Performed by: _____ Date(s) Performed _____

Product/Reagent Name: _____

Lot. No.: _____

qPCR Machine:_____

Scope: To test the AzureSeq Validation Kit for LOD. (20ul Reactions size)

Equipment Required: Before performing the assay make sure that the equipment in the list below is within calibration and check the box. Record the asset no of the equipment used in appropriate areas.

Equipment	Asset #	Calibrated (Yes ☑ or No ☒)	Comments
		☐	
		☐	
		☐	
		☐	
		☐	
		☐	
		☐	
		☐	
		☐	
		☐	
		☐	
		☐	

☐ 1. Allow all reagents to thaw on ice for **30±10min**

☐ 2. Set up the qPCR protocol as following:

a. 50°C for 15 min
b. 95°C for 2 min
c. 95°C for 3 sec \
d. 60°C for 30 sec / **45 Cycles (Step c-d)**

☐ 3. Set up the qPCR Plate as shown below on the qPCR Machine.

- Set up the wells as following:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Tube 1			Tube 2			Tube 3			Tube 4		
B	Tube 5			Tube 6		Tube 7		Tube 8		Tube 9		Tube 10
C	Tube 10	Tube 11		Tube 12		Tube 13		Tube 14				
D	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1
E	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 2	Tube 2	Tube 2	Tube 2
F	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2
G	Tube 2	Tube 2	Tube 2	Tube 2	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3
H	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3

- Select the following channels for each well.

Target	Channel	Excitation/Emission (nm)
N1	FAM	490/520
N2	HEX* /VIC/JOE	530560
Rnase P	Texas Red* /ROX	590/610

*Preferred Channel

☐ 4. Dilute the CoVi Primer/Probe Mix 3 down to 4.45X by adding **367uL** of RNase Free Water directly into the stock tube (100uL), and then check the box on the tube label.
CoVi Primer/Probe Mix 3 Lot No._____ Exp. Date_____
Sterile Water/RNase Free Water Lot No._____ Exp. Date_____

☐ 5. Cap and label as 4.45X CoVi Primer/Probe Mix 3 and vortex for approximately 10 seconds.

☐ 6. Label a 2mL Eppendorf tubes as MM.

☐ 7. Add reagents in Table 1 in order shown to the Eppendorf tube labeled as MM.

Table 1: Reagent MM setup

Lot No.	Exp. Date	Component	Volume per rxn (uL)	MM 100 rxns to add (uL)	Added ☐
		4.45X COVID Multiplex Primers/Probe (from step 4)	4.5	**450**	☐
		2X InhibiTaq Multiplex MM	10	**1000**	☐
		Empirical RT	0.5	**50**	☐

☐ 8. Mix the Reagent MM by pipetting up and down ten times or vortexing.

☐ 9. Spin down the Reagent MM in a centrifuge for approximately 5 seconds.

☐ 10. Label a qPCR Plate according to plate layout in step 3.

☐ 11. Add **15uL** of MM to all labeled tubes according to plate layout in step 3.

☐ 12. In appropriate nuclease free tubes, make the SARS-CoV-2 RNA dilutions according to the table below:

	Stock Initial RNA dilution (80 cp/uL)		1X LoD of 20 copies expected				
Tube #	Volume from 80cp/uL Tube (uL)	Added?	Volume Dilution Buffer (uL)	Added?	Volume Total (uL)	Copies/uL	Copies/reaction (5uL into 20uL total)
1	15	☐	135	☐	150	8	40
2	7.5	☐	142.5	☐	150	4	20
3	3.75	☐	146.25	☐	150	2	10
4	1.25	☐	98.75	☐	100	1	5
5	0.25	☐	99.75	☐	100	0.2	1
6	1.1	☐	18.9	☐	20	4.4	22
7	1.2	☐	18.8	☐	20	4.8	24
8	1.3	☐	18.7	☐	20	5.2	26
9	1.4	☐	18.6	☐	20	5.6	28
10	1.5	☐	18.5	☐	20	6	30
11	1.6	☐	18.4	☐	20	6.4	32
12	1.7	☐	18.3	☐	20	6.8	34
13	1.8	☐	18.2	☐	20	7.2	36
14	1.9	☐	18.1	☐	20	7.6	38

☐ 13. Add **5uL** RNA dilutions to all labeled tubes according to attached qPCR Plate layout below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Tube 1			Tube 2			Tube 3			Tube 4		
B	Tube 5			Tube 6		Tube 7		Tube 8		Tube 9		Tube 10
C	Tube 10	Tube 11		Tube 12		Tube 13		Tube 14				
D	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1
E	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 1	Tube 2	Tube 2	Tube 2	Tube 2
F	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2
G	Tube 2	Tube 2	Tube 2	Tube 2	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3
H	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3

☐ 14. Seal the qPCR plate and spin down the plate using a Plate Centrifuge for approximately 30 seconds.

☐ 15. Place the qPCR plate in the qPCR machine and start the run.

☐ 16. Once the run is complete and export the data for analysis.

☐ 17. Print the amplification plots and text reports and attach to the document.

☐ 18. Number of Attachments: _____

Discussion:

Reviewed by:

Name _____ Signature _____ Date _____

Comments: