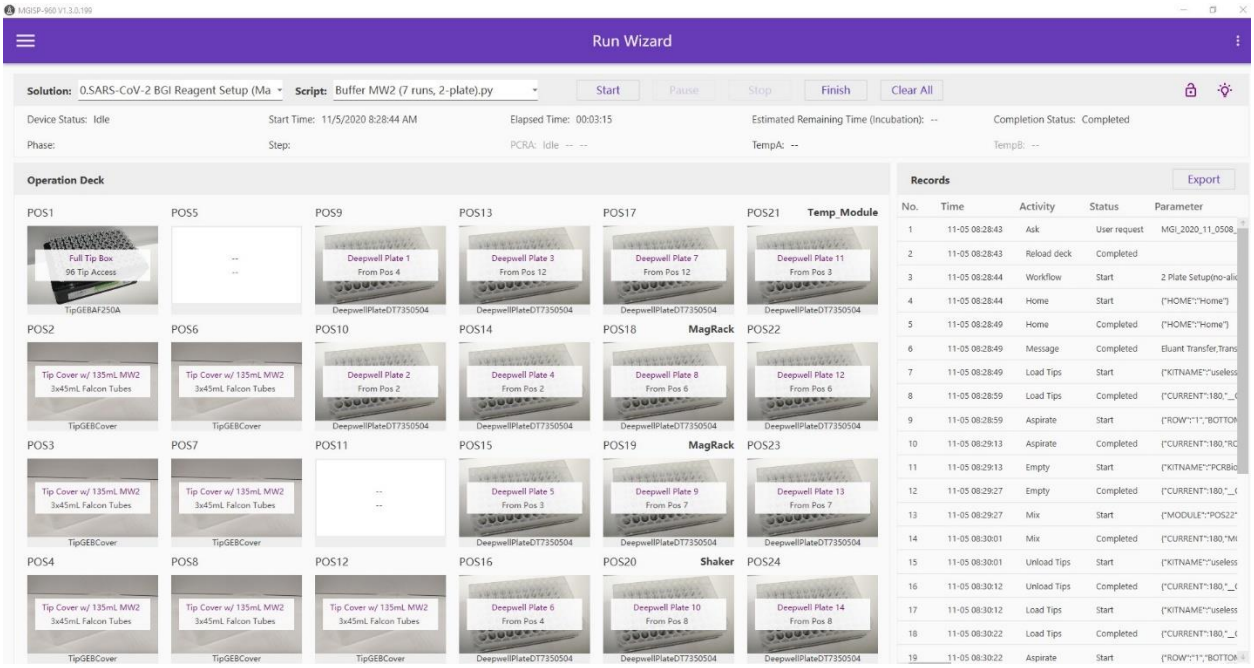
1. From the **Run Wizard** in the main menu, choose **0.SARS CoV2 BGI Reagent Setup (Massive)** in the **Solution** drop-down menu.
2. Choose **RNase Free Water (14 runs, 2-plate).py** from **Script** drop-down menu to dispense water.
3. Load tips, deep well plates and the tip covers according to the positions on the screen.
4. Measure and transfer 45ml RNase Free Water into the tip cover at position 3 and 4.
5. Press **Start** button to dispense RNase free water.
6. After RNase free water is dispensed, pour the unused RNase Free Water back into the original container.
7. Seal the deep well plates with RNase Free Water and leave them on the bench at room temperature.



8. Similarly, choose **buffer MW1 (14 runs, 2-plate).py** from **Script** drop-down menu to dispense MW1 buffer.



9. Similarly, choose **Buffer MW2 (14 runs, 2-plate).py** from **Script** drop-down menu to dispense MW2 buffer.



Off board preparation

1. Using the adjustable multichannel pipette, aliquot/pipet 200 µL of samples into a labeled deep well plate.
2. The negative control must be placed in the first well (A1) and the positive control in the last well (H8)

MGI # - Patient's samples from the space rack should be labeled in the same way as deep well plates.

e.g. MGI 1-1, MGI 1-2

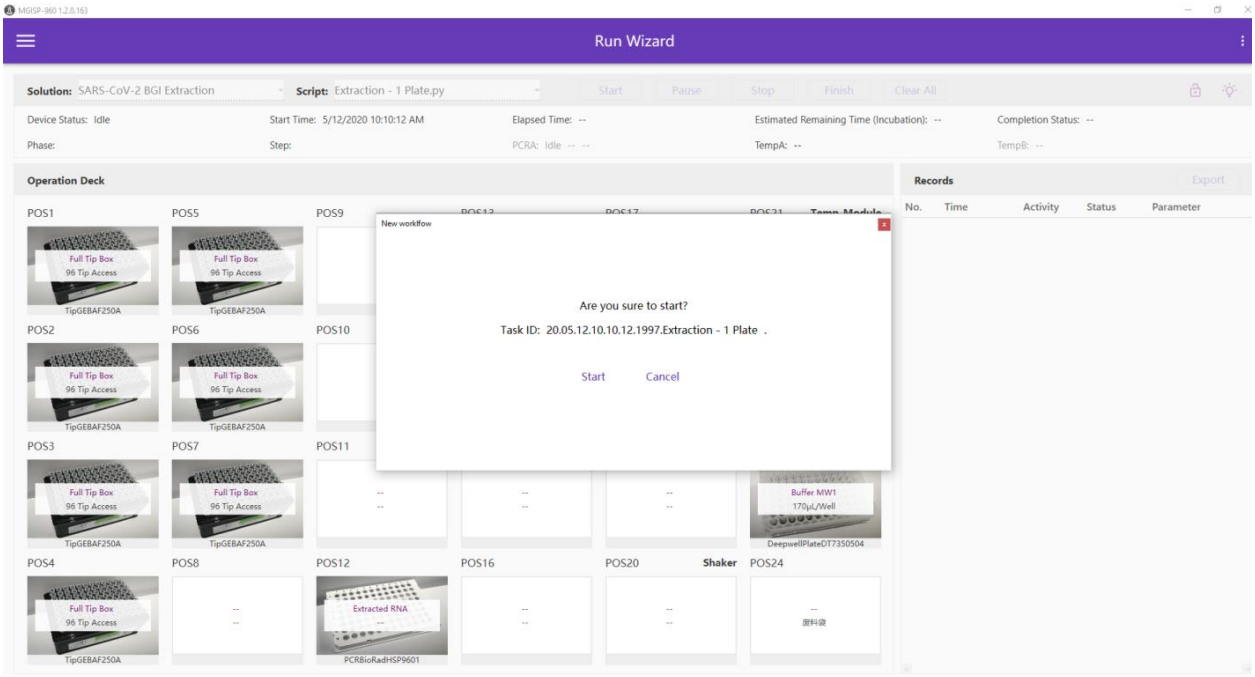
MGI 2-1, MGI 2-2

MGI 3-1, MGI 3-2

3. Uncap each column of samples one by one and set aside the caps. Adjust the pipette to draw the volume into the pipette. Adjust the pipette again and deliver samples into the deep well plate. Cap each tube and put them on a different space rack at the same locations as on the original sample space rack.
4. Put a gauze wet with disinfectant on the counter to catch any drips, also each time you pipetted a line touch the gauze to decontaminate your gloves. Repeat until one plate is done. Load the sample deep well plate into the MGISP according to the plate map. Do the second plate and load accordingly.
5. Make the following Lysis buffer mixture into two of 50 mL tube if doing 2 extractions of 96, only 1 if doing 1 set of 96.

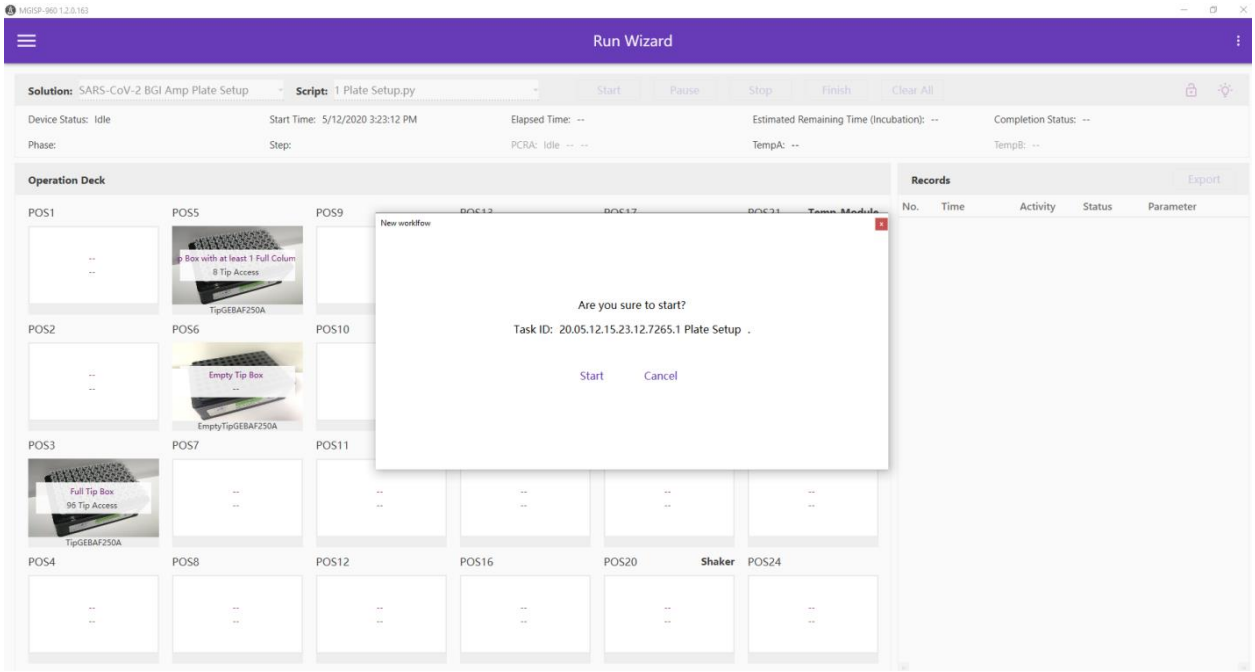
REAGENT	VOLUME (1 PLATE)	PER TEST
Buffer MLB	16,000 µL	160 µL
Absolute Alcohol	20,000 µL	200 µL
Proteinase K	1,500 µL	15 µL
Magnetic Beads	1,500 µL	15 µL
Enhancer	100 µL	1 µL

6. Pour into a reagent trough/ boat. Using a multichannel pipette dispense 360 µL into a clean deep well plate labeled as Lysis Buffer. Repeat until done. Load the lysis buffer plate according to the plate map. Repeat for another deep well plate if extracting 2 sets of 96 samples.
7. For SARS-CoV-2 Extraction, go to Script and choose **Extraction 1 plate.py** if doing 1 plate of 96. Choose **Extraction 2 plate.Py** if doing 2 plates of 96.
8. Check that all reagents/tips/ samples are loaded as the plate map. Label the Biorad PCR plate/s MGI machine#-Sample#
   e.g. MGI1-1, MGI1-2
9. Start
10. Make your worklist according to the sample position into the deep well plate.



11. After extraction clean the platform, bag the left over sample deep well plates and secure the PCR plate/s.
12. For SARS-CoV-2 Amplification set up: From the Script drop down menu choose **1 Plate setup.py** if doing 1 plate set up. Choose **2 plate setup.py** if doing 2 plate set up.
13. Prepare PCR Master mix using reaction mix and enzyme mix in the clean room.
    Option 1: pipette 264 µL of master mix into each well of the first column (A1-H1) of a deep well plate, MGI will dispense the master mix from well A1-H1 into a Biorad plate PCR plate on board.
    Option 2: Manually pipette 20 µL of master mix into the PCR plate as detailed in **PCR Set-up** section below.
14. Load the PCR end product, tips and Master mix into the MGI SP

15. Press **Start**.



16. Seal the plate and load it into the Biorad Thermocycler.
17. If there are NO Biorad thermocycler available for
    - <=30 mins: put your plate in the clean fridge/reagent fridge near Virology Seniors Room;
    - >30 mins: put your plate in the -20^{o}C freezer beside Virology Seniors Room
18. Leave the Worklist and a note (location of the plate) on the bench for the MLA and MLT to follow up.

PCR Set-up

In the Clean Room: (Change into dedicated clean room gown and gloves, work in Biosafety Cabinet)

Remove the required boxes of BGI Real time fluorescent RT-PCR kit for detecting 2019-nCoV (50 reactions), for examples, 2 boxes if doing one extraction plate and 4 boxes if doing 2 extraction plates.

Storage at >4^{0}C should not exceed 2 hours.

Prepare the number of Master Mix reactions required: Number of Test samples + 4 Controls + one extra test
Work quickly.
Mix gently. DO NOT VORTEX.
Make only enough master mix for the tests you are running.

Number of Reactions	**1**
2019-nCoV Reaction Mix	18.5
2019 nCoV Enzyme Mix	1.5
Volume of Master Mix	**20**
Sample/Control Volume	**10**

No. of Test reactions	**BGI nCoV PCR Mix**	
	2019-nCoV Reaction Mix (µL)	2019 nCoV Enzyme Mix (µL)
1	**18.5**	**1.5**
50 (48rxn)	**925**	**75**
100 (1 plate)	**1850**	**150**
200 (2 plates)	**3700**	**300**

<u>To be loaded manually</u>:

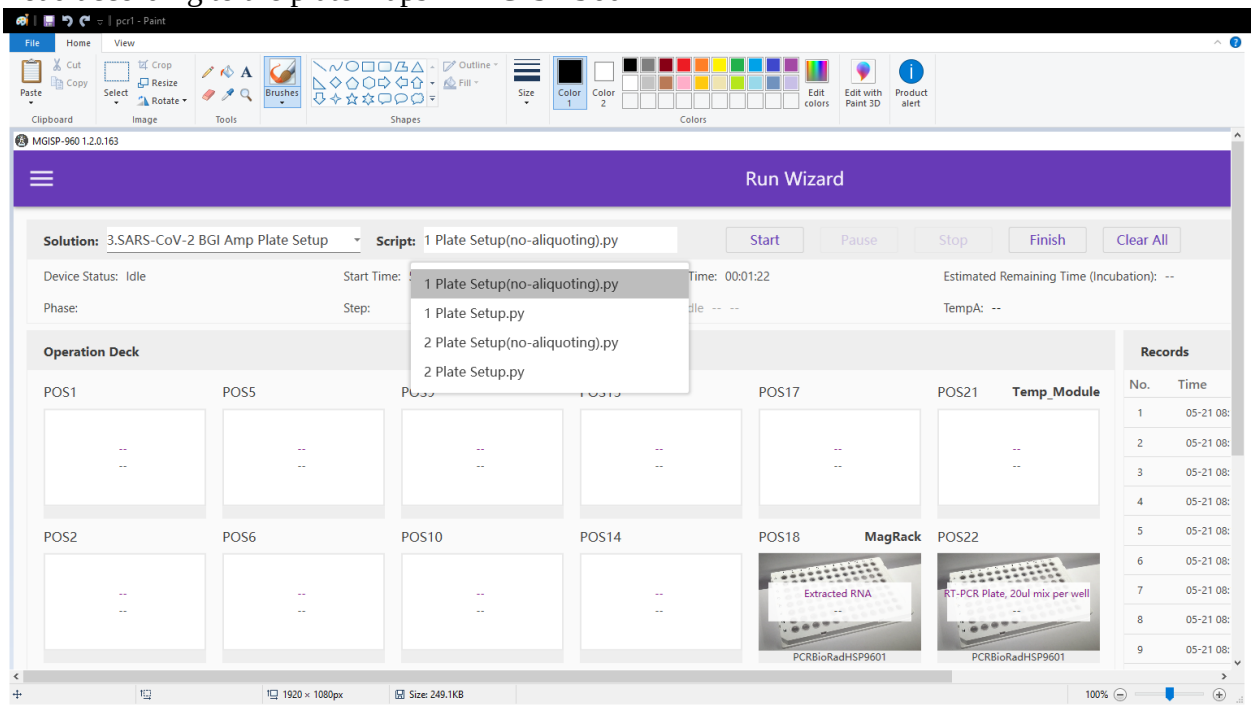
- Place 96-well plate onto pre-cooled block

- Pipette **20** µL prepared PCR Master Mix into each reaction well of the Biorad Hard Shell PCR plate using the multichannel pipette or repeater pipette.

On the MGI SP 960:

From the drop down menu

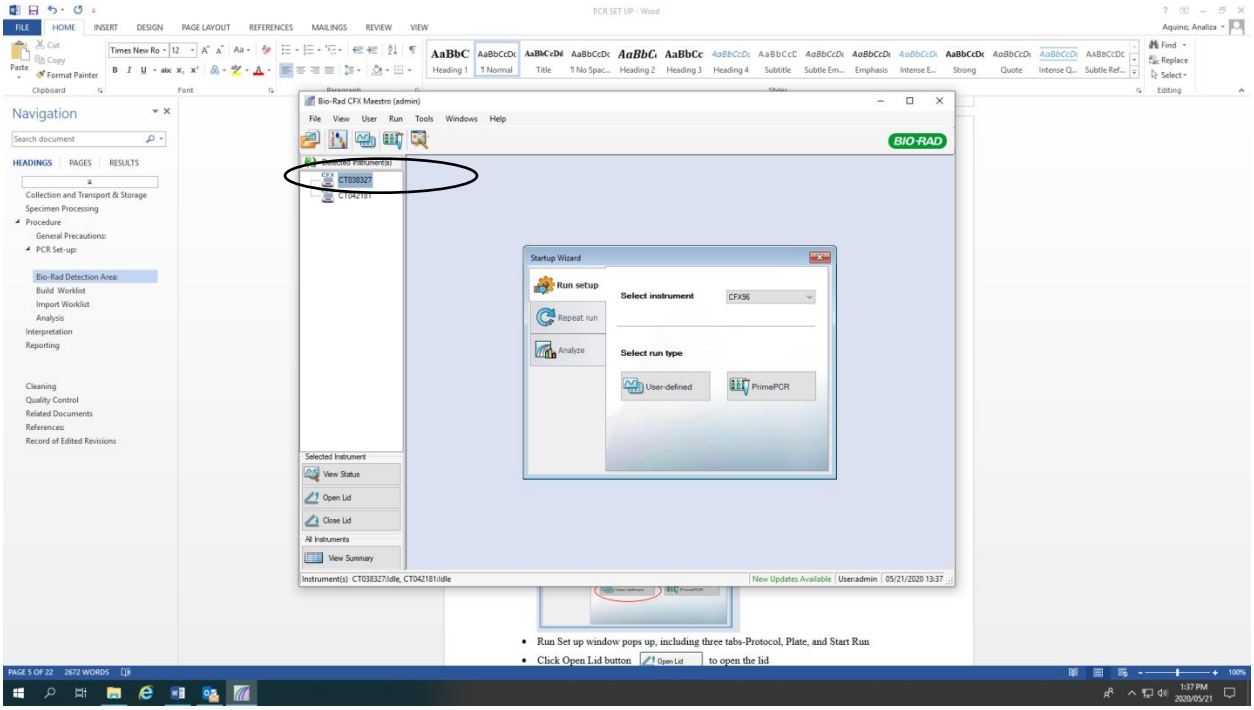
Solution: Choose **SARS-CoV-2 Amp Plate Set up**
Script: 1 plate setup (no aliquoting).py
If doing 1 extraction plate
From Script choose **2 plate setup (no aliquoting).py** if doing 2 extraction plates
Label your Mastermix according to run MGI number and run number.
Load according to the plate maps in MGISP 960



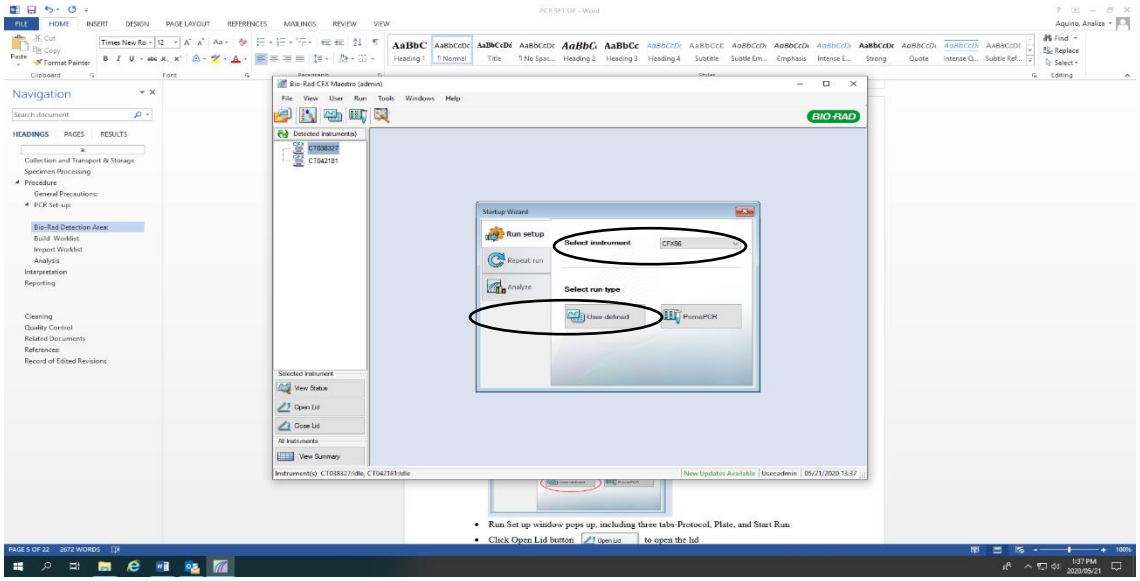
Bio-Rad Detection Area:

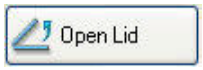
- Double click **Bio-Rad CFX Maestro** icon 

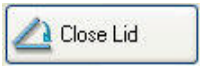
- Startup Wizard window pops up
- Make sure **CFX96 instrument** is selected



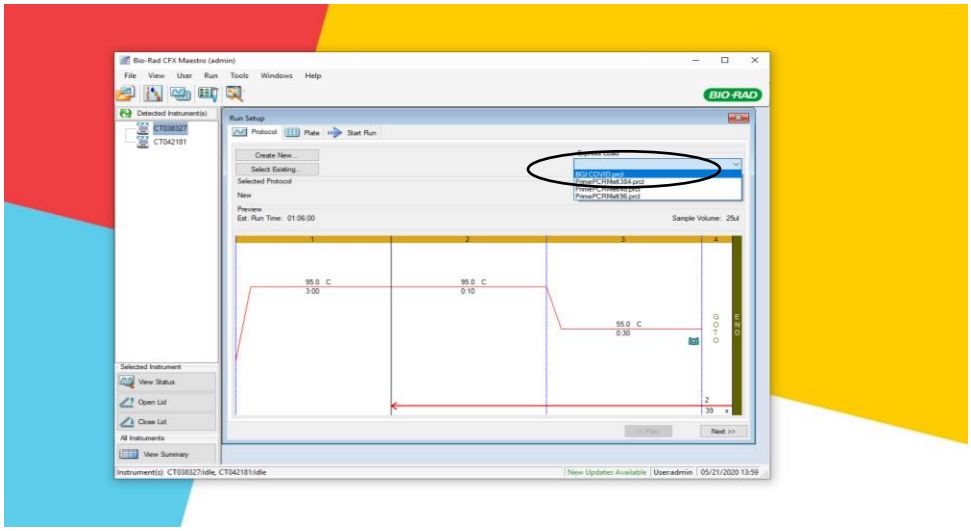
- Under Select run type, click User-defined button



- Run Set up window pops up, including three tabs-Protocol, Plate, and Start Run
- Click Open Lid button 

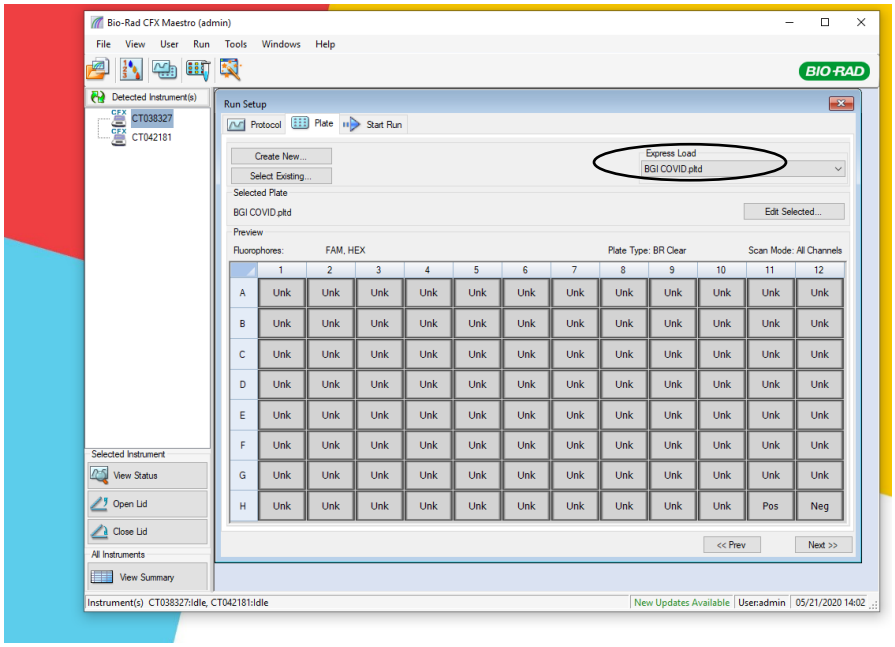
to open the lid
- Load sealed plate to the block
- Click Close Lid button 

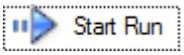
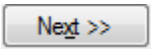
to close the lid
- WARNING! Do NOT manually close the motorized lid
- Under Protocol tab, select **BGI COVID.pcrd** from Express Load pull-down menu

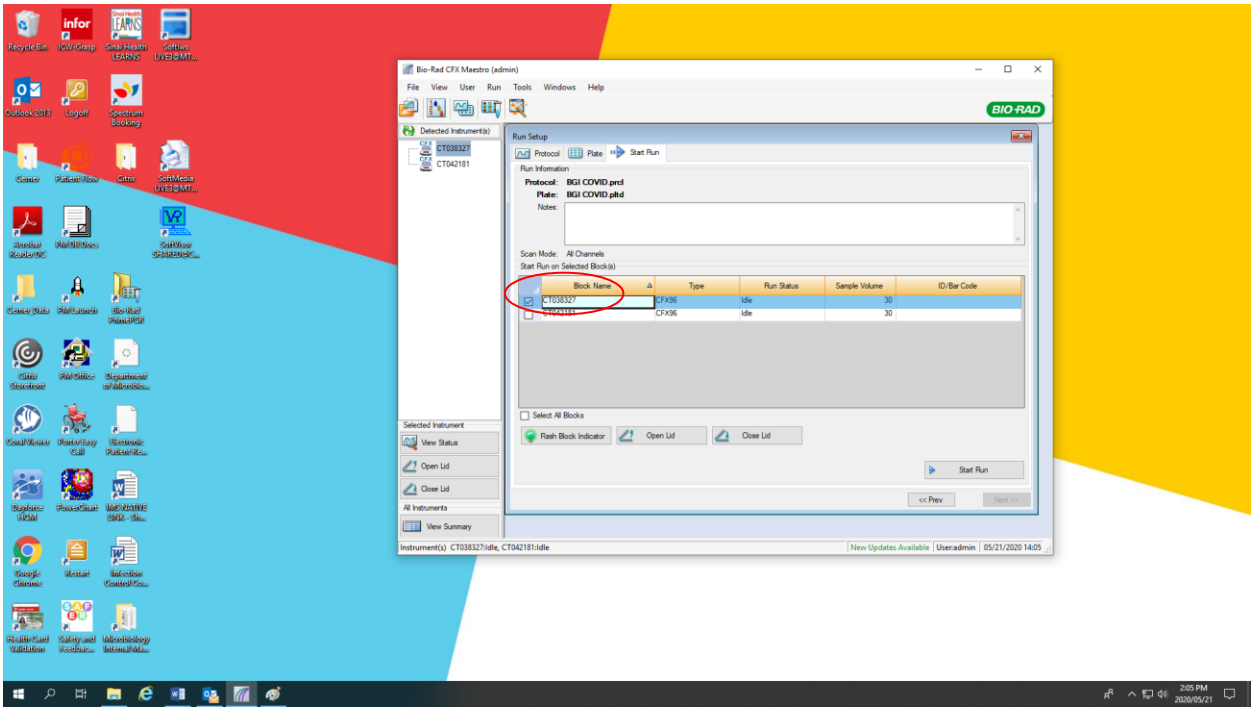


Click Plate tab | Plate | on the top or click Next button Next >> on the bottom right side to load the plate profile

- Select plate profile BGI COVID.pltd from the Express Load pull-down menu



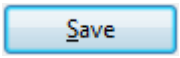
- Click Start Run tab  on the top or click Next button  on the bottom right side



- Confirm the following information:
  Protocol: **BGI COVID.pcrd**
  Plate: **BGI COVID.pltd**
  Scan Mode: **All Channels**
  CFX Thermocycler instrument number: checked on

- If any information above needs to be edited, click Prev button 
- If all information is correct, click Start Run button 
- Save Optical Data File [CT014845] window pops up
- Change "admin" to MGI 1-Run no.
- Save the file in the designated folder:

T:Microbiology>Virology>Bio Rad COVID MGI 1-1>Save Run>

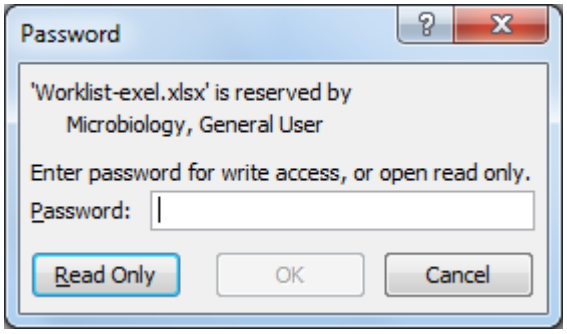
- Click Save button 

Build Worklist

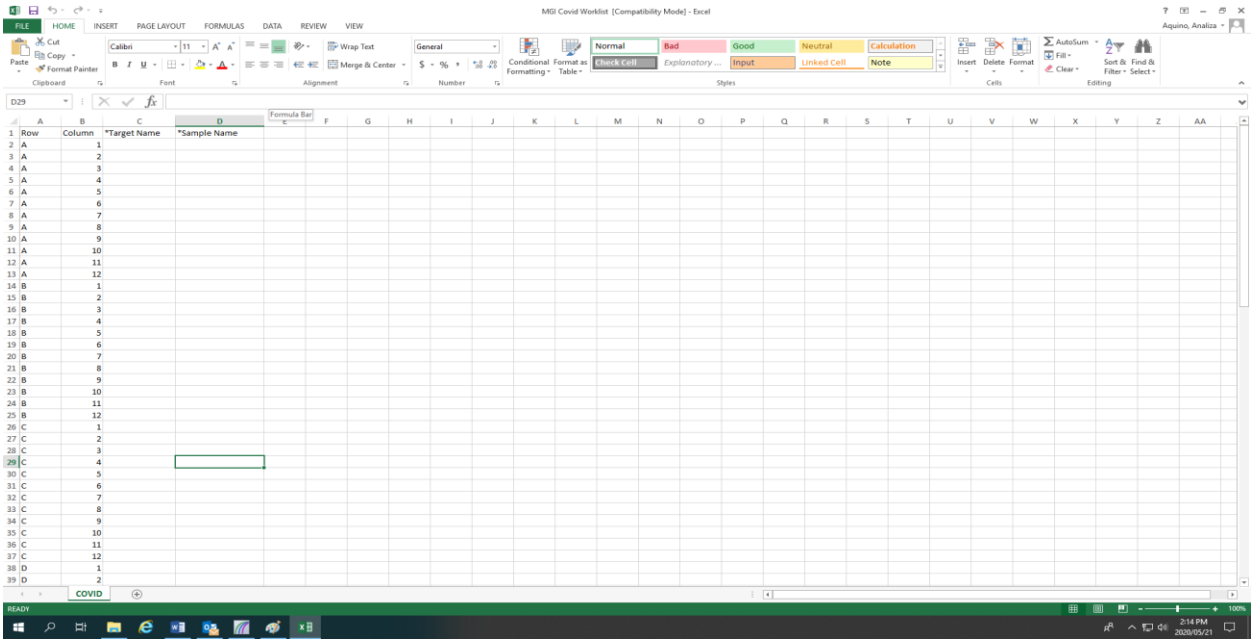
- Open the worklist file according to the following path:

T:Microbiology>Virology>Bio Rad CFX96 PCR>Worklist Master copy

- Password window pops up, click **Read only** button



- Scan the samples' information

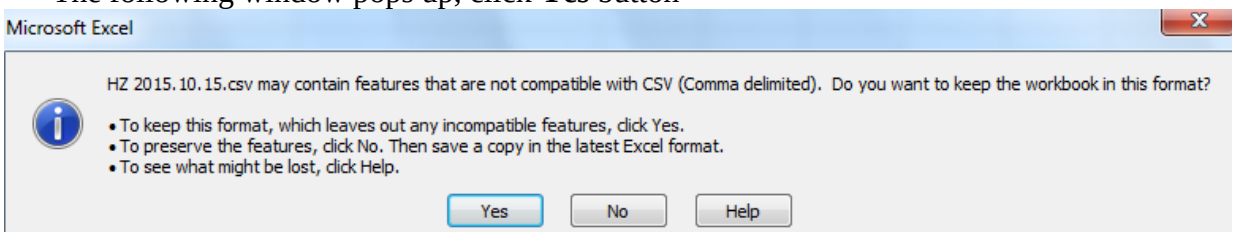


- **File > Save as CSV (Comma delimited) at**

T:Microbiology>Virology>Bio Rad MGI 1-1>Import Worklist

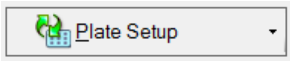
File name: MGI 1-1yyyy.mm.dd.run No. eg.MGI 1-1_2020.05.20.1
Save as type: **CSV** (Comma delimited)
Note: Worklist can only be imported as CSV type.

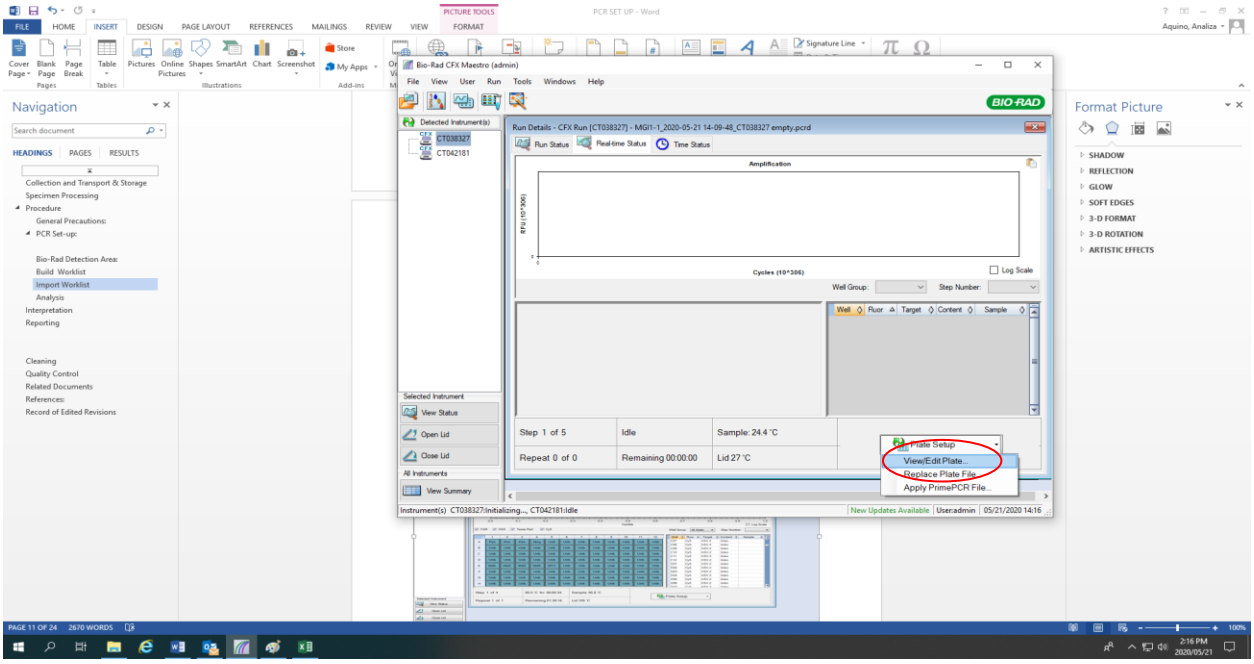
- Click **Save** button
- Click **OK**
- The following window pops up, click **Yes** button



- **Close** current excel file without saving.

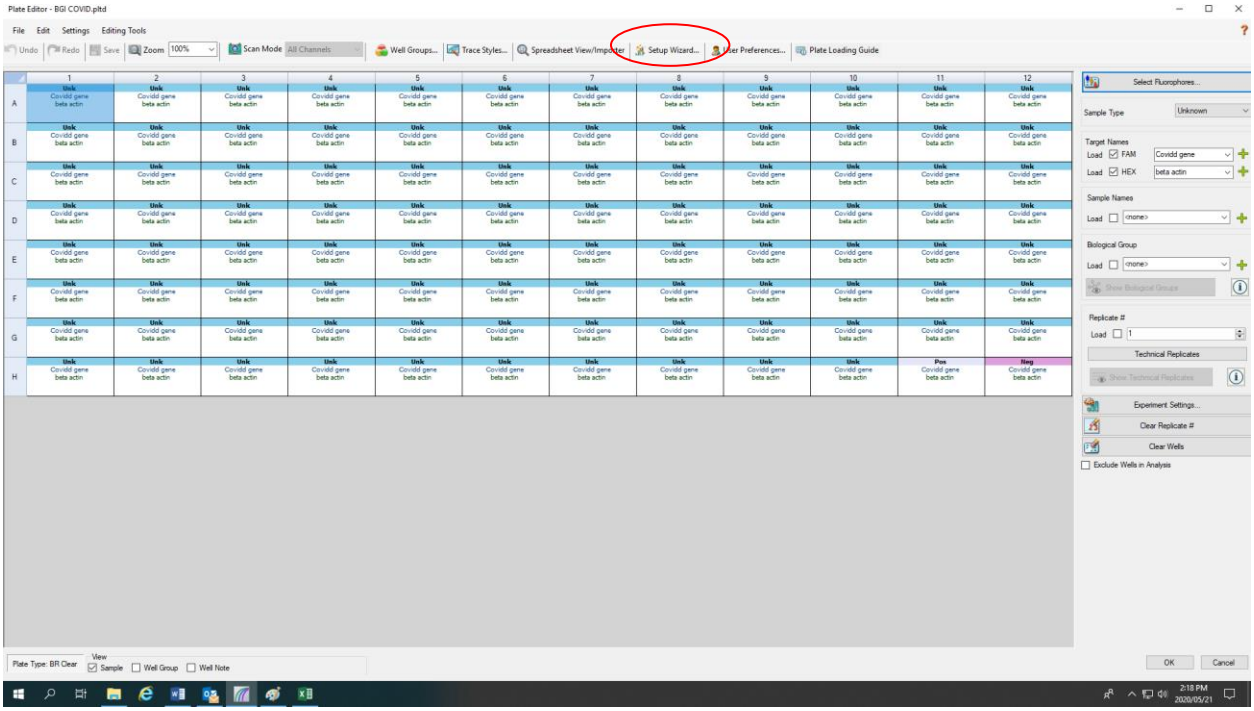
Import Worklist

- Click **Realtime Status** tab
- Select **View/Edit Plate…** from **Plate Setup** pull-down menu 



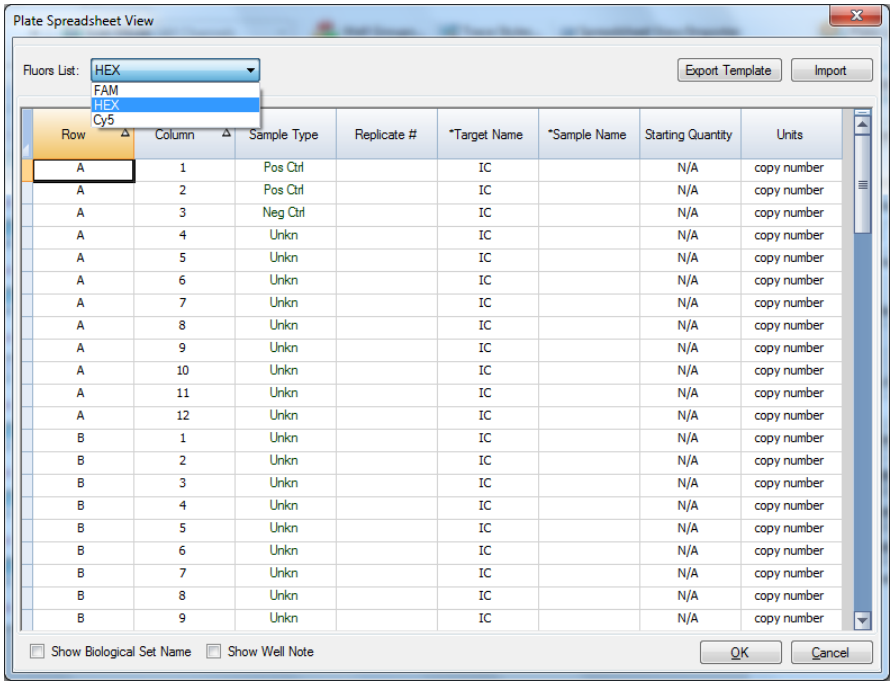
- Plate Editor window pops up

- Click **Spreadsheet View/Importer** button 



- Plate Spreadsheet View window pops up

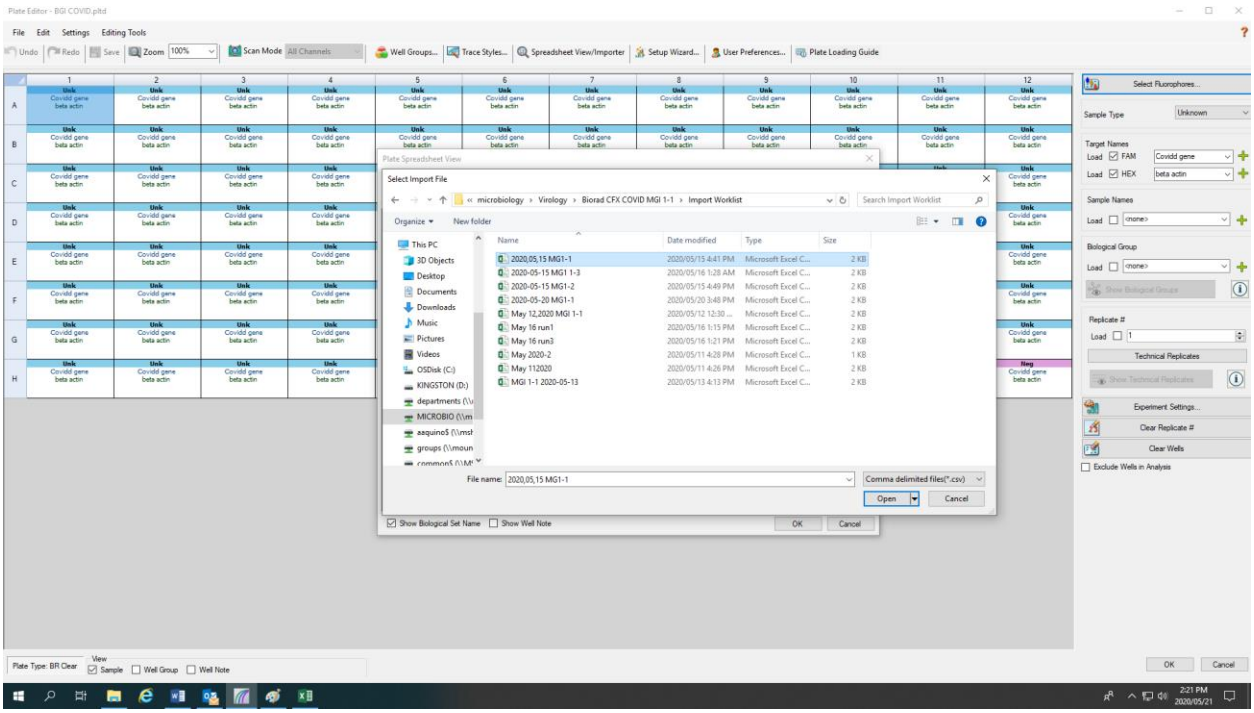
- Select **HEX** from **Fluors List** pull-down menu

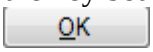


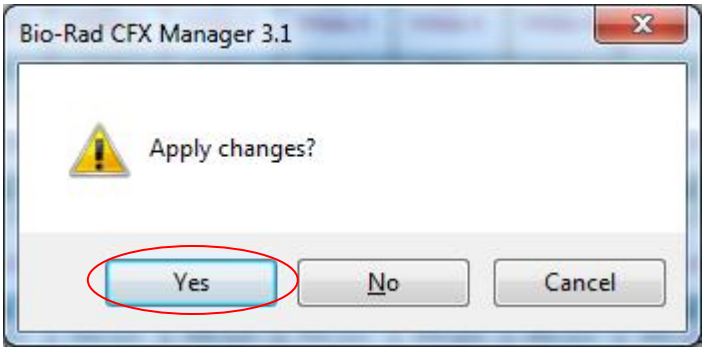
- Click **Import** button 

- **Select Import File** window pops up

T:Microbiology>Virology>Bio Rad MGI 1-1 yyy-mo-date>Import Worklist

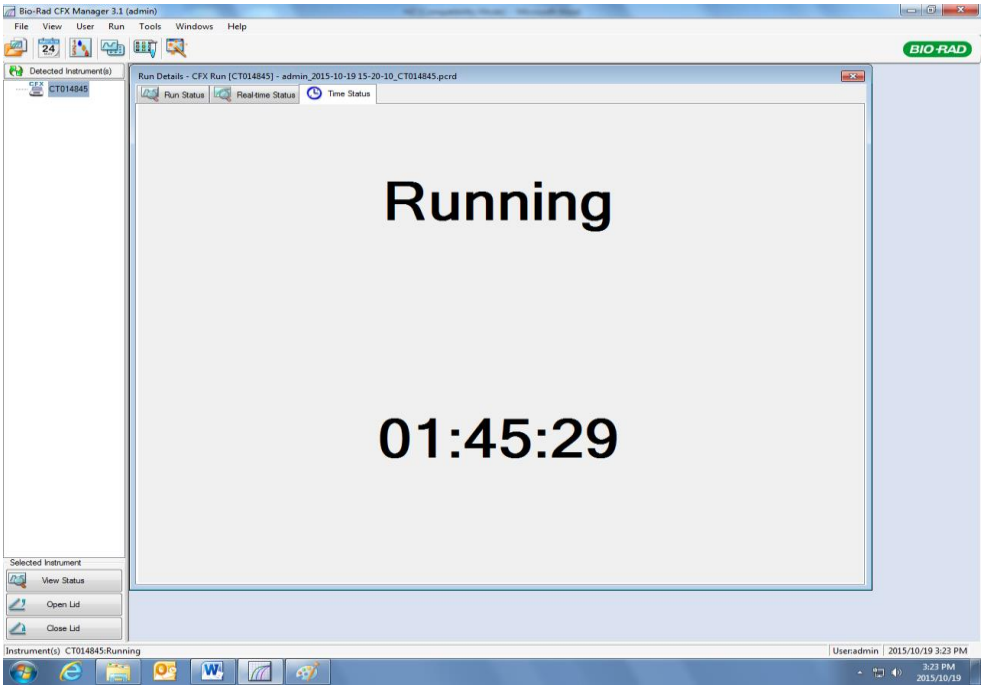


- Select appropriate worklist and click **Open,** all samples and controls information are imported
- Click **OK** button
- Select all wells by clicking top left side corner
- Type **beta actin** as target Name for **HEX channel**
- Hit **Enter key** on the key board
- Click **OK** button 
- Apply changes, click yes.



Click **Time Status** tab 

from the Run Details window



Analysis

After the PCR is completed:

- Change the channel name from **Fluorophore** to **Target**
- Mark **beta actin** box under the graph area with Covid gene unmarked, click any point to the left of all the beta actin curves, drag the cursor to select all the graphs above the threshold. Examine and mark on the worksheet any sample and control with no beta actin or Ct of beta actin greater than 35.

For ALL samples from any client on the BGI on a 96 well plate:

- IF there is a widespread range of beta-actin curves
- IF >5 are invalid

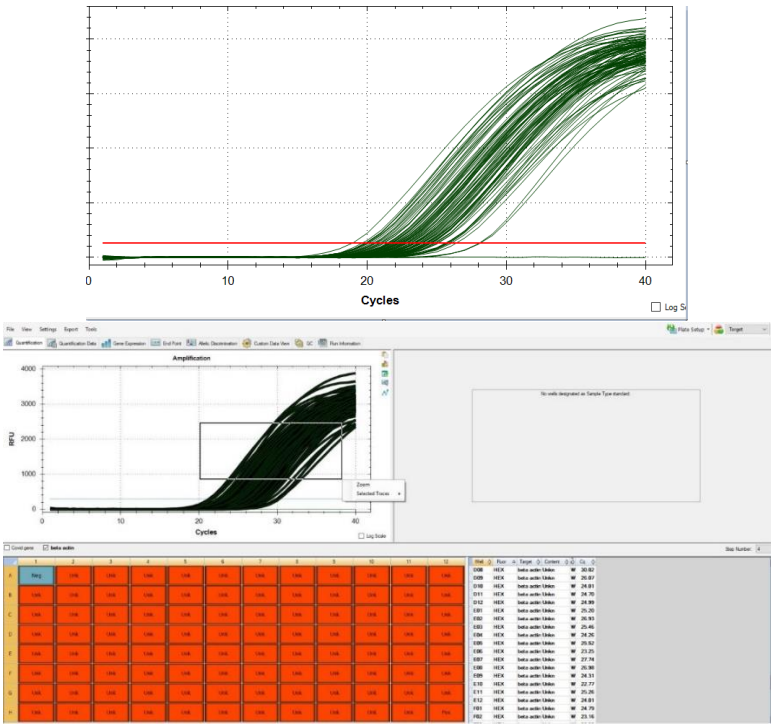
Then:

- Adjust threshold of **beta-actin** to **100 RFU** line.
- Repeat remaining invalids on Seegene

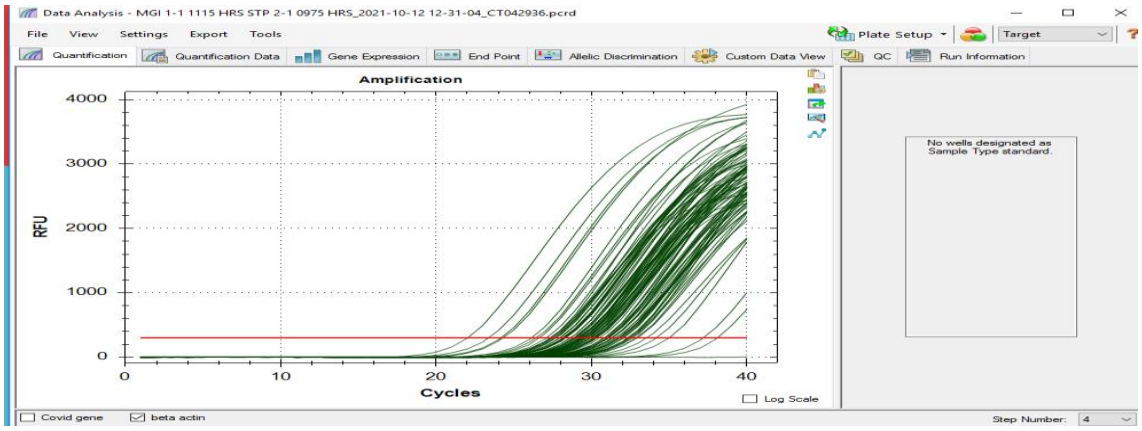
NOTE: **If there is NOT a widespread range of beta-actin curves and more than 5 invalids, DO NOT adjust curve, repeat invalids on Seegene.**

- ***Please consult microbiologists or/and seniors if you have any concerns about the adjustment of beta-actin threshold***

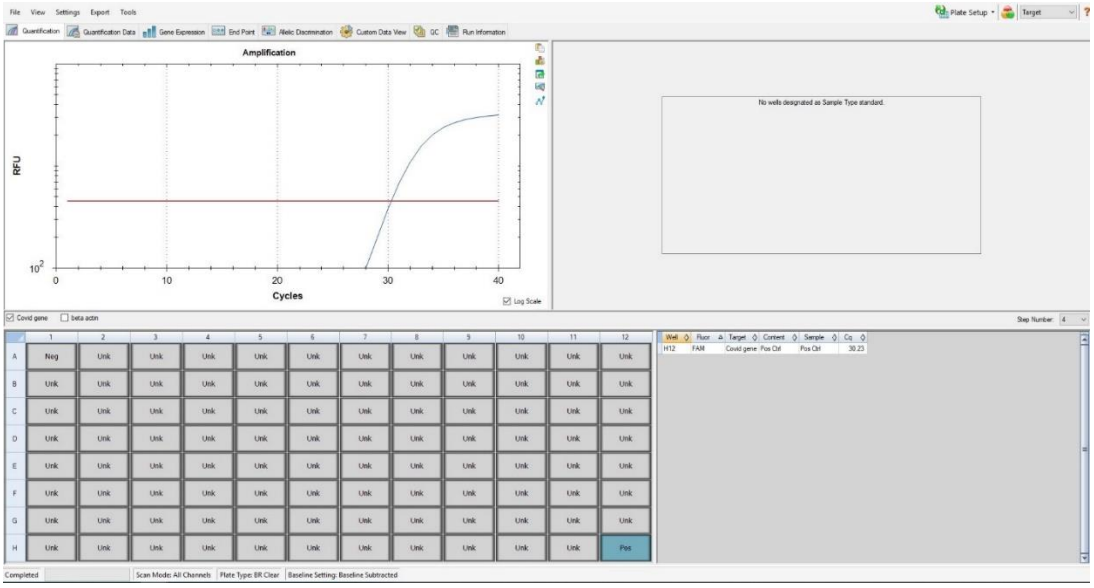
Example for a tight-spread range of beta-actin curves:



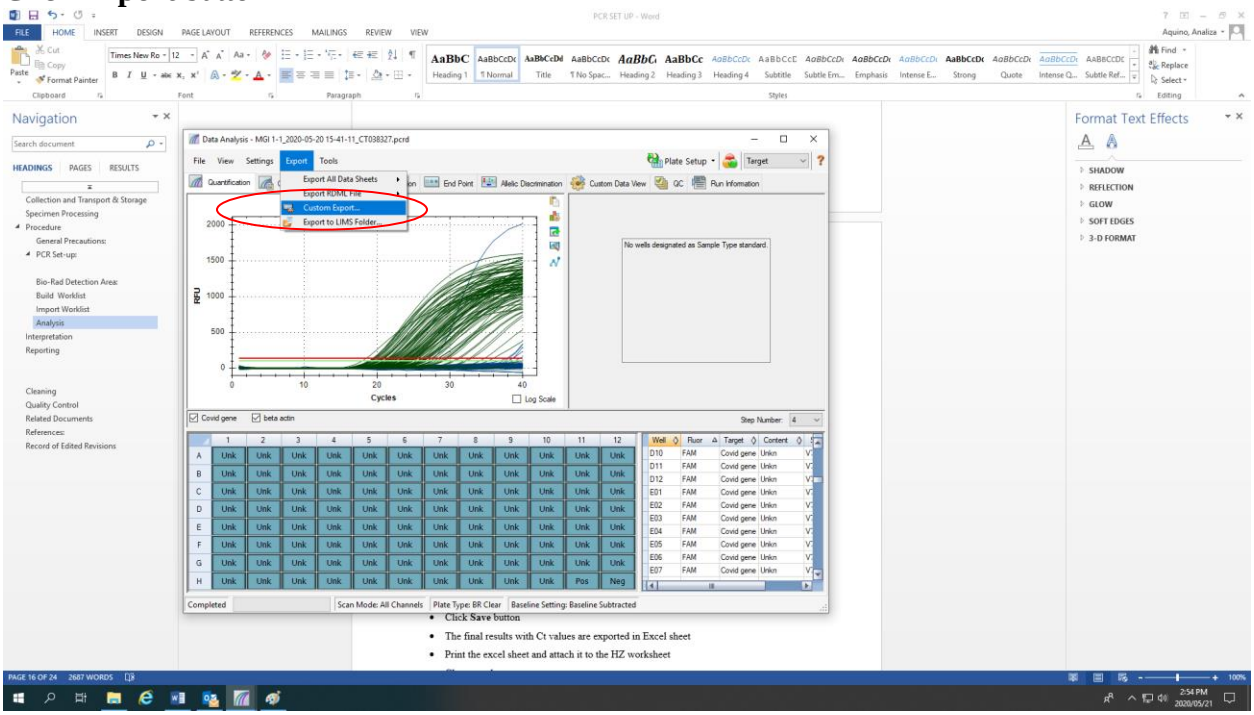
Example for a wide-spread of beta-actin curves:



- Mark **Covid gene** box and **log scale** box under the graph area with beta actin unmarked. Click positive control only, and drag the threshold line to the mid-point of the exponential amplification (linear portion of the sigmoidal amplification curve), and then unmark log scale and select all the samples. Examine and mark on the worksheet the result of all samples and controls for Covid gene.



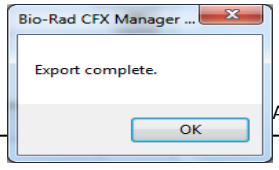
- **A positive PCR is observed if there is a rise or amplification in the Target channel e.g. FAM (Covid gene), & HEX (beta actin). The graph should be <u>exponential and sigmoidal</u> in shape. Conversely a negative PCR is a flat line or no signal.**
- Click **Export**
- Select **Custom Export…**
- Click **Export** button



- "Save as" Window pops up

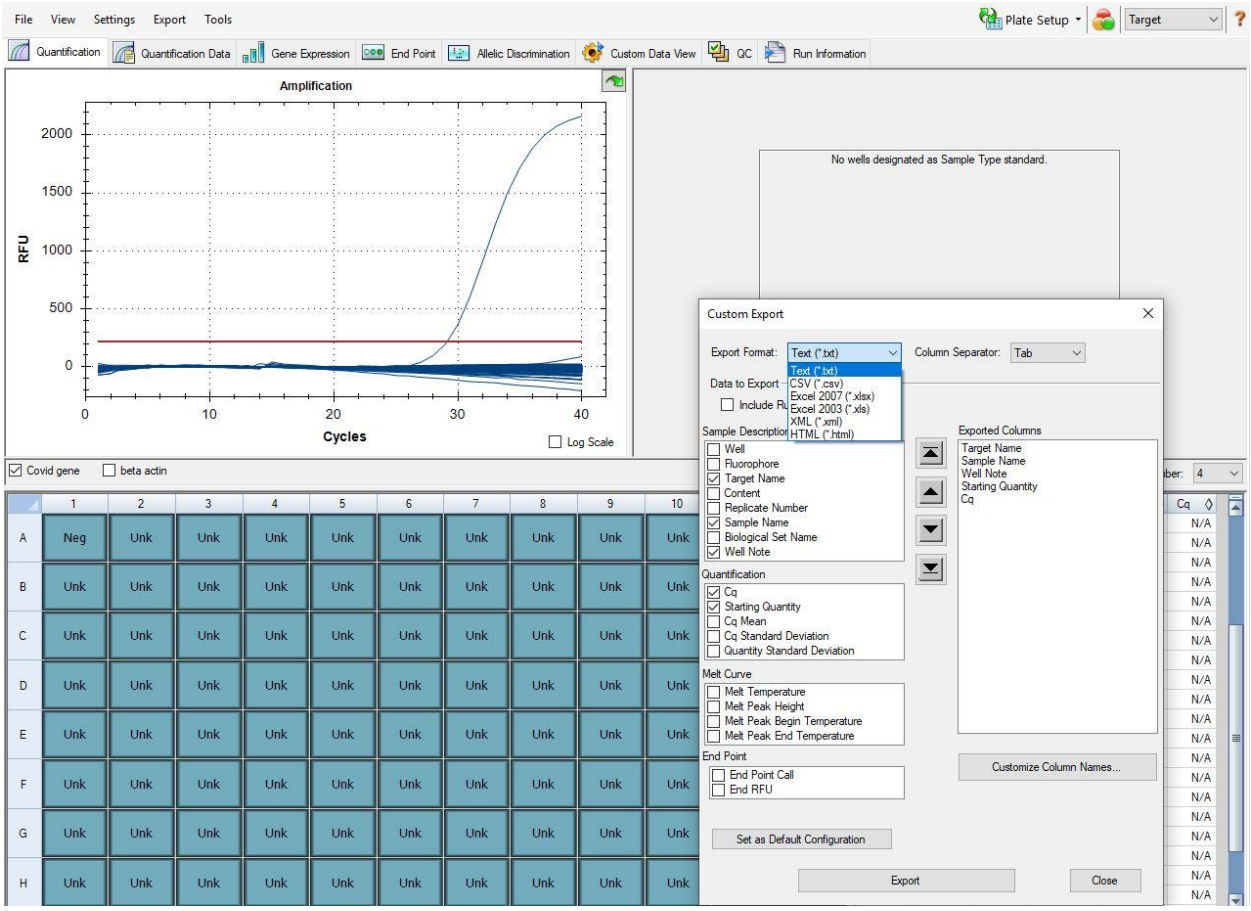
  T:\microbiology\Virology\Biorad CFX COVID MGI 1-1\COVID Excel results

- Click **Save** button
- The final results with Ct values are exported in Excel sheet
- Print the excel result sheet and attach it to the COVID Worksheet.
- Close excel.
- Click OK button.



- Click **Close** button.
- **LIS Interfacing**: exclude from analysis any sample that is not expected to cross over to LIS, e.g. low positive sample from Seegene repeated as negative in the current MGI run.
  Click **Export** > **Custom Export**
  Choose **Export Format** as **Text (*txt)**
  Click **Export** button and save the text file to T:\microbiology\Virology\MGI
  Close the text file that is automatically opened.

- Close Biorad CFX96 Maestro Real-Time System. **Do you want to save the changes to MGI 1-1yyyy.mm.dd CT038327.pcrd?**
- Press **Yes**.
- Shut down computer
- Shut down Biorad CFX 96 Thermocycler.


File View Settings Export Tools
Plate Setup
Target
Quantification
Quantification Data
Gene Expression
End Point
Allelic Discrimination
Custom Data View
QC
Run Information
Amplification
RFU
Cycles
Log Scale
Covid gene
beta actin
No wells designated as Sample Type standard.
Custom Export
Export Format:
Text (*.txt)
CSV (*.csv)
Excel 2007 (*.xlsx)
Excel 2003 (*.xls)
XML (*.xml)
HTML (*.html)
Column Separator:
Tab
Data to Export
Sample Description
Well
Fluorophore
Target Name
Content
Replicate Number
Sample Name
Biological Set Name
Well Note
Quantification
Cq
Starting Quantity
Cq Mean
Cq Standard Deviation
Quantity Standard Deviation
Melt Curve
Melt Temperature
Melt Peak Height
Melt Peak Begin Temperature
Melt Peak End Temperature
End Point
End Point Call
End RFU
Exported Columns
Target Name
Sample Name
Well Note
Starting Quantity
Cq
Customize Column Names...
Set as Default Configuration
Export
Close

Interpretation

Quality Control (QC) Samples

Assess QC samples for run validity prior to interpreting patient samples.

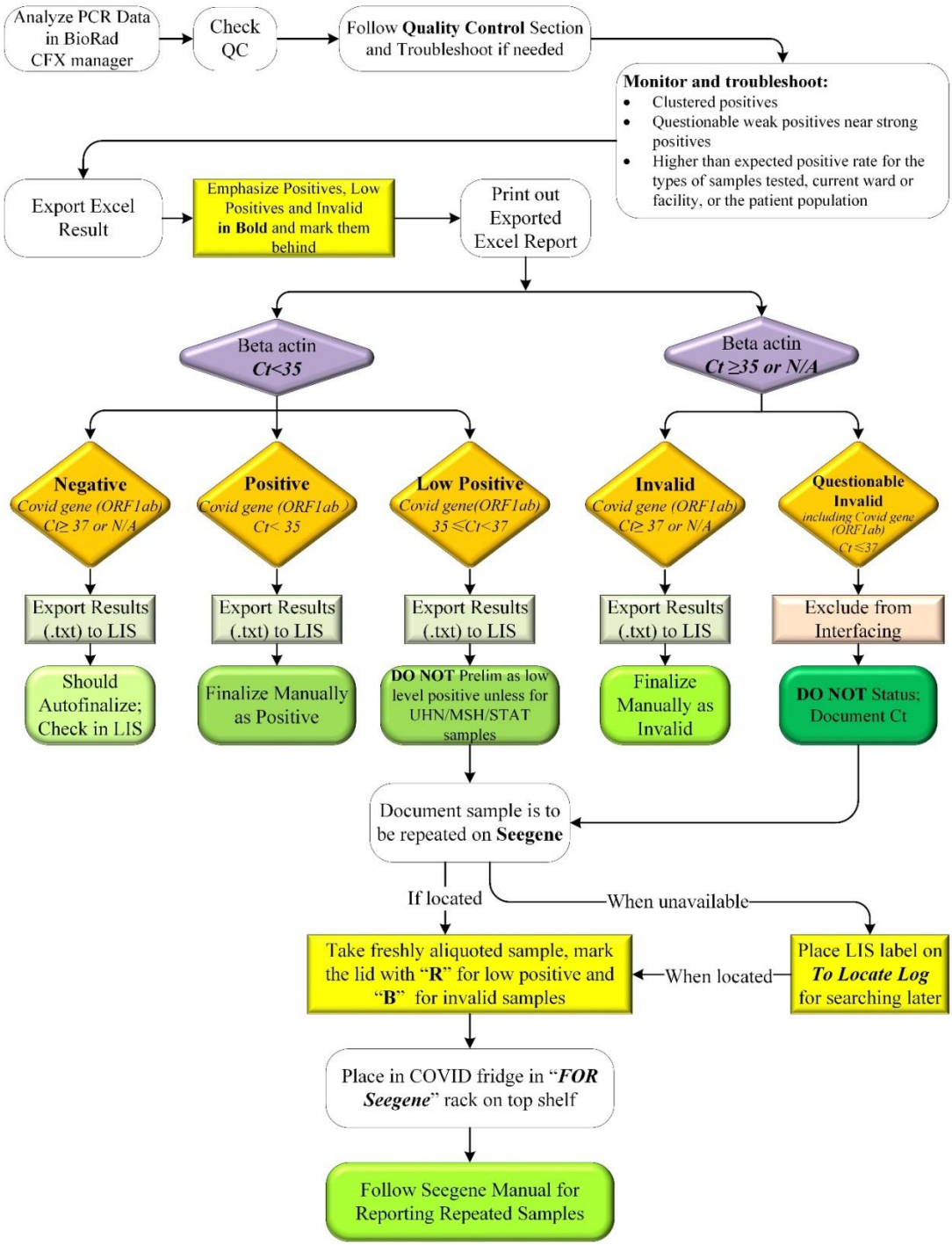
QC Sample	VIC channel	FAM channel	Interpretation
No template control	No amplification	No amplification	Pass – Proceed to sample analysis
Positive control	Sigmoidal amplification curve and Ct value is <35	Sigmoidal amplification curve and Ct value is <37	Pass – Proceed to sample analysis
No template control	Sigmoidal amplification curve and Ct value is <35	Sigmoidal amplification curve and Ct value is <37	Fail - Repeat all positive and "repeat" samples from the run and report the negatives as negative. ***Note: if curve is not sigmoidal, contact microbiologist-on-call for further instructions.***
Positive control	No amplification or Ct value is >35	No amplification or Ct value is >37	Fail - Do not report sample results. Repeat run

Test Samples

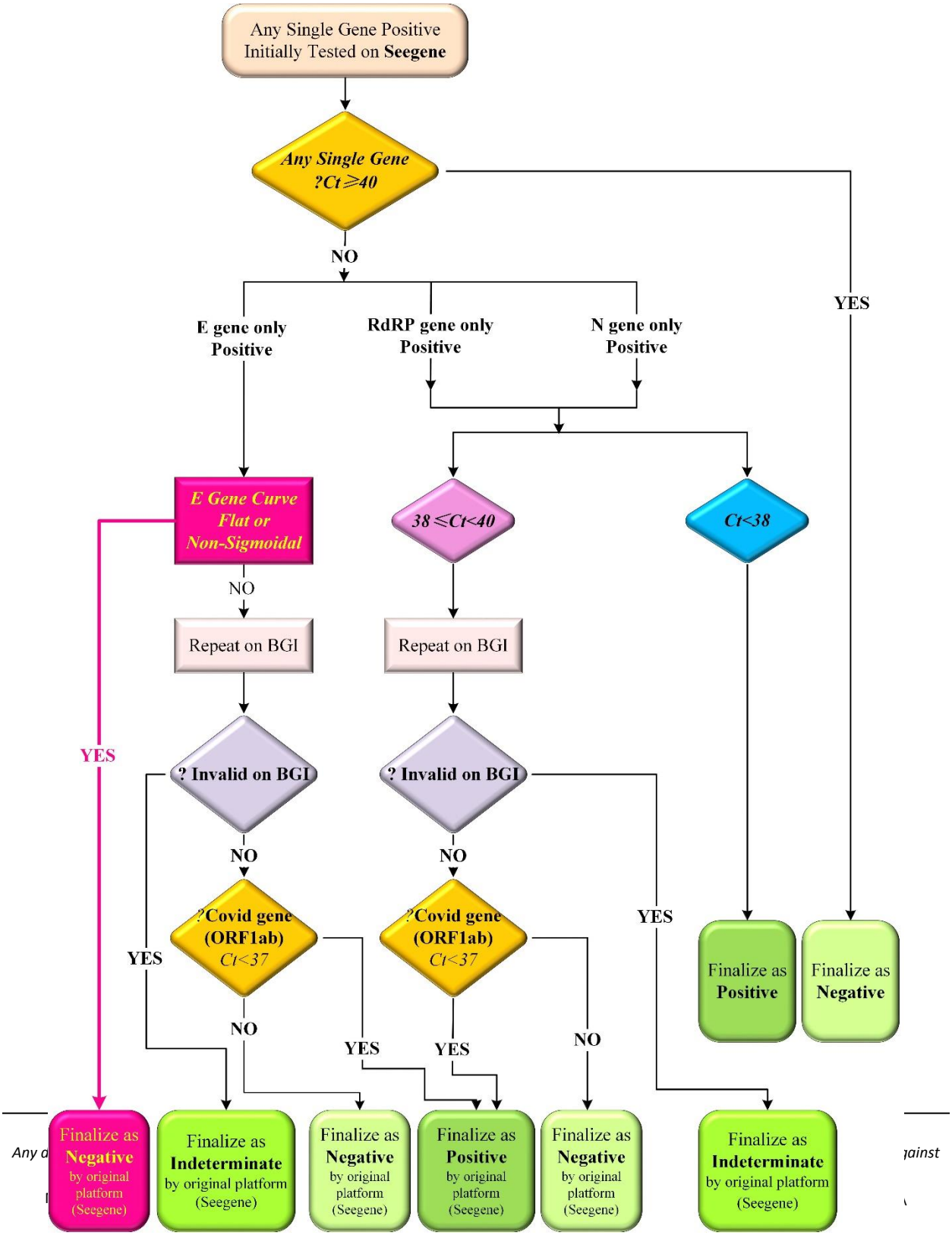
Note: Interpretations below are based on **Sigmoidal** amplification curves.

Sample	FAM (COVID gene-ORF1ab)	HEX (beta actin)	INTERPRETATION
A	Ct value ≥37	Ct value <35	SARS-CoV-2 RNA NOT Detected
B	Ct value <35	Ct value <35	SARS-CoV-2 RNA Detected
C	35.0≤Ct<37.0	Ct value <35	SARS-CoV-2 RNA Detected – Low level *Repeat on Seegene*
D	Ct value ≥37	Ct value ≥35	INVALID
E	Ct<37.0	Ct value ≥35	Questionable Invalid *Repeat on Seegene*
Repeat	Follow General BGI Reporting Process and BGI Reporting Process of Repeat Samples		FINAL RESULT for REPEAT of LOW LEVEL DETECTION

General BGI Reporting Process


Analyze PCR Data in BioRad CFX manager
Check QC
Follow Quality Control Section and Troubleshoot if needed
Monitor and troubleshoot:
Clustered positives
Questionable weak positives near strong positives
Higher than expected positive rate for the types of samples tested, current ward or facility, or the patient population
Export Excel Result
Emphasize Positives, Low Positives and Invalid in Bold and mark them behind
Print out Exported Excel Report
Beta actin Ct<35
Beta actin Ct ≥35 or N/A
Negative Covid gene (ORF1ab) Ct≥ 37 or N/A
Positive Covid gene (ORF1ab) Ct< 35
Low Positive Covid gene(ORF1ab) 35 ≤Ct<37
Invalid Covid gene (ORF1ab) Ct≥ 37 or N/A
Questionable Invalid including Covid gene (ORF1ab) Ct ≤37
Export Results (.txt) to LIS
Export Results (.txt) to LIS
Export Results (.txt) to LIS
Export Results (.txt) to LIS
Exclude from Interfacing
Should Autofinalize; Check in LIS
Finalize Manually as Positive
DO NOT Prelim as low level positive unless for UHN/MSH/STAT samples
Finalize Manually as Invalid
DO NOT Status; Document Ct
Document sample is to be repeated on Seegene
If located
When unavailable
Take freshly aliquoted sample, mark the lid with "R" for low positive and "B" for invalid samples
When located
Place LIS label on To Locate Log for searching later
Place in COVID fridge in "FOR Seegene" rack on top shelf
Follow Seegene Manual for Reporting Repeated Samples

BGI Reporting Process of Repeat Samples


Any Single Gene Positive Initially Tested on Seegene
Any Single Gene ?Ct ⩾40
YES
NO
E gene only Positive
RdRP gene only Positive
N gene only Positive
E Gene Curve Flat or Non-Sigmoidal
38 ⩽Ct<40
Ct<38
NO
Repeat on BGI
Repeat on BGI
YES
? Invalid on BGI
? Invalid on BGI
NO
NO
?Covid gene (ORF1ab) Ct<37
?Covid gene (ORF1ab) Ct<37
YES
YES
YES
NO
NO
YES
YES
Finalize as Positive
Finalize as Negative
Finalize as Negative by original platform (Seegene)
Finalize as Indeterminate by original platform (Seegene)
Finalize as Negative by original platform (Seegene)
Finalize as Positive by original platform (Seegene)
Finalize as Negative by original platform (Seegene)
Finalize as Indeterminate by original platform (Seegene)

Reporting

1. **PRELIMINARY POSITIVE RESULTS** (for **UHN/MSH/STAT** patients only**)**

- Result in **the isolate window** as **COVID-19 virus**
- In the Isolate comment, choose **\DPCR** & **\indM (Low Prelim MGI)**
- Status as **PRELIMINARY**
- Inform all positive results according to **Isolate Notification and Freezing Table**

Example:

COVID-19 virus
DETECTED by real-time PCR.
* * * * * * * * * * * * * * * * * * * *
(Low level detection)
Sample is being retested. Further report to follow.
Testing performed using the BGI Real-Time Fluorescent
RT-PCR 2019-nCoV Assay.
NOTE: The BGI Real-Time Fluorescent RT-PCR 2019-nCoV Assay
has been approved by Health Canada for Emergency Use Access
(EUA) and has been verified by the University Health
Network/Sinai Health Microbiology Laboratory

2. FINAL RESULTS

2.1 NEGATIVE RESULTS

- Result in the test window using COVID-19 Virus Not Detected test comment
- Choose from the keypad: }-NCB (Neg BGI)
- Status as FINAL
- When BGI .txt file for a run is exported to LIS, all negative samples should auto-finalize.

Example:

COVID-19 virus NOT detected by real-time PCR.
* *
Testing performed using the BGI Real-Time Fluorescent
RT-PCR 2019-nCoV Assay.
NOTE: The BGI Real-Time Fluorescent RT-PCR 2019-nCoV Assay
has been approved by Health Canada for Emergency Use Access
(EUA) and has been verified by the University Health
Network/Sinai Health Microbiology Laboratory.

2.2 POSITIVE RESULTS

- Result in **the isolate window** as **COVID-19 virus**
- In the Isolate comment, choose **\DPCR** & **\CVB+ (BGI)**
- Status as **FINAL**
- Inform all positive results according to **Isolate Notification and Freezing Table**

Example:

COVID-19 virus

DETECTED by real-time PCR

* *

Testing performed using the BGI Real-Time Fluorescent RT-PCR 2019-nCoV Assay.
NOTE: The BGI Real-Time Fluorescent RT-PCR 2019-nCoV Assay has been approved by Health Canada for Emergency Use Access (EUA) and has been verified by the University Health Network/Sinai Health Microbiology Laboratory.

2.3 INVALID RESULTS

- Result in the test window using COVID-19 virus PCR test unable to be completed test comment
- Choose from the keypad: }**INCB (BGI INV)**
- Status as **FINAL**
- Inform all invalid results according to **Isolate Notification and Freezing Table**

Example:

COVID-19 virus PCR test unable to be completed.
* * * * * * * * * * * * * * * * * * * *
No gene targets were detected including the human beta-actin gene suggesting inhibition of the BGI Real-Time Fluorescent RT-PCR 2019-nCoV Assay PCR reaction or inadequate sampling. Please submit another sample for testing if clinically indicated.
NOTE: The BGI Real-Time Fluorescent RT-PCR 2019-nCoV Assay has been approved by Health Canada for Emergency Use Access (EUA) and has been verified by the University Health Network/Sinai Health Microbiology Laboratory.

3. **FINAL RESULT for REPEAT of LOW LEVEL DETECTION**

   Follow BGI Reporting Process for Repeat Samples

3.1 CONFIRMED POSITIVE RESUTLS

Follow FINAL RESULTS for POSITIVE reporting procedure.

- Finalize as COVID-19 virus Detected instead of "Low level detection confirmed".

3.2 INDETERMINATE RESUTLS

- **Suppress** COVID-19 virus isolate
- Choose from the keypad: }**INDC (Indeterm)**
- Status as **FINAL**
- Inform all invalid results to the ward /clinic according to **Isolate Notification and Freezing Table**

Example:

INDETERMINATE for COVID-19 virus.
* *
Results should be interpreted within the context of the
clinical signs, symptoms, and history of the patient.
INDETERMINATE result may indicate the presence of low
levels of virus, non-specific reactivity of the assay,
or other unrecognized factors. Please submit a follow-up
sample if clinically indicated.
Testing performed using the Seegene Allplex 2019-nCoV Assay.
NOTE: The Seegene Allplex 2019-nCoV Assay has been approved
by Health Canada for Emergency Use Access (EUA) and has
been verified by the University Health Network/Sinai
Health Microbiology Laboratory
* *

3.3 NSQ to Repeat RESUTLS

- Initially COVID-19 virus **Detected – Low level** but **Non-Sufficient Quantity to repeat**
- Update Isolate Comment from the keypad: \DPCR + \liq
- Status as **FINAL**
- Inform all invalid results according to **Isolate Notification and Freezing Table**

Example:

COVID-19 virus

DETECTED by real-time PCR.

* * * * * * * * * * * * * * * * * * * *

(Low level detection-insufficient quantity of specimen to confirm)

* * * * * * * * * * * * * * * * * * * *

Testing performed using the Seegene Allplex 2019-nCoV Assay.
NOTE: The Seegene Allplex 2019-nCoV Assay has been approved by Health Canada for Emergency Use Access (EUA) and has been verified by the University Health Network/Sinai Health Microbiology Laboratory

Cleaning

At shift end, perform cleaning protocol as outlined below:

Clean Room: Wipe down with RNAse Away or NucleoClean on paper towel, followed by distilled water, and then 70% alcohol
Biological Safety Cabinet
Pipettes
Bench tops

Specimen Preparation Area: Wipe down with Working 1% hypochloride (made daily), followed by distilled water, and then 70% alcohol
Biological Safety Cabinet (BSC), pipettes, centrifuge, and bench top.
Seal and discard BSC waste
Wash racks
Space Racks, and Multi-channel Pipette: Wipe down surfaces with RNAse Away or NucleoClean on KimWipe, followed by UltraPure water, and then 70% alcohol Wipe

Amplification Area: Wipe down surfaces with RNAse Away or NucleoClean on KimWipe, followed by UltraPure water, and then 70% alcohol Wipe
Seal & discard reaction microtubes into biohazard waste after each run.
Perform the cleaning procedure according the daily maintenance sheet.

MGI SP 960: wipe any spills with 70% Alcohol and do a post clean.

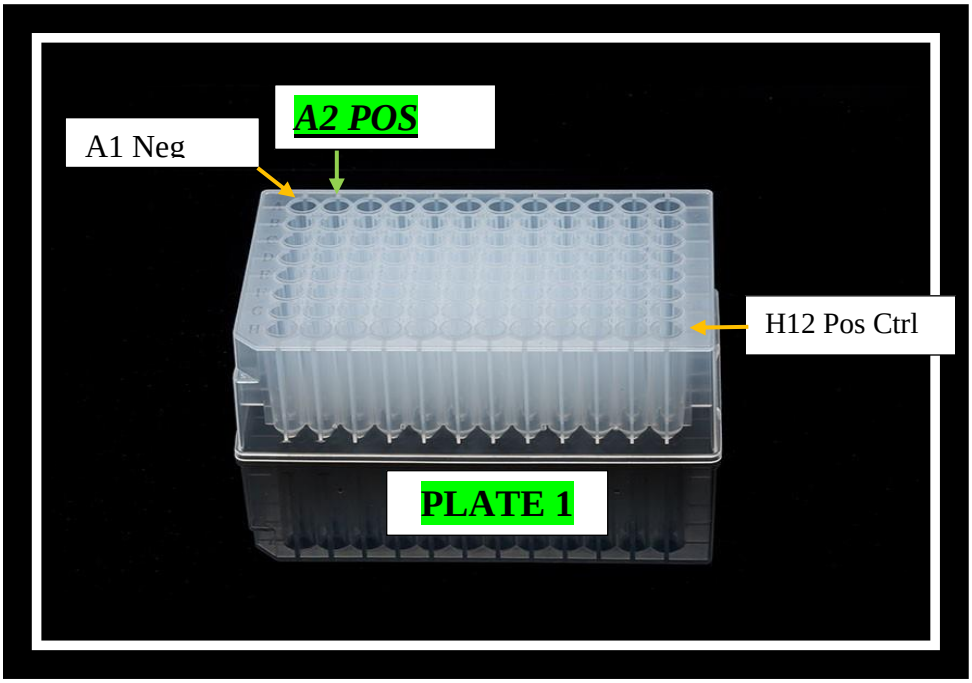
Quality Control

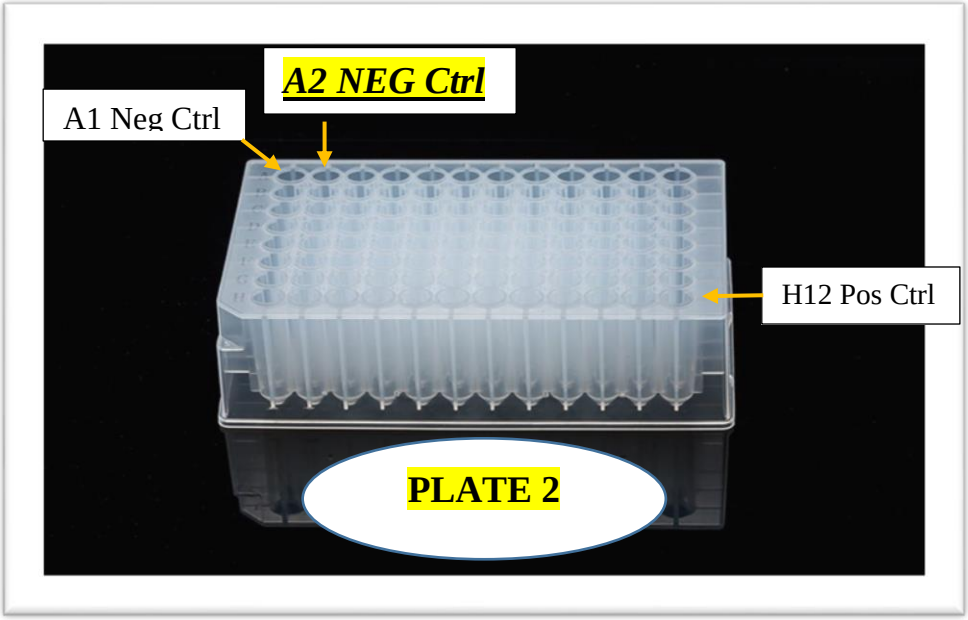
Reagent QCs:
An External Control (external to MGI BGI assay) is used to monitor the isolation, amplification and detection procedures. The result must correspond to expected value supplied by the manufacturer.
A positive and negative external control for Covid gene should be extracted on the MGI run

- with each new lot/new shipment of RNA/DNA MGI Easy Extraction kit and BGI *Real-Time Fluorescent RT-PCR* 2019-nCoV Assay
  and
- after major maintenance

Daily QCs:
Every run:

- Each patient specimen must show beta actin to monitor both extraction and PCR inhibition.
- Both a Positive and Negative control including target and beta actin will be included.
- For QC result acceptability refer to interpretation section.
- As a way of avoiding plates swap from extraction to reporting, please follow the graph below for addition controls:
  Plate 1: add POS control in A2 well
  Plate 2: add NEG control in A2 well


A1 Neg
A2 POS
H12 Pos Ctrl
PLATE 1


A1 Neg Ctrl
A2 NEG Ctrl
H12 Pos Ctrl
PLATE 2

Report all failed QCs to senior/charge technologist.

Failed QC:
Test is invalid without satisfactory QC results.

a. Do not release results pending resolution of QC failure.
b. Inform charge/senior technologist.
c. Record in Reagent Log Chart, Instrument Maintenance Log or Incident Report where appropriate.
d. If the QC failure was due to a simple matter of position reversal or misplacement, the run can be released (positive QC material yielded positive result, negative yielded negative result).

e. If negative QC material yielded positive result, it may be due to cross-contamination from adjacent positive sample within the run or carry-over contamination from previous runs via equipment or the environment. Review procedure and equipment to establish and eliminate potential sources of contamination.
g. The extent and nature of contamination can also be evaluated by comparing the positive rate of the run with its expected positive rate.
h. If the contamination is extensive, it is necessary to quarantine/discard potentially contaminated reagents and consumables and disinfect equipment and environment before repeating the run.
i. If a carry-over contamination is suspected (e.g. two or more runs with negative QC being positive or patient samples have higher than expected positive rate and these samples are often non-repeatable positives), it is necessary to have a thorough environmental disinfection followed by swabbing to monitor.
j. Successful ending to a carry-over contamination may be indicated by QC results and patient positivity rate falling back to the expected normal range and three negative environmental swabs.

Troubleshooting

1. Technical Support

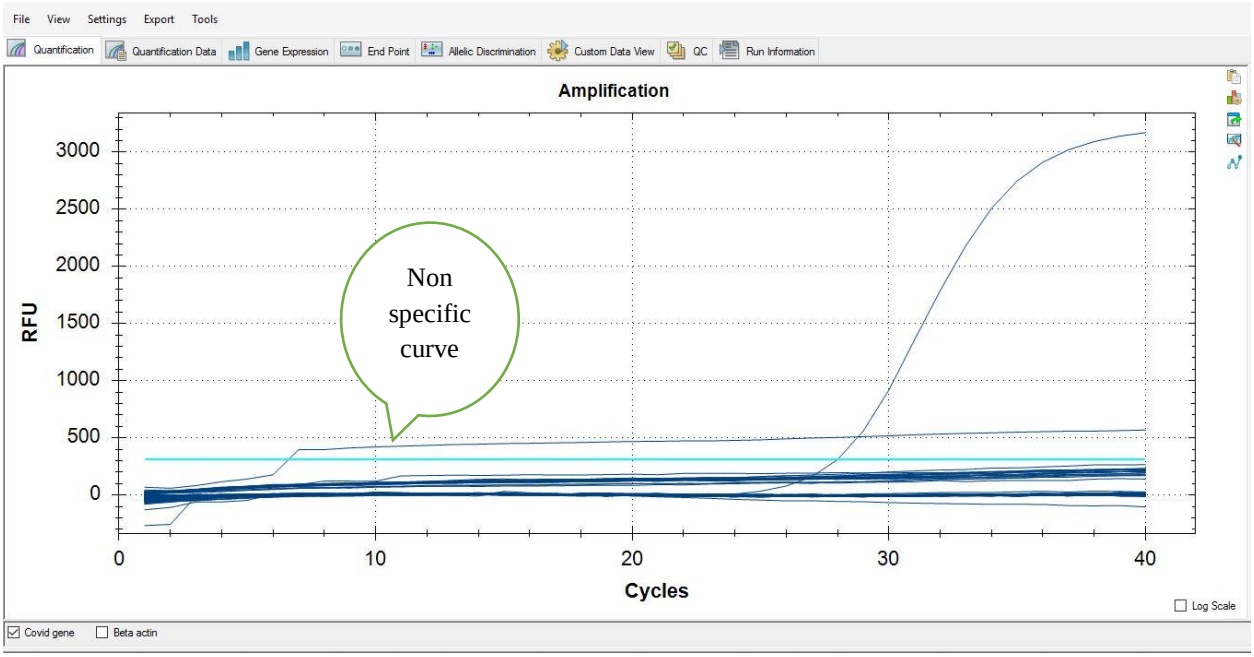
Collect information by taking pictures of reagents/supplies and run/area of issue, printing screen and exporting logs) and send them to MGI Canada Service Support (MGICSUPPORT@mgiamericas.com)

For details, please refer to **MGISP960 Troubleshooting Guide**.

For urgent support, phone BGI technician support staff immediately.

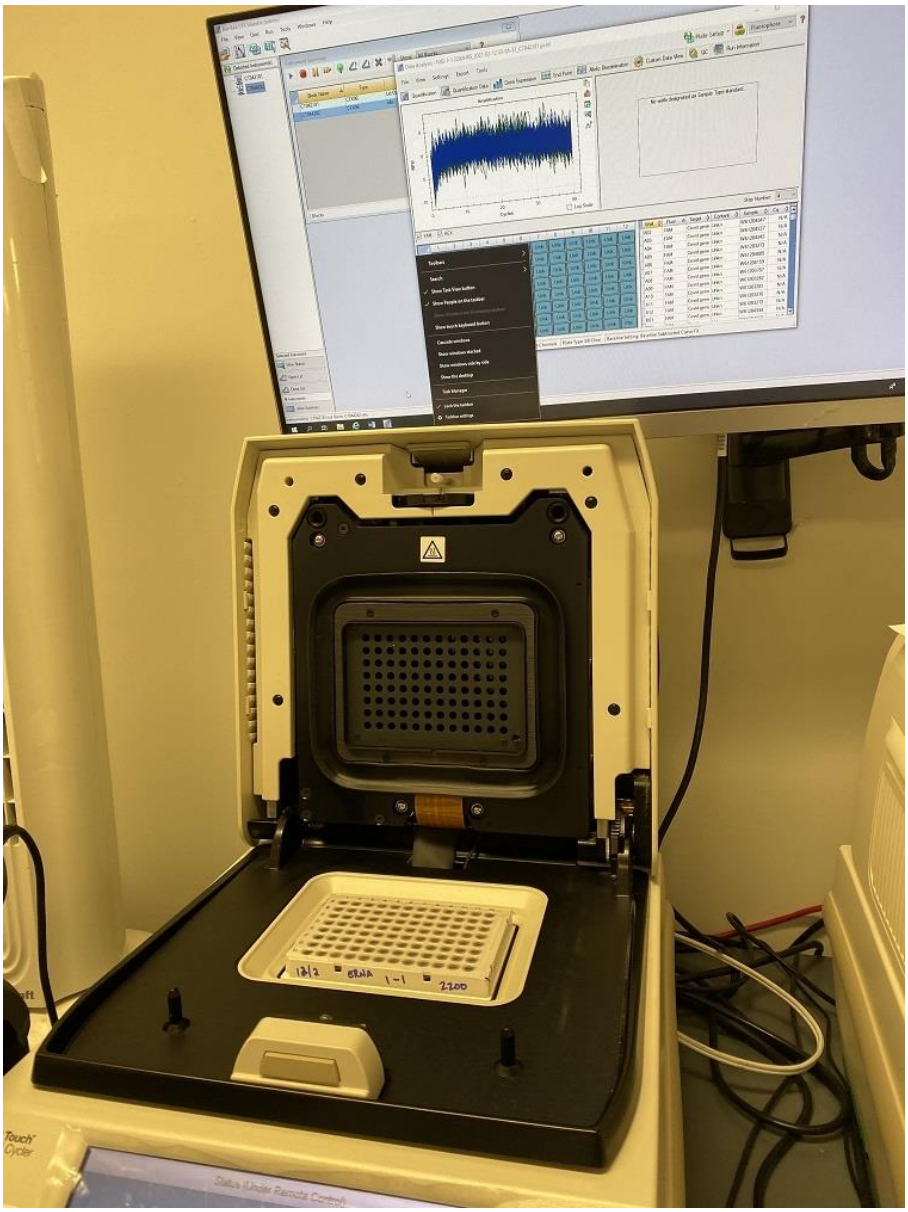
2. Common Troubleshooting Tips

Non-Specific curves:
The non-specific curve (exemplified in the following picture) should be excluded from interfacing and reported as negative manually. If you still have concerns, please let the senior in your shift or microbiologist on call know.

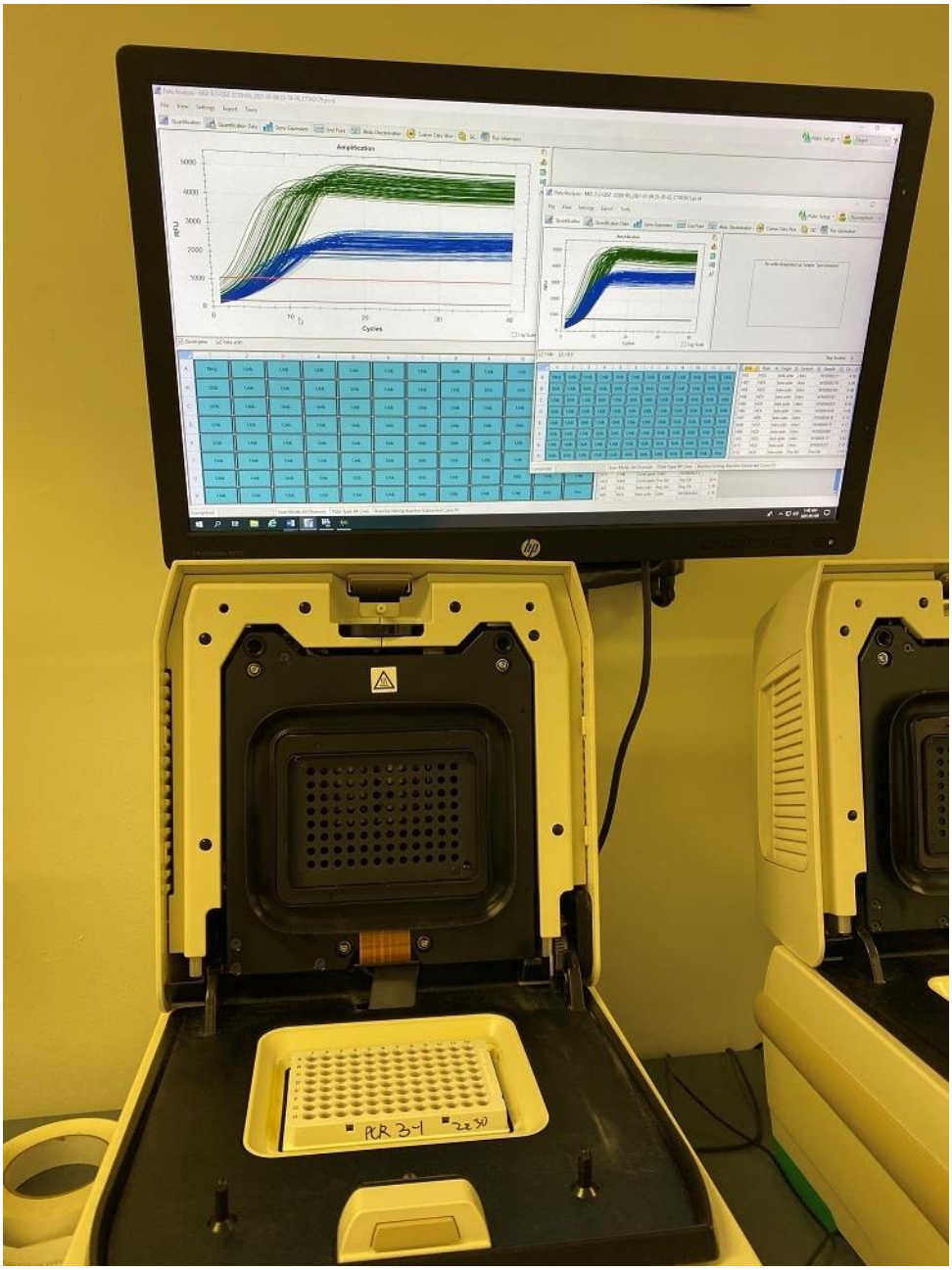


3. Failed run, possible Root cause and Graph

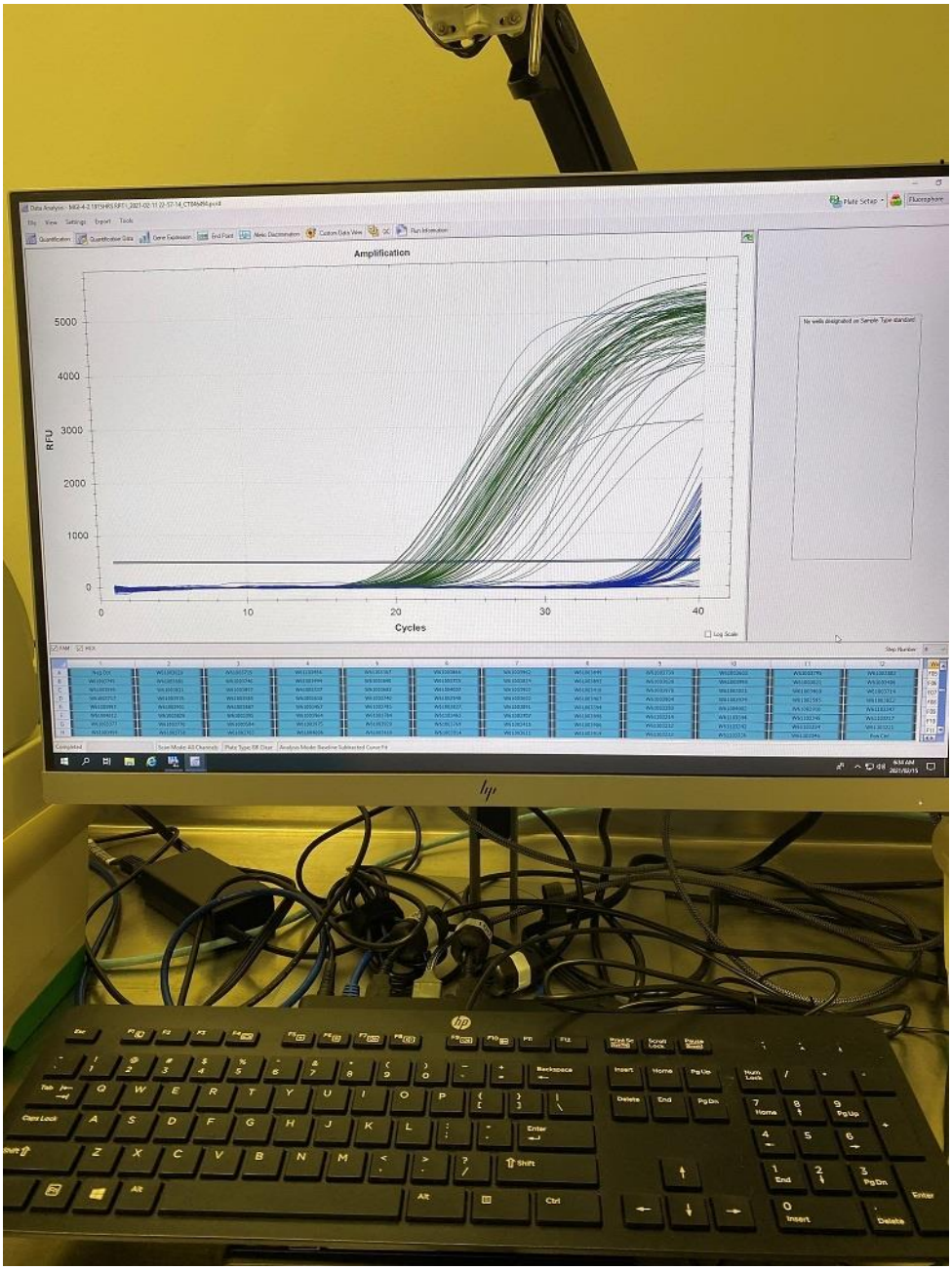
a. Eluate plate use in PCR



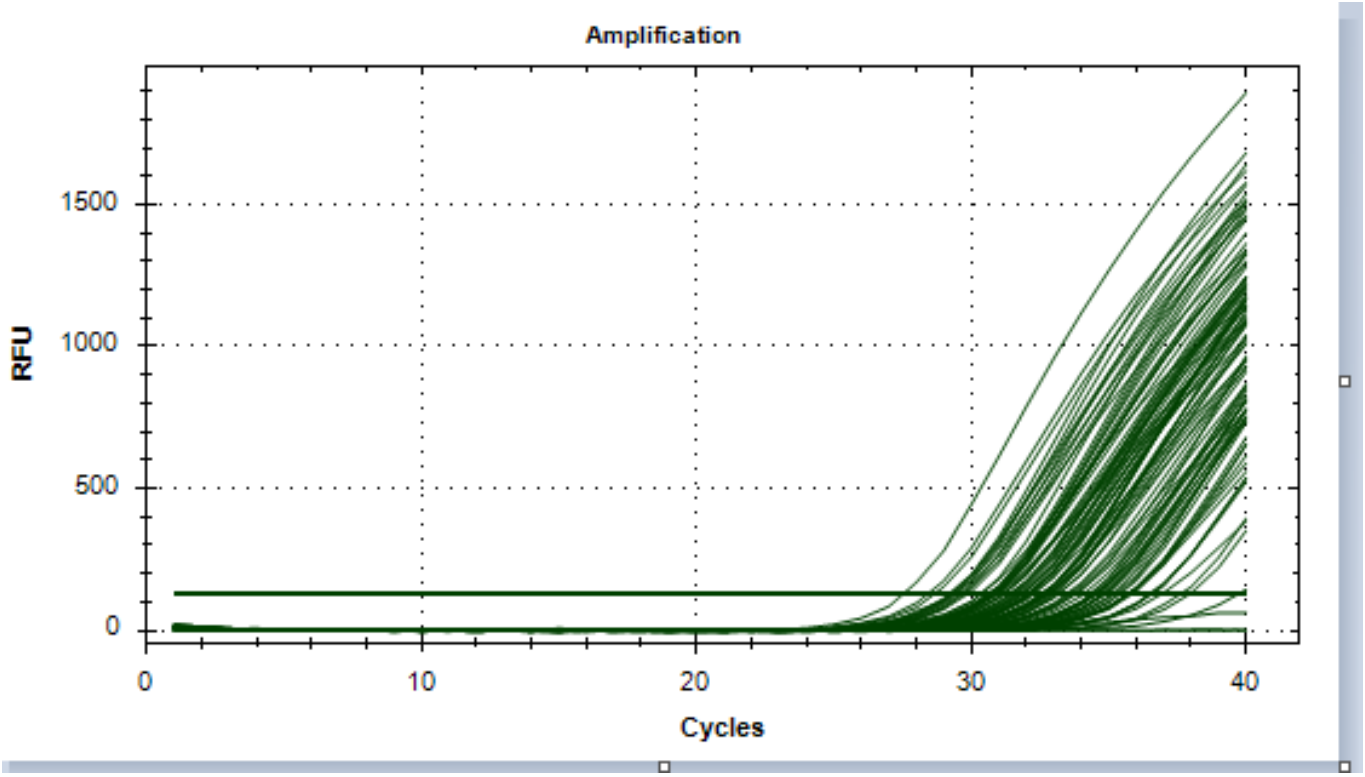
b. Plate not sealed



c. Contaminated run



d. Fanning curves (uneven distribution of Mastermix)



4. MGI workflow checklist

Many manuals steps are needed in the MGI testing process. All the steps in the procedure should be followed to avoid potential sample mix-ups and plate swaps.

The following MGI workflow checklist is used to check and monitor all critical steps from aliquoting to reporting.

MGI WORK FLOW CHECKLIST

DATE:

STEPS TO CHECK *	Initial §	Comments
Aliquoting		Please export STP worklist to STP folder if using MGI STP 7000
MIANT 32-1/STP-1		
MIANT 32-2/STP-2		
Manual Aliquot		
CONTROLS ADDED by		
Plate 1 (2 Pos, 1 Neg)		
Plate 2 (1 Pos, 2 Neg)		
Lysis Buffer prep by		
Reagents (H_2O/MW1/MW2) volume checked by		
MGI extraction started by		Check if all plates are in correct positions
Eluate/Mastermix volume checked by		
Import Worklist and Time Stamp		Convert STP/manual worklist to CSV
MGI 1-1 (Worklist created/time stamped by)	/	
MGI 1-2 (Worklist created/time stamped by)	/	
MGI 2-1 (Worklist created/time stamped by)	/	
MGI 2-2 (Worklist created/time stamped by)	/	
MGI 3-1 (Worklist created/time stamped by)	/	
MGI 3-2 (Worklist created/time stamped by)	/	
MGI 4-1 (Worklist created/time stamped by)	/	
MGI 4-2 (Worklist created/time stamped by)	/	
MGI 5-1 (Worklist created/time stamped by)	/	
MGI 5-2 (Worklist created/time stamped by)	/	
MGI 6-1 (Worklist created/time stamped by)	/	
MGI 6-2 (Worklist created/time stamped by)	/	
Biorad PCR/Reporting		
Master Mix Prep		
Load & Check PCR Plate Plates		Make sure plate 1 & 2 at correct positions
Worklist Imported by		
Analyzed by		
Biorad checked by		Open Biorads & check correct plates loaded before exporting
Exported by		
Reported by		

***Please initial each step and pay attention to newly added critical check points as highlighted.**

§ No need to initial in shaded area

5. MGI Reagent Preparation Log

Insufficient reagent volume during reagent preparation may cause run failure of various forms. Use of the following log sheet facilitates good documentation and tracking of MGI reagents.

Month/Year _____ **Reviewed Monthly By Senior (initial)_____**

Day	MW2 Plates	MW1 Plates	H_2O Plates	Tech Initials/Shift	Lysis Buffer Vials	Tech Initials/Shift	NEGATIVE CONTROL Boxes	Tech Initials/Shift
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

Related Documents

Virology Accessioning Manual
Biorad Worksheet
Qualitative PCR External QC
Nucleic Acid Extraction – Biomerieux NucliSENS easyMAG
Pipetting by epMOTION Manual
Training Checklist

References

MGI Easy DNA/RNA Extraction
BGI Real-time fluorescent RT PCR kit for detecting nCoV

Record of Edited Revisions

Manual Section Name: SARS-CoV-2 PCR by MGI

Page Number / Item	Date of Revision	Signature of Approval
Addition of neg and pos control positions on plate	July 17, 2020	Dr. T. Mazzulli
Changed No template control interpretation when CT<37 from Repeat run **to** Repeat all positive and "repeat" samples and report the negative as negative.	September 14, 2020	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Changed the analyzing method for the graphs of target gene and beta actin; Added the massive procedure for making extraction reagents; Added LIS interfacing procedure.	November 18, 2020	Dorna Zareianjahromi
• Addition of General Seegene Reporting Process, BGI Reporting Process for Repeat Samples; • Updated BGI reporting rules, preliminary, final and repeat sample report statements in test window and isolate window • Addition of troubleshooting	January 08, 2021	Dorna Zareianjahromi
Minor formatting change	April 11, 2021	Jessica Bourke
MGI workflow checklist added in the trouble shooting session Graphs for additional controls added	May 17, 2021	Oliver Li
MGI workflow checklist updated with volumes of reagents/eluates/mastermix to be checked	June 14, 2021	Oliver Li
MGI Reagent Preparation Log (form) added in Troubleshooting section	July 22, 2021	Oliver Li
Updated technical support by adding details as to how to collect information of runs of issue	September 20, 2021	Oliver Li

Page Number / Item	Date of Revision	Edited by:
Added steps to follow when there is no BioRad thermocycler available in **Off board preparation** section	September 23, 2021	Oliver Li
Updated BGI reporting flowchart for repeating samples	October 22, 2021	Qin Liu
Added criteria for the adjustment of beta-actin threshold	October 27, 2021	Qin Liu
Updated E gene only in "General BGI Reporting Process"	November 05, 2021	Oliver Li
Minor formatting changes	November 08, 2021	Oliver Li
Added trouble shooting for failed run: Possible root cause and Graph	March 11, 2022	Qin Liu