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Prepared by QA Committee		
Issued by: Laboratory Manager	Revision Date: 3/11/2022	
Approved by Laboratory Director:	Next Review Date: 3/11/2024	
Microbiologist-in-Chief		

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# **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°C and process specimens as soon as possible.
- If delay of more than one day is expected, aliquot specimens and store at  $\leq -20^{\circ}$ C.

## Materials, Equipments and Facilities

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

- Specimen Preparation area: Biosafety Cabinet (MIBCT7 or MIBCT8)
- BIO-RAD CFX96 <sup>TM</sup> Real-Time System

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- 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  $\mu$ L, 10 to 200  $\mu$ L, 100 to 1000  $\mu$ L
- 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  $\mu$ L 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 <u>never used in Clean Room.</u>
- 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.

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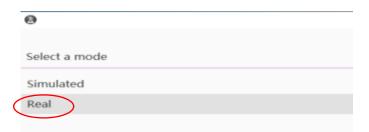
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# **Extraction Set up**

- 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.

0	
Select a mode	
Simulated	
Real	
	Real
	Create

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

Authentication				×
	Or Authenti	cation		
	Verify	Exit		
			$\subset$	User Ently

6. MGI SP960 is in the Initializing process.

≣	Home
	Initialize
Running Records	
Current Mode: Real	
🖾 IO board 🔤 Robot 🔤 Shaker 🗔 Scanner 🗔 Remote	
TempA  TempB  TempC  TempD  TempE  TempF	
PCRA     PCRB	

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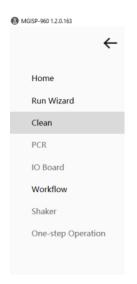
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MGISP-960 1.2.0.163	
	Home
	Initialize successfully.
Running Records	
Current Mode: Real	
🖾 IO board 🛛 Robot 🖾 Shaker 🗆 Scanner 📄 Remote	
TempA TempB TempC TempD TempE TempF PCRA PCRB	
Module Information	

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



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9. Select Pre Clean.

# Click Start button.

MGISP-960	1.2.0.163
=	Clean
Clean:	Pre-clean <u>Start</u> Stop

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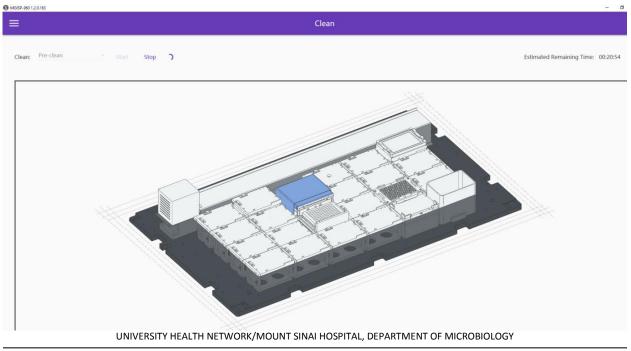
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10. Confirm the operation listed in the confirmation window Click **Continue** button.

0	Confirm the following information:			×
	00:00:08 Clo	ose Buzzer	n:	
	1. Empty Operation Deck			
	2. Lock PCR Lid Manually			
	3. Close Door			
	Note: If there is no PCR equipment, ignore the operatio	n relate <mark>d</mark>	to PC	R
	Continue Stop			

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



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MGISP-960 1.2.0.163
~
Home
Run Wizard
Clean
PCR
IO Board
Workflow
Shaker
One-step Operation

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

Solution:		
	Script:	• Start
Device St SARS-CoV-2 BGI Amp Plate Setup	îme:	Elapsed Time:
Phase: SARS-CoV-2 BGI Extraction		PCRA: Idle

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

≣		Run Wizard	
Solution: SARS-CoV-2 BGI Reagent Setup	Script: RNase Free Water.py	Start Pause	
Device Status: Idle	Start Time: 5/12/2020 8:47:31 AM	Elapsed Time:	Estim
Phase:	Step:	PCRA: Idle	Temp

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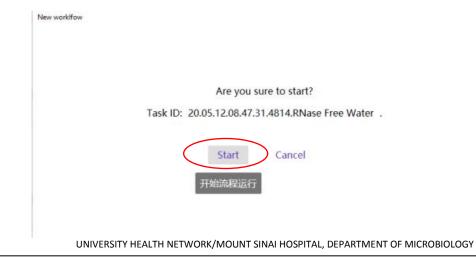
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- 5. Load tips, deep well plates and the tip lid according to the position on the screen.
- 6. Measure and transfer 45ml RNase Free Water into the tip lid at position 2.

Operation Deck	
POS1	POS5
Full Tip Box 96 Tip Access	Deepwell Plate 1
TipGEBAF250A	DeepwellPlateDT7350504
POS2	POS6
Tip Cover w/ 45mL RNF Water 1x45mL Falcon Tube	Deepwell Plate 2
TipGEBCover	DeepwellPlateDT7350504
	POS7
POS4	POS8
-	

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

7. Press **Start** button.



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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

🙆 Require input

Number of Extraction Pl	ates 1		
Name Value		Comment	
	_		
			Continue
Click Cont	inue button		
Click Cont	<b>inue</b> button		
	<b>inue</b> button		
	<b>inue</b> button		
() Require input	Value		
Require input Comment	Value	Comment	
Require input Comment Number of loading plate	Value 1	Comment	

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- 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

Device Status: Idle Phase:	Start Step:	Time:		
	Step:		Elapse	
			PCRA:	
Operation Deck				
POS1	POS5	POS9	POS13	
Full Tip Box 96 Tip Access TipGEBAF250A	Deepwell Plate 1	- 4		
POS2	POS6	POS10	POS14	
Tip Cover w/ 135mL MW1 3x45mL Falcon Tubes TipGEBCover	Deepwell Plate 2	8	-	
POS3	POS7	POS11	POS15	
	Deepwell Plate 3			

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

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

Comment Value		
Number of Extraction Plates 1	<u> </u>	
Name Value	Comment	

Continue

Stop

# 14. Choose number of loading plate(s) (from 1 to 3) for MW1.

# Click Continue button

Comment	Value			
Number of loading pla	ite			
	1			
Name Value	2	Comment		
	3			
			Continue	Stop

- 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.*

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- 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.

				Run Wizar	d		
Solution: SARS-CoV-2 BG	6I Reagent Setup 🔹	Script: Buffer MW2.py	*	Start			Finish
Device Status: Idle	Start 1	Fime: 5/12/2020 8:40:12 AM	Elapsed Time	e: 00:03:56		Estimated	d Remaining Time (In
Phase:	Step:		PCRA: Idle			TempA:	
Operation Deck							
POS1	POS5	POS9	POS13	POS17		POS21	Temp_Module
Full Tip Box 96 Tip Access	Deepwell Plate 1	Deepwell Plate 5	-				
TipGEBAF250A POS2	DeepwellPlateDT7350504 POS6	POS10	POS14	POS18	MagRack	POS22	
Tip Cover w/ 135mL MW2 3x45mL Falcon Tubes TipGEBCover	Deepwell Plate 2	Deepwell Plate 6	÷		-		2
POS3	POS7	POS11	POS15	POS19	MagRack	POS23	
Tip Cover w/ 135mL MW2 3x45mL Falcon Tubes	Deepwell Plate 3				44		**
TipGEBCover POS4	DeepwellPlateDT7350504 POS8	POS12	POS16	POS20	Shaker	POS24	
Tip Cover w/ 135mL MW2 3x45mL Falcon Tubes	Deepwell Plate 4	I	2		2		

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

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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.

🚯 Require input

Comment	Value	
Number of loading p	lates	
	1	
Name Value	2	Comment
	3	
		Continuo Ston

- 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.

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#### **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.

				Run Wizard						
Solution: 0.SARS-CoV-2	BGI Reagent Setup (Ma	• Script: RNase-Free Water (1	4 runs, 2-plate).pj +	Start Pause	Stop Finish	Clear Al	I			ð Ý
Device Status: Idle	St	art Time: 11/5/2020 8:28:44 AM	Elapsed Time: 0	0:03:15	Estimated Remaining Time (Incu	bation):	Con	npletion Status:	Completed	
Phase:	St	ep:	PCRA: Idle		TempA:		Terr	ірВ:		
Operation Deck						Rec	ords			Export
POS1	POS5	POS9	POS13	POS17	POS21 Temp_Module	No.	Time	Activity	Status	Parameter
-maximilian			AND 885222625	144492224664	1. A & & & & & & & & & & & & & & & & & &	1	11-05 08:28:43	Ask	User request	MGI_2020_11_05
Full Tip Box		Deepwell Plate 1	Deepwell Plate 3	Deepwell Plate 7	Deepwell Plate 11	2	11-05 08:28:43	Reload deck	Completed	
96 Tip Access		From Pos 3	From Pos 3	From Pos 3	From Pos 3	3	11-05 08:28:44	Workflow	Start	2 Plate Setup(no
TipGEBAF250A		DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	4	11-05 08:28:44	Home	Start	["HOME":"Home
POS2	POS6	POS10	POS14	POS18 MagRack	POS22	5	11-05 08:28:49	Home	Completed	("HOME":"Home
		1448222222		1449922244	AND REPORTED FOR	6	11-05 08:28:49	Message	Completed	Eluant Transfer, T
**		Deepwell Plate 2	Deepwell Plate 4	Deepwell Plate 8	Deepwell Plate 12	7	11-05 08:28:49	Load Tips	Start	("KITNAME":"use
**		From Pos 3	From Pos 3	From Pos 3	From Pos 3	8	11-05 08:28:59	Load Tips	Completed	("CURRENT":180
		DeepwellPlateD17350504	DeepweliPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	9	11-05 08:28:59	Aspirate	Start	["ROW":"1","BOT
POS3	POS7	POS11	POS15	POS19 MagRack	POS23	10	11-05 08:29:13	Aspirate	Completed	("CURRENT":180
			Same a State Ballie	NATES STATISTICS	A A A A A A A A A A A A A A A A A A A	11	11-05 08:29:13	Empty	Start	("KITNAME":"PC
Tip Cover w/ 135mL RNF Water			Deepwell Plate 5	Deepwell Plate 9	Deepwell Plate 13	12	11-05 08:29:27	Empty	Completed	("CURRENT":180
3x45mL Falcon Tube			From Pos 4	From Pos 4	From Pos 4	13	11-05 08:29:27	Mix	Start	("MODULE":"PO
TipGEBCover			DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	14	11-05 08:30:01	Mix	Completed	("CURRENT":180
POS4	POS8	POS12	POS16	POS20 Shaker	POS24	15	11-05 08:30:01	Unload Tips	Start	("KITNAME":"use
			and a second states	NA A DA DA DA DA	1.5444492525	16	11-05 08:30:12	Unload Tips	Completed	("CURRENT":180
Tip Cover w/ 135mL RNF Water	-	-	Deepwell Plate 6	Deepwell Plate 11	Deepwell Plate 14	17	11-05 08:30:12	Load Tips	Start	("KITNAME": "use
3x45mL Falcon Tube	-		From Pos 4	From Pos 4	From Pos 4	18	11-05 08:30:22	Load Tips	Completed	{"CURRENT":180
TipGEBCover			DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	19	11-05 08:30:22	Aspirate	Start	("ROW":*1","BOT

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

				Run Wizard						
Solution: 0.SARS-CoV-2	BGI Reagent Setup (Ma 👻	Script: Buffer MW1 (14 runs	, 2-plate).py 🔹	Start Pause	Stop Finish	Clear A				ð ý
Device Status: Idle	Start T	ime: 11/5/2020 8:28:44 AM	Elapsed Time: 0	0:03:15	Estimated Remaining Time (In	cubation):	Con	pletion Status:	Completed	
Phase:	Step:		PCRA: Idle		TempA:		Tem	рВ;		
Operation Deck						Rec	ords			Export
POS1	POS5	POS9	POS13	POS17	POS21 Temp_Module	No.	Time	Activity	Status	Parameter
		The land of the lot of	The second second	Contraction of the local division of the	The local day is the second	1	11-05 08:28:43	Ask	User request	MGI_2020_11_0508_
Full Tip Box	-	Deepwell Plate 1	Deepwell Plate 5	Deepwell Plate 8	Deepwell Plate 8	2	11-05 08:28:43	Reload deck	Completed	
96 Tip Access		From Pos 13	From Pos 13	From Pos 7	From Pos 7	3	11-05 08:28:44	Workflow	Start	2 Plate Setup(no-alic
TipGEBAF250A		DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	4	11-05 08:28:44	Home	Start	{"HOME":"Home"}
POS2	POS6	POS10	POS14	POS18 MagRack		5	11-05 08:28:49	Home	Completed	("HOME":"Home")
and the second second		THE CASE OF THE PARTY OF	THE REAL PROPERTY AND	The second second second	TOT CANADA AND AND AND AND AND AND AND AND AN	6	11-05 08:28:49	Message	Completed	Eluant Transfer, Trans
Tip Cover w/ 135mL MW1		Deepwell Plate 2	Deepwell Plate 6	Deepwell Plate 9	Deepwell Plate 12	7	11-05 08:28:49	Load Tips	Start	("KITNAME": "useless
3x45mL Falcon Tubes	-	From Pos 2	From Pos 2	From Pos 2	From Pos 7	8	11-05 08:28:59	Load Tips	Completed	("CURRENT":180,"0
TipGEBCover		DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	9	11-05 08:28:59	Aspirate	Start	("ROW":"1","BOTTON
POS3	POS7	POS11	POS15	POS19 MagRack	POS23	10	11-05 08:29:13	Aspirate	Completed	("CURRENT":180,"RC
			A + + + + + + + + + + + + + + + + + + +	100000000000	14444492225	11	11-05 08:29:13	Empty	Start	("KITNAME": "PCRBio
Tip Cover w/ 135mL MW1	Tip Cover w/ 135mL MW1		Deepwell Plate 7	Deepwell Plate 10	Deepwell Plate 13	12	11-05 08:29:27	Empty	Completed	("CURRENT":180,"(
3x45mL Falcon Tubes	3x45mL Falcon Tubes		From Pos 3	From Pos 3	From Pos 3	13	11-05 08:29:27	Mix	Start	("MODULE":"POS22"
TipGEBCover	TipGEBCover		DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	14	11-05 08:30:01	Mix	Completed	("CURRENT":180,"MC
POS4	POS8	POS12	POS16	POS20 Shaker	POS24	15	11-05 08:30:01	Unload Tips	Start	("KITNAME": "useless
				100000000000	14440222222	16	11-05 08:30:12	Unload Tips	Completed	("CURRENT":180,"(
Tip Cover w/ 135mL MW1	Tip Cover w/ 135mL MW1		Deepwell Plate 8	Deepwell Plate 11	Deepwell Plate 14	17	11-05 08:30:12	Load Tips	Start	("KITNAME": "useless
3x45mL Falcon Tubes	3x45mL Falcon Tubes		From Pos 4	From Pos 4	From Pos 4	18	11-05 08:30:22	Load Tips	Completed	("CURRENT":180,"(
TipGEBCover	TipGEBCover		DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	19	11-05 08:30:22	Aspirate	Start	("ROW":"1","BOTTON

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

				Run Wizard						
Solution: 0.SARS-CoV-2	BGI Reagent Setup (Ma 👻	Script: Buffer MW2 (7 runs,	2-plate).py	Start Pause	Stop Finish	Clear A	1			ð ý
Device Status: Idle	Start Ti	ime: 11/5/2020 8:28:44 AM	Elapsed Time: 0	0:03:15	Estimated Remaining Time (Ir	cubation):	Con	npletion Status:	Completed	
Phase:	Step:		PCRA: Idle		TempA:		Tem	рВ:		
Operation Deck						Rec	ords			Export
POS1	POS5	POS9	POS13	POS17	POS21 Temp_Module	No.	Time	Activity	Status	Parameter
CARNER STATE		Total State State State State	Contract of the Party of the Pa	Constant and the second	Contraction of the Party of the	1	11-05 08:28:43	Ask	User request	MGI_2020_11_0508
Full Tip Box		Deepwell Plate 1	Deepwell Plate 3	Deepwell Plate 7	Deepwell Plate 11	2	11-05 08:28:43	Reload deck	Completed	
96 Tip Access		From Pos 4	From Pos 12	From Pos 12	From Pos 3	3	11-05 08:28:44	Workflow	Start	2 Plate Setup(no-a
TipGEBAF250A		DeepwellPlateD17350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	4	11-05 08:28:44	Home	Start	("HOME": "Home")
POS2	POS6	POS10	POS14	POS18 MagRack	POS22	5	11-05 08:28:49	Home	Completed	("HOME":"Home")
		TOTO COLUMN STATE	TOTAL CONTRACTOR	The second second second	TOCOLOGICAL STREET,	6	11-05 08:28:49	Message	Completed	Eluant Transfer, Tra
Tip Cover w/ 135mL MW2	Tip Cover w/ 135mL MW2	Deepwell Plate 2	Deepwell Plate 4	Deepwell Plate 8	Deepwell Plate 12	7	11-05 08:28:49	Load Tips	Start	{"KITNAME":"usele
3x45mL Falcon Tubes	3x45mL Falcon Tubes	From Pos 2	From Pos 2	From Pos 6	From Pos 6	8	11-05 08:28:59	Load Tips	Completed	("CURRENT":180,"
TipGEBCover	TipGEBCover	DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	9	11-05 08:28:59	Aspirate	Start	("ROW":"1","BOTT
POS3	POS7	POS11	POS15	POS19 MagRack	POS23	10	11-05 08:29:13	Aspirate	Completed	("CURRENT": 180,")
				14444493555	14405022222	11	11-05 08:29:13	Empty	Start	{"KITNAME":"PCRI
Tip Cover w/ 135mL MW2	Tip Cover w/ 135mL MW2		Deepwell Plate 5	Deepwell Plate 9	Deepwell Plate 13	12	11-05 08:29:27	Empty	Completed	("CURRENT": 180,"
3x45mL Falcon Tubes	3x45mL Falcon Tubes	-	From Pos 3	From Pos 7	From Pos 7	13	11-05 08:29:27	Mix	Start	("MODULE":"POS
TipGEBCover	TipGEBCover		DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	14	11-05 08:30:01	Mix	Completed	("CURRENT":180,"
POS4	POS8	POS12	POS16	POS20 Shaker	POS24	15	11-05 08:30:01	Unload Tips	Start	{"KITNAME":"usel
				100000000000	ANN 88.822777	16	11-05 08:30:12	Unload Tips	Completed	{"CURRENT": 180,"
Tip Cover w/ 135mL MW2	Tip Cover w/ 135mL MW2	Tip Cover w/ 135mL MW2	Deepwell Plate 6	Deepwell Plate 10	Deepwell Plate 14	17	11-05 08:30:12	Load Tips	Start	{"KITNAME":"usek
3x45mL Falcon Tubes	3x45mL Falcon Tubes	3x45mL Falcon Tubes	From Pos 4	From Pos 8	From Pos 8	18	11-05 08:30:22	Load Tips	Completed	("CURRENT": 180,"
TipGEBCover	TipGEBCover	TipGEBCover	DeepwellPlateDT7350504	DeepwellPlateDT7350504	DeepwellPlateDT7350504	19	11-05 08:30:22	Aspirate	Start	{"ROW":"1","BOT

## **Off board preparation**

- 1. Using the adjustable multichannel pipette, aliquot/pipet 200  $\mu$ L 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

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- 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.
- 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.

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🔕 MGISP-960 1.2.0.163									- 0
				Run \	Wizard				:
Solution: SARS-Co	/-2 BGI Extraction	Script: Extraction	- 1 Plate.py						ê ŵ
Device Status: Idle		Start Time: 5/12/2020 10:	10:12 AM	Elapsed Time:		Estimated Remaining Time (In	cubation):	Completion Status:	
Phase:		Step:		PCRA: Idle		TempA:		TempB:	
Operation Deck							Records		
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POS2	POS6	POS10	Ta	sk ID: 20.05.12.10.10.12	2.1997.Extraction - 1 F	Plate .			
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Full Tip Box 96 Tip Access	Full Tip Bo 96 Tip Acce			Start	Cancel				
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TipGEBAF250A POS4	TipGEBAF25 POS8	POS12	POS16	POSZ	20 Shaker	DeepwellPlateDT7350504 POS24			
exession and the		STITUT	1111						
Full Tip Box		Extracte	RNA	-					
96 Tip Access	-					度料袋			
TipGEBAF250A		PCRBioRad	HSP9601						

- 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  $\mu$ L 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

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15. Press Start.

					Run Wizard						
olution: SARS-CoV-2	BGI Amp Plate Setup -	Script: 1 Plate Setup.p	y.	-	Start Pause	Stop	Finish	Clear All			ê <sup>:</sup>
evice Status: Idle	Star	t Time: 5/12/2020 3:23:12 P	M	Elapsed Time:		Estimated F	Remaining Time (Incu	ubation):	Completion State	us:	
hase:	Step	):		PCRA: Idle		TempA:			TempB:		
peration Deck								Records			
OS1	POS5	POS9 New wo	00612		PO517	00021	Town Module	No. Time	Activity	Status	Parameter
-	p Box with at least 1 Full Cok 8 Tip Access		rkitow.								
	TipGEBAF250A				Are you sure to start?						
OS2	POS6	POS10		Task ID: 20.	05.12.15.23.12.7265.1 Plate Set	up.					
-	Empty Tip Box	5			Start Cancel						
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OS3	POS7	POS11									
Full Tip Box 96 Tip Access	-			** **							
TipGEBAF250A OS4	POS8	POS12	POS16		POS20 Shake	r POS24					

- 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°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)

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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<sup>o</sup>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	BGI nCoV	PCR Mix
reactions	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

To be loaded manually:

• Place 96-well plate onto pre-cooled block

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• 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

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MGISP-960 1.2.0.163								
≡				Run Wizard				
Solution: 3.SARS-CoV-2 B	GI Amp Plate Setup 🔹 s	cript: 1 Plate Setup(no-aliqu	ioting).py	Start		Stop Finish	Clear A	II
Device Status: Idle	Start Ti	ne: 1 Plate Setup(no-aliqu	uoting).py	Time: 00:01:22		Estimated Remaining Time	Incubation):	
Phase:	Step:	1 Plate Setup.py	0.17	dle		TempA:		
Operation Deck		2 Plate Setup(no-aliqu	ioting).py				Rec	ords
		2 Plate Setup.py						
POS1	POS5	Puss	10313	POS17		POS21 Temp_Modu	e No.	Time
							1	05-21 08:
							2	05-21 08:
-				-			3	05-21 08:
							4	05-21 08:
POS2	POS6	POS10	POS14	POS18	MagRack	POS22	5	05-21 08:
					HIII		6	05-21 08:
				Extracted	RNA	RT-PCR Plate, 20ul mix per well	7	05-21 08:
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				PCRBioRadi	HSP9601	PCRBioRadHSP9601	9	05-21 08:
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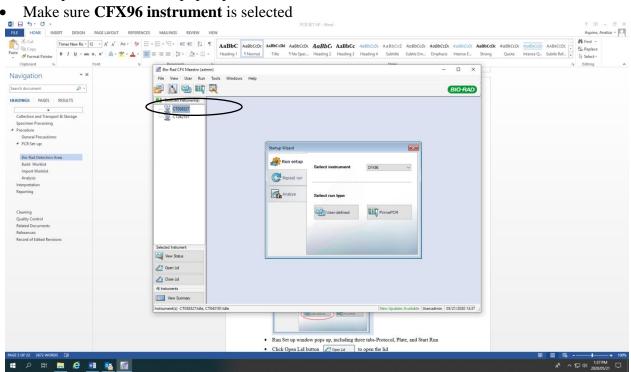
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## **Bio-Rad Detection Area:**

- Double click Bio-Rad CFX Maestro icon
- Startup Wizard window pops up



• Under Select run type, click User-defined button

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DINGS PAGES RESULTS	Detected instrument(s)			-	
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Specimen Processing					
Procedure					
General Precautions: # PCR Set-up:					
• РСКЗИ-ар	Sta	rtup Wizard			
Bio-Rad Detection Area:					
Build Worklist	d	Run setup Select instrument CFX36			
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	Louis and a second a second second	(NEED THAT ARE ADDRESS OF THE PARTY OF			
		un Set up window pops up, including three tabs Proto	col. Plate, and Start Run		

- Run Set up window pops up, including three tabs-Protocol, Plate, and Start Run
- Click Open Lid button
   Den Lid
   to open the lid
- Load sealed plate to the block

•

Click Close Lid button

Close Lid 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

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Bio-Rad CFX Maestro	(admin)			- 🗆 ×	
File View User Ri	un Tools Windows Help				
🥔 🔝 🗠 🗉	V 🔍			BIO RAD	
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	Selected Protocol		PrimePCRMet 384 pro		
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Click Plate tab |  $\square$  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

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CFX Thermocycler	instrument number: checked or	1
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- If all information is correct, click Start Run button 👂 gat Run
- 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>

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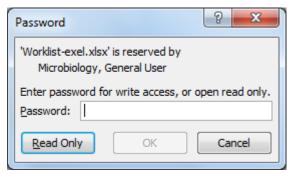
Click Save button Save

# **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

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• 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

Microsoft Excel
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1	HZ 2015.10.15.csv may contain features that are not compatible with CSV (Comma delimited). Do you want to keep the workbook in this format? • To keep this format, which leaves out any incompatible features, dick Yes. • To preserve the features, dick No. Then save a copy in the latest Excel format. • To see what might be lost, dick Help.
	Yes No Help

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

**Import Worklist** 

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



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• Plate Editor window pops up

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CUEN English Contraction Contr	Policy # MI_MD_COVMGI	Page 32 of 67
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													Exclude Wells in Analysis

# • Click Spreadsheet View/Importer button == Spreadsheet View/Importer

• Plate Spreadsheet View window pops up

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# • Select <u>HEX</u> from Fluors List pull-down menu

s List: HEX FAM		•				Export Ten	nplate Impo
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- Click Import button Import
- Select Import File window pops up

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

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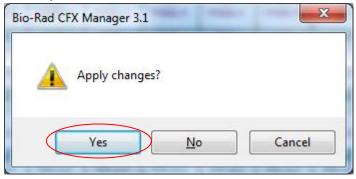
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Quality Manual         Section: Molecular Diagnostics Procedures	Version: 3.12 CURRENT Subject Title: SARS-CoV-2 PCR by MG	I
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- Select appropriate worklist and click **Open**, all samples and controls 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.



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Click Tin	ne Status tab C Time Status from the Run Details win	ndow
Bio-Rad CFX Manager 3.1		
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## 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.

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# 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

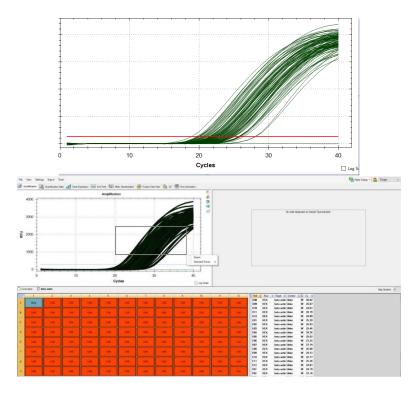
Then:

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

#### <u>NOTE:</u> 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:

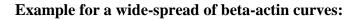


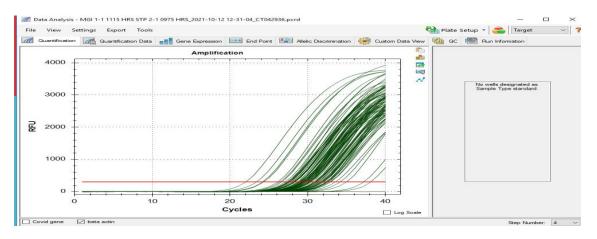
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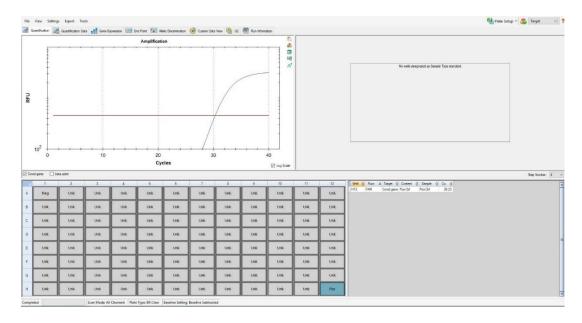
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• Mark **Covid gene** box and **log scale** box under the graph area with beta actin unmarked. Click positive control only, and drag the threshold line to the mid-point of the exponential amplification (linear portion of the sigmoidal amplification 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.



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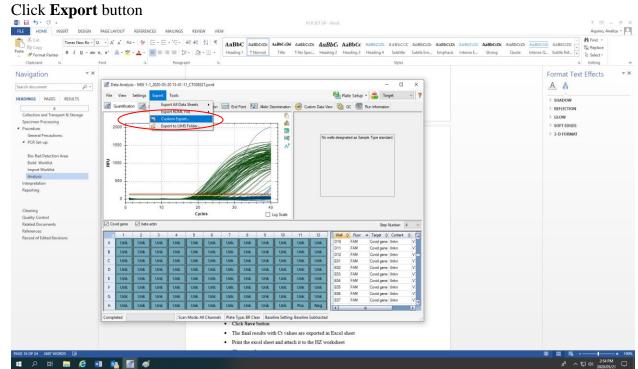
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- A positive PCR is observed if there is a rise or amplification in the Target channel e.g. FAM (Covid gene), & HEX (beta actin). The graph should be <u>exponential and sigmoidal</u> in shape. Conversely a negative PCR is a flat line or no signal.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

•

• Select Custom Export...



• "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.

Bio-Rad CFX Manager	
Export complete.	
ОК	ALTH

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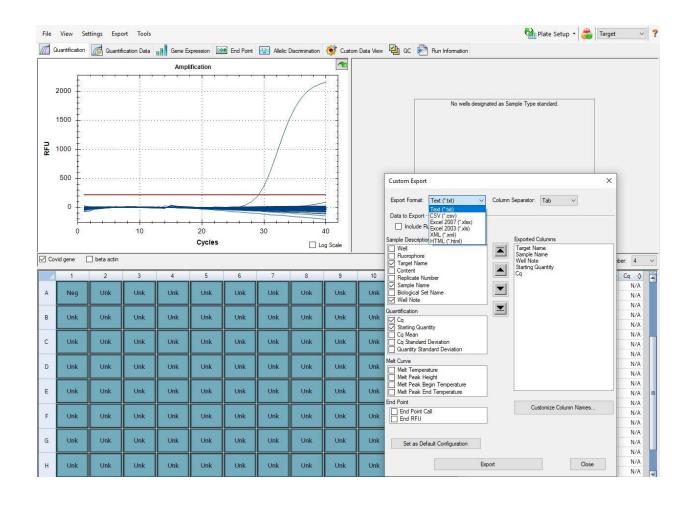
- Click **Close** button.
- LIS Interfacing: exclude from analysis any sample that is not expected to cross over to LIS, e.g. low positive sample from Seegene repeated as negative in the current MGI run. Click Export > Custom Export
   Choose Export Format as Text (\*txt)
   Click Export button and save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

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## **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. <i>Note: if curve is not</i> <i>sigmoidal, contact</i> <i>microbiologist-on-call for</i> <i>further instructions.</i>
Positive control	No amplification or Ct value is >35	No amplification or Ct value is >37	Fail - Do not report sample results. Repeat run

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## **Test Samples**

Note: Interpretations below are based on **<u>Sigmoidal</u>** amplification curves.

Sample	FAM (COVID gene-ORF1ab)	HEX (beta actin)	INTERPRETATION
A	Ct value ≥37	Ct value <35	SARS-CoV-2 RNA NOT Detected
В	Ct value <35	Ct value <35	SARS-CoV-2 RNA Detected
С	35.0≤Ct<37.0	Ct value <35	<u>SARS-CoV-2 RNA Detected – Low</u> <u>level</u> Repeat on Seegene
D	Ct value ≥37	Ct value ≥35	INVALID
Е	Ct<37.0	Ct value $\geq$ 35	Questionable Invalid Repeat on Seegene
Repeat	Follow <u>General BGI R</u> and <u>BGI Reporting Proces</u>		FINAL RESULT for REPEAT of LOW LEVEL DETECTION

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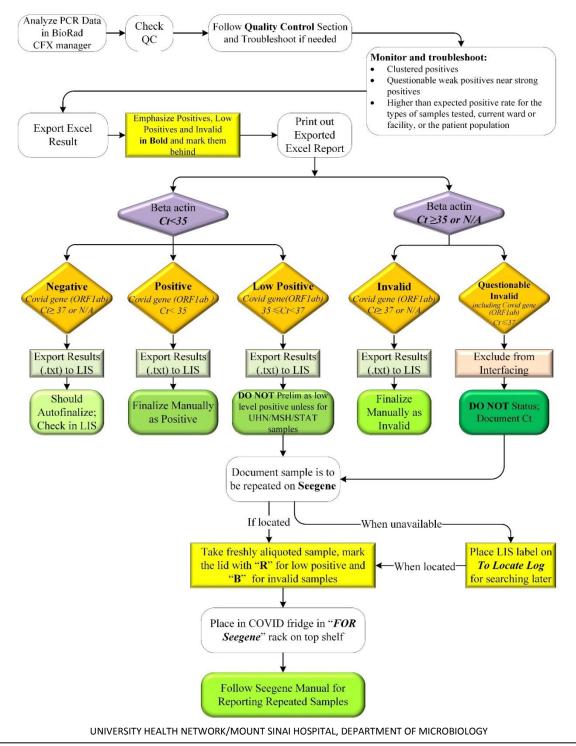
**General BGI Reporting Process** 

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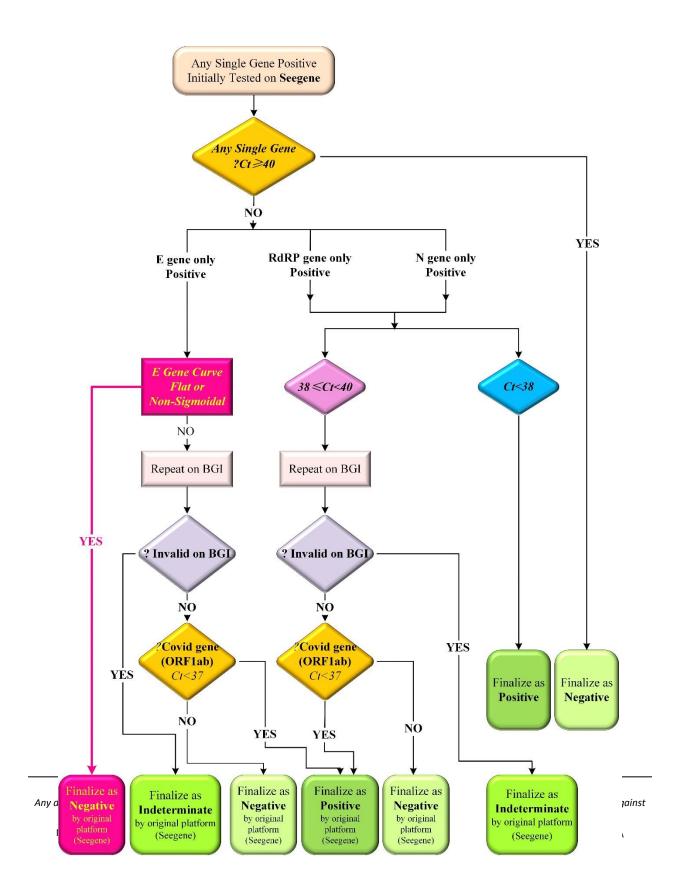
**BGI Reporting Process of Repeat Samples** 

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## **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

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## 2. FINAL RESULTS

## **2.1 NEGATIVE RESULTS**

- Result in the test window using <u>COVID-19 Virus Not Detected</u> 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.

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#### **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.

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## **2.3 INVALID RESULTS**

- Result in the test window using <u>COVID-19 virus PCR test unable to be completed</u> 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.

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

Follow BGI Reporting Process for Repeat Samples

## **3.1 CONFIRMED POSITIVE RESUTLS**

## Follow <u>FINAL RESULTS for POSITIVE</u> 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:

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

\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*

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## **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.

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

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#### <u>Cleaning</u>

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.

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## **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

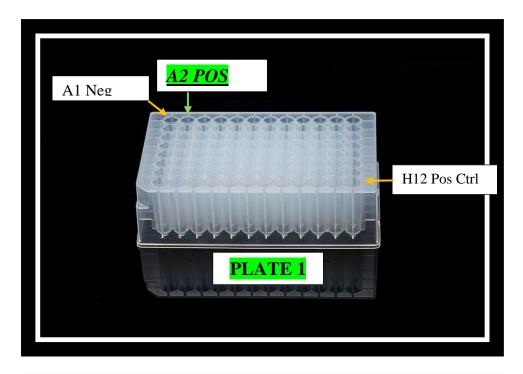
Plate 2: add NEG control in A2 well

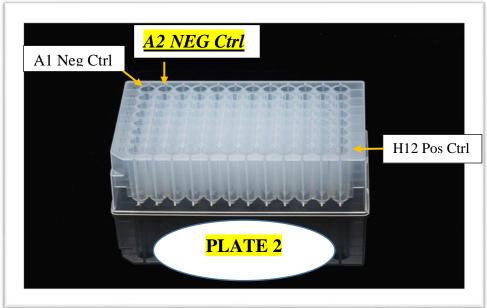
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Report all failed QCs to senior/charge technologist.

## 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.

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## **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 (<u>MGICSUPPORT@mgiamericas.com</u>)

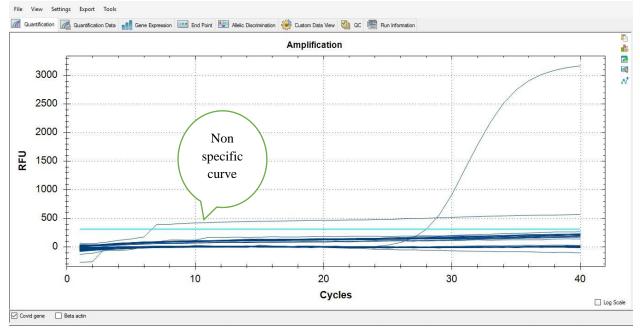
For details, please refer to MGISP960 Troubleshooting Guide.

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.



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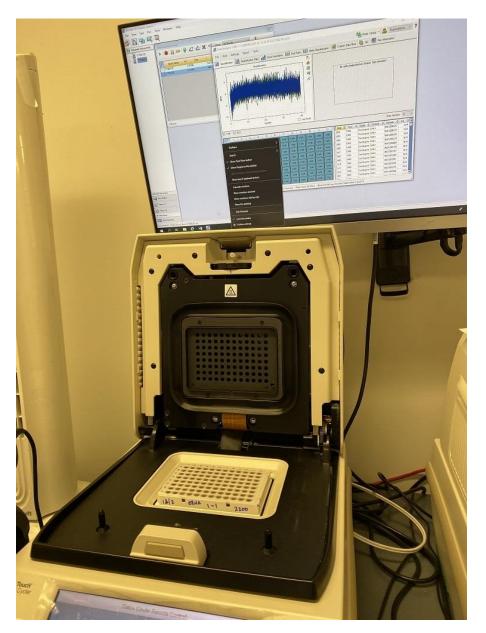
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## 3. Failed run, possible Root cause and Graph

a. Eluate plate use in PCR



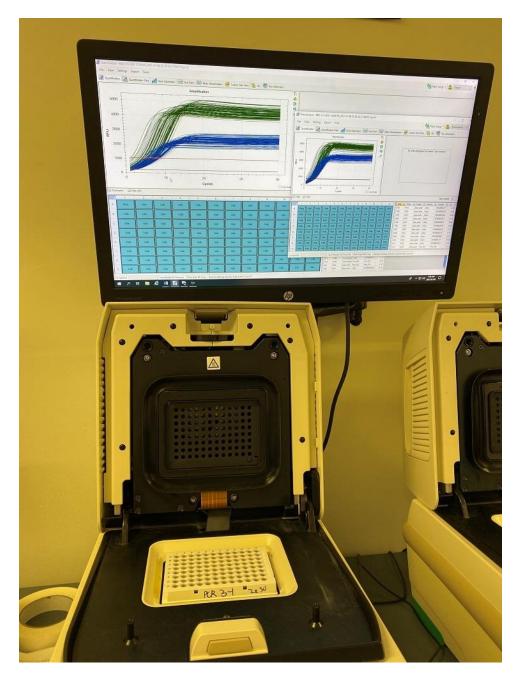
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## b. Plate not sealed



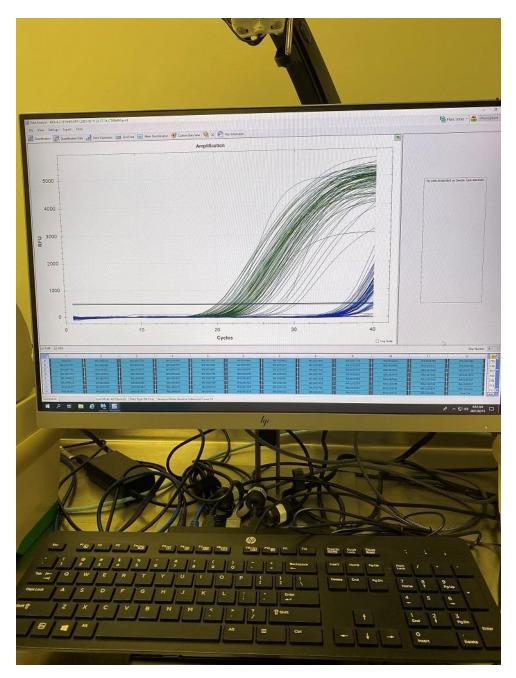
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## c. Contaminated run



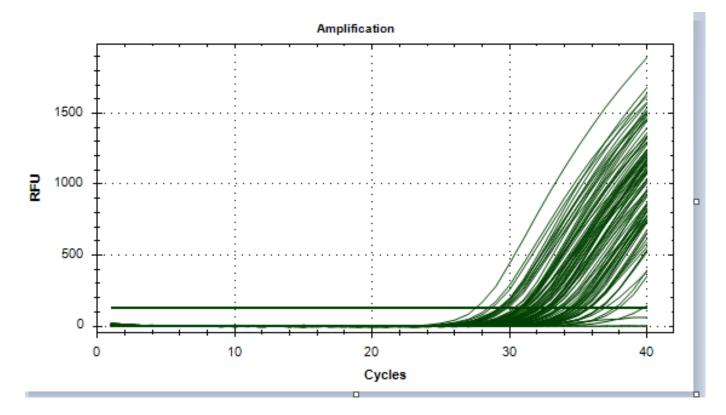
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d. Fanning curves (uneven distribution of Mastermix)



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#### 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.

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# 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 <sub>2</sub> O/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)	/	
<b>Biorad PCR/Reporting</b>		
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

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## 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 <sub>2</sub> O Plates	Tech Initials/Shift	Lysis Buffer Vials	Tech Initials/Shift	NEGATIVE CONTROL Boxes	Tech Initials/Shift
1								
2								
3								
4								
5								
6								
7								
8								
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12								
13								
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#### **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

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## **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	September 14, 2020	Dr. T. Mazzulli
from Repeat run to Repeat all positive and "repeat"		
samples and report the negative as negative.		

# 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
<ul> <li>Addition of General Seegene Reporting Process, BGI Reporting Process for Repeat Samples;</li> <li>Updated BGI reporting rules, preliminary, final and repeat sample report statements in test window and isolate window</li> <li>Addition of troubleshooting</li> </ul>	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

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Page Number / Item	Date of Revision	Edited by:
Added steps to follow when there is no BioRad	September 23, 2021	Oliver Li
thermocycler available in <b>Off board preparation</b> section		
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	March 11, 2022	Qin Liu
and Graph		

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