





































- Commonly run scripts are also listed in **Recent Protocols** in the main Bindit screen.
5. When the instrument is ready, press **Start**.
    - a. The instrument will prompt you to save the run with a unique name.
    - b. The user has the option to assign sample names, as well as record reagent lot numbers, to the plate prior to starting the run.
    - c. The deck will rotate and inform the user which plate to load.
    - d. Make sure the plate orientation is correct by aligning the plate's A1 well to **A1** on the deck.
    - e. After placing a plate on the deck, press **Start** in order to place the next plate.
    - f. Repeat until all plates are loaded.
    - g. Press **Start** after all plates are loaded to initiate the rest of the run.
  6. Discard all plastic consumables after each run by following the instructions on the software or control panel located on the KingFisher Flex.

### STOPPING POINT

Use RNA right away or cover the sample elution plate with an adhesive plate sealer and store at 2-8°C until needed for PCR set-up. For longer term storage, store plate at the -80°C using a foil adhesive seal.

## 3.4 Positive control preparation

### IMPORTANT

Prepare the positive control sample in **Lab Area 3**.

Prepare the positive control by diluting the Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 (1x10<sup>6</sup> copies/μL) to a working stock of 50 copies/μL, as shown in [Table 3.3](#). Make multiple aliquots at once and store at -80°C.

### WARNING

Exercise caution when performing the serial dilutions. Risk of lab contamination is high due to the highly concentrated RNA control being used.

1. Prepare a 100-fold dilution by pipetting 990 μL of nuclease-free water into a microfuge tube, then adding 10 μL of SARS-CoV-2 RNA Control. Mix well, then centrifuge briefly.
2. Prepare a 10-fold dilution by pipetting 90 μL of nuclease-free water into a microfuge tube, then adding 10 μL of the step 1 dilution. Mix well, then centrifuge briefly.
3. Prepare a 10-fold dilution by pipetting 90 μL of nuclease-free water into a microfuge tube, then adding 10 μL of step 2 dilution. Mix well, then centrifuge briefly.
4. Pipette 50 μL of nuclease-free water into a microfuge tube, then add 50 μL of Step 3 dilution. Mix well, then centrifuge briefly.

**Table 3.3 Positive Sample Control Dilution**

	Step	Final concentration (copies/ $\mu$ L)	Amount of SARS-CoV-2 RNA Control transferred ( $\mu$ L)	Source	Nuclease-free water	Final Volume ( $\mu$ L)
<b>Dilutions</b>	<b>1</b>	10,000	10	Stock	990	1,000
	<b>2</b>	1,000	10	Step 1	90	100
	<b>3</b>	100	10	Step 2	90	100
	<b>4</b>	50	50	Step 3	50	100

Label with correct copies/ $\mu$ L and store each dilution in aliquots at  $-80^{\circ}\text{C}$ . When needed to make new positive controls, take one of the tubes and dilute as per table above, from the specific copy number down.

### 3.5 RT-PCR amplification

#### IMPORTANT

Prepare the RT-PCR cocktail and add cocktail and samples to the reaction plate in **Lab Area 2**. Thermocycle the RT-PCR reaction plate in **Lab Area 3**. Maintain an RNase-free environment and keep samples on ice during use. Make sure all reagents are thawed completely at room temperature and enzymes are kept on ice. Make sure reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept on ice.

1. Turn on the PCR thermal cycler with the program shown in [Table 3.6](#), so that the heated cover can come to the correct operating temperature and there is no time delay between the RT-PCR reaction plate being prepared and cycling being started.
2. Prepare the RT-PCR cocktail in a 1.5 mL tube placed on ice or a cold block by adding reagents in the order in which they are listed in [Table 3.4](#).  
Prepare more cocktail than the number of RT-PCR reactions to be performed. Either prepare for one or more extra reactions or use a percentage extra to ensure sufficient overage is present to overcome typical pipetting variation.

**Table 3.4 RT-PCR Reaction**

Reagent	Per Reaction ( $\mu$ L)
10X PCR Buffer	0.500
MgCl <sub>2</sub> , 25 mM	0.400
dUTP/dNTP Mix	0.100
UNG (heat labile)	0.050
RNase Inhibitor	0.125
PCR Enzyme	0.200
MMLV Enzyme	0.125
SARS-CoV-2 PCR Primer	0.500
<b>RT-PCR Cocktail Final Volume</b>	<b>2.000</b>
Sample RNA/Positive Control/ Negative Control	3.000
<b>RT-PCR Reaction Final Volume</b>	<b>5.000</b>

3. Pulse vortex the tube briefly 3 times and briefly centrifuge.
4. Dispense 2.0  $\mu\text{L}$  RT-PCR cocktail into each well of a new microtiter plate.
5. Add 3.0  $\mu\text{L}$  of either sample RNA, positive control, or negative control to each well of the plate.

**Table 3.5 Samples and Controls**

Component	Volume per reaction		
	Sample reaction	Positive control reaction	Negative control reaction
RT-PCR Cocktail	2.0 $\mu\text{L}$	2.0 $\mu\text{L}$	2.0 $\mu\text{L}$
Purified sample RNA (from RNA Extraction section above)	3.0 $\mu\text{L}$	--	--
Positive control (from Positive Control Preparation section above)	--	3.0 $\mu\text{L}$	--
Purified negative control (from RNA Extraction section above)	--	--	3.0 $\mu\text{L}$
<b>Total volume</b>	<b>5.0 <math>\mu\text{L}</math></b>	<b>5.0 <math>\mu\text{L}</math></b>	<b>5.0 <math>\mu\text{L}</math></b>

6. Seal the RT-PCR reaction plate, briefly pulse vortex 1-2 times, then centrifuge at 1000 x g for 15 seconds.
7. Visually inspect the individual wells from the bottom of the reaction plate to confirm uniform and adequate cocktail solution is present in every well before continuing.
8. Thermocycle the RT-PCR reaction plate using the conditions in [Table 3.6](#). On a standard cycler with a ramp rate of approximately 3-4°C/s this program takes approximately 1 hour and 45 minutes.

**Table 3.6 RT-PCR Thermal Cycling Conditions**

Step		Temperature	Time	Number of Cycles
1	UNG incubation	25°C	5 minutes	1 cycle
2	RNA reverse transcription into cDNA	55°C	10 minutes	1 cycle
3	Polymerase activation	95°C	2 minutes	1 cycle
4	PCR to amplify specific fragments	95°C	30 seconds	40 cycles of steps 4-6
5		60°C	30 seconds	
6		72°C	30 seconds	
7	Final extension	72°C	5 minutes	1 cycle
8	Sample preservation	10°C	--	Hold

## STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

### 3.6 SAP reaction

#### IMPORTANT

Prepare the SAP cocktail in **Lab Area 2**. Add the SAP cocktail to the RT-PCR reaction plate and thermocycle the plate in **Lab Area 3**. Make sure all reagents are thawed completely and enzymes are kept on ice. Make sure all reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept on ice.

1. Prepare the SAP cocktail in a 1.5 mL tube on ice or a cold block as shown in [Table 3.7](#). Prepare more cocktail than the number of SAP reactions to be performed. Either prepare for one or more extra reactions or use a percentage extra to ensure sufficient overage is present to overcome typical pipetting variation.

**Table 3.7 SAP Cocktail**

Reagent	Per reaction (µL)
HPLC-grade water	1.53
SAP Buffer	0.17
Shrimp Alkaline Phosphatase (SAP)	0.30
<b>SAP Cocktail Final Volume</b>	<b>2.00</b>

2. Pulse vortex the tube briefly 3 times and briefly centrifuge.
3. Centrifuge the RT-PCR reaction plate at 1000 x g for 15 seconds.
4. Dispense 2 µL of SAP cocktail into each well of the reaction plate.
5. Seal the reaction plate, briefly pulse vortex 1-2 times, then centrifuge at 1000 x g for 15 seconds.
6. Visually inspect the individual wells from the bottom of the reaction plate to confirm uniform and adequate solution is present in every well before continuing.
7. Thermocycle the reaction plate using the conditions in [Table 3.8](#). On a standard cycler with a ramp rate of approximately 3-4°C/s this program takes approximately 15-20 minutes.

**Table 3.8 SAP Cycling Conditions**

Step	Temperature	Time	Number of Cycles	
<b>1</b>	Dephosphorylation	37°C	10 minutes	1 cycle
<b>2</b>	Enzyme inactivation	85°C	5 minutes	1 cycle
<b>3</b>	Sample preservation	10°C	--	Hold

#### STOPPING POINT

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

### 3.7 iPLEX Pro extension reaction

#### IMPORTANT

Prepare the extension reaction cocktail in **Lab Area 2**. Add the extension reaction cocktail to the reaction plate and thermocycle the plate in **Lab Area 3**. Make sure all reagents are thawed completely and enzymes are kept on ice. Make sure all reagents are homogenized before taking aliquots. If plates were stored frozen prior to this step, make sure they are thawed completely, gently homogenized, spun down, and kept on ice.

1. Prepare the extension cocktail in a 1.5 mL tube on ice or a cold block, as shown in [Table 3.9](#). Prepare more cocktail than the number of extension reactions to be performed. Either prepare for one or more extra reactions per extension or use a percentage extra to ensure sufficient coverage is present to overcome typical pipetting variation.

**Table 3.9 Extension Cocktail**

Reagent	Per reaction (µL)
HPLC-grade water	0.62
iPLEX Buffer Plus, GPR	0.20
iPLEX Termination Mix	0.20
iPLEX Pro Enzyme	0.04
SARS-CoV-2 Extend Primer	0.94
<b>Extension Cocktail Final Volume</b>	<b>2.00</b>

2. Pulse vortex the tube briefly 3 times and briefly centrifuge.
3. Centrifuge the reaction plate at 1000 x g for 15 seconds.
4. Dispense 2 µL of extension reaction cocktail into each well of the reaction plate.
5. Seal the reaction plate, briefly pulse vortex 1-2 times, then centrifuge at 1000 x g for 15 seconds.
6. Visually inspect the individual wells from the bottom of the reaction plate to confirm uniform and adequate solution is present in every well before continuing.
7. Thermocycle the reaction plate using the conditions in [Table 3.10](#). On a standard cycler with a ramp rate of approximately 3-4°C/s this program takes approximately 45 minutes.

**Table 3.10 Extension Thermal Cycling Conditions**

	Step	Temperature	Time	Number of Cycles
1	Initial denaturation	95°C	30 seconds	1 cycle
2	Denaturation	95°C	5 seconds	40 cycles of steps 2-4
3	Annealing/Extension	52°C	15 seconds	
4	Denaturation	80°C	5 seconds	
5	Final extension	72°C	3 minutes	1 cycle
6	Sample preservation	10°C	--	Hold

**STOPPING POINT**

If not proceeding directly to the next step, the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing for more than 24 hours). Do not store for more than 2 weeks.

---

**3.8 Water addition**

1. Add 41 µL HPLC-grade water to each well of the reaction plate.
2. Seal the plate and centrifuge at 1000 x g for 1 minute.

**STOPPING POINT**

If not proceeding directly to processing the plate on the MassARRAY System ([Chapter 4](#)), the reaction plate should be sealed, and stored at 4°C (if storing for less than 24 hours), or at -20°C (if storing more than 24 hours). Do not store for more than 2 weeks.

---

## Data Acquisition on the MassARRAY System with Chip Prep Module 96

### 4.1 Create an input file

1. Double-click the Chip Linker icon on the desktop.
2. In the dialog box that appears, enter your username, password, and server.
3. Click **Connect**. The Chip Linker window appears.
4. Select a plate in the Chip Linker directory tree.
5. Select **iPLEX** as the terminator chemistry.
6. Select **Genotype+Area** for the process method.
7. Select **Nanodispenser 96 to 96** as the dispenser method.
8. Enter an experiment name.
9. Enter the SpectroCHIP Array barcode or other SpectroCHIP Array identifier.
10. Click **Add**. The input information appears in the Chip Linker table.
11. If a second SpectroCHIP Array will be processed, repeat step 4 to step 10 for the second SpectroCHIP Array.
12. Click **Create** to create an input XML file. This file will be selected for use when you set up the automatic run.

### 4.2 Prepare the instrument

1. Double-click the **Start All** icon to start MassARRAY Caller, Analyzer Control, Chip Prep Controller (CPC), and SpectroACQUIRE.
2. In the Status section in the Run Setup tab of SpectroACQUIRE check the Waste Tank, System Fluid, and Resin buttons; they should be green/Okay. If any are red (Waste Tank Full, System Fluid Empty, Resin Low or Empty) perform the necessary maintenance.
3. Click **Chip prep module Deck In/Out** at the top of the SpectroACQUIRE window. The deck will extend.
4. If there are SpectroCHIP Arrays in the completed chips position on the deck remove them.
5. Allow the calibrant to equilibrate to room temperature for 5 minutes (if it has been refrigerated) or 10 minutes (if it has been frozen). Pipette 75  $\mu$ L of calibrant into the calibrant vial and place in the calibrant vial holder on the deck.
6. Load the SpectroCHIP Arrays.
  - a. Orient the Chip holder so that the bevelled corner is at the top right.
  - b. Open a new SpectroCHIP Array pouch and insert the new SpectroCHIP Array into the chip holder in position 1 (on the left); orient the SpectroCHIP Array such that the Agena logo and barcode are at the bottom. Make sure that the SpectroCHIP Array is properly seated so that the chip holder and SpectroCHIP Array surface are flush.

- c. If you are processing two plates, insert another new SpectroCHIP Array into the chip holder in position 2 (on the right). If you are only processing one plate, place a previously completed SpectroCHIP Array in position 2, as the MassARRAY Analyzer requires both SpectroCHIP Array positions to be filled to function properly.
  - d. Place the full chip holder in the chip holder tray, new chips position, on the deck.
7. Load microtiter plates (MTPs). Up to two 96-well microtiter plates of analyte may be loaded.



### WARNING

The Chip Prep Module is configured at installation for the particular plates you will be using. If at any point you wish to change the plates you are using, contact Agena Bioscience Customer Support to update instrument configuration. Using plates with different well depth without reconfiguration can damage the instrument or compromise assay performance.

- a. Centrifuge the plates at 1000 x g for 1 minute.
  - b. Place the first MTP on MTP holder 1 (on the left). This corresponds to chip position 1. Orient the plate such that well A1 is in the front left corner.
  - c. If a second MTP is being processed, place it on MTP holder 2 (on the right). This corresponds to chip position 2.
8. Click **Chip prep module Deck In/Out** again to retract the deck.

## 4.3 Set up and start the run



### WARNING

Confirm that instrument settings are correct, as shown in the tables below, prior to each run.

1. Select the **Run Setup** tab in SpectroACQUIRE.
2. In the Experiment Setup section:
  - a. Under MTP 1, click on the browse button next to Experiment Name and select the XML input file created earlier.
  - b. In the Wells to Process field, select **Automatic**.
  - c. Repeat steps 1 and 2 for MTP 2 if running two plates. Otherwise, select **None** in the Experiment Name and Wells to Process fields for MTP 2.
  - d. Enter the settings for the SpectroCHIP type being used, as shown in [Table 4.1](#).

**Table 4.1 Experiment Setup Settings**

Setting	Value
Use Autotune	Selected
Start Dispense Condition	600
Resin Volume	13
Sample Volume	n/a

3. In the Analyzer Setup section:
  - a. Select **Tools > Load Parameters** on the SpectroACQUIRE toolbar, then select the appropriate parameter file based on the SpectroCHIP type. Acquisition parameters will automatically populate.
  - b. Make sure all settings in this section are as shown in [Table 4.2](#).



**Table 4.2 Analyzer Setup Settings**

Setting	Value
Parameter file name	iPLEX_CPM.par
Shots (n)	30
Maximum acquisitions	9
Minimum good spectra	5
Maximum good spectra	5
Turn Off HV After Analysis	Selected
Analyze Calibrant Pads	Selected
Filter Saturated Shots	Selected
Chip Type	SpectroCHIP CPM-96

4. In the Chip Prep Module Setup section:
  - a. Check the **Normal Operation** box.
  - b. Check **MTP Barcodes Required**, if desired.
  - c. Select **iPLEX** in the Chemistry drop-down menu.
5. In the Temperature Control section:
  - a. If the MTP and calibrant will not be removed from the instrument shortly after the run is completed, select the **MTP Cool** box, and check the **Auto** box.
  - b. Check the **Chip Heat** box and enter **30** in the Setpoint field.
6. If desired, enter your email and check the **When Chip prep module is finished** and **When MA4 is finished** boxes to receive email notifications.
7. Click **Start Chip prep module** to start the run.

#### 4.4 Remove plates, calibrant, and SpectroCHIP Arrays when run is complete

1. Once the run is completed, click **Chip prep module Deck In/Out** to move the deck out and remove the MTPs and calibrant vial. Store remaining calibrant refrigerated or frozen for future use.
2. Click **Remove Old Chips from MA4** in the Run Setup tab. The instrument will move the completed SpectroCHIP Arrays from the MassARRAY Analyzer to the completed chips position on the Chip Prep Module deck, and then extend the deck so you may remove the SpectroCHIP Arrays from the completed chips position.

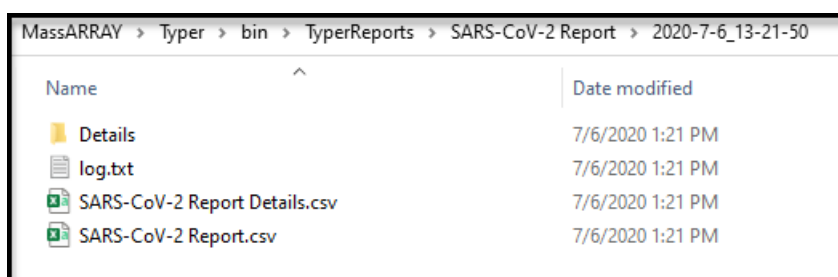
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## Analysis and Results

### 5.1 Analyze the data

1. Open MassARRAY Typer Analyzer and in the Project Explorer pane double click on the SpectroCHIP Arrays of interest. The SpectroCHIP Arrays will be added to the Chip List.
2. Load the SpectroCHIP Arrays by checking the box next to the SpectroCHIP Array names in the Chip List.
3. Select **File > Reports > SARS-CoV-2 Report-v1** in the MassARRAY Typer Analyzer menu bar.

When the report is complete, the *SARS-CoV-2 Report Details.csv* will automatically open, and the results will be made available in a date- and time-stamped folder in the MassARRAY/Typer/bin/TyperReports/SARS-CoV-2 Report folder.



### 5.2 Interpretation of the results

#### Interpretation of MassARRAY SARS-CoV-2 Panel Controls

The results from testing control samples are interpreted according to the criteria described in [Table 5.1](#).

**Table 5.1 Interpretation of MassARRAY SARS-CoV-2 Panel Controls**

Control Type	External Control Name	N1	N2	N3	ORF1	ORF1ab	MS2
Negative***	Negative Control	ND	ND	ND	ND	ND	D
Positive**	Positive Control	D	D	D	D	D	ND
Extraction	MS2 Control	n/a	n/a	n/a	n/a	n/a	D*

ND=Not Detected; D=Detected

\*Can be ND if SARS-CoV-2 is Detected in at least  $\geq 2$  assays.

\*\*PC will pass QC only if it is Detected by all targets

\*\*\*NC will pass QC only if it is Not Detected by all targets

### Examination and interpretation of patient specimen results

Interpretation of the results is performed by the Agena Bioscience SARS-CoV-2 Report software.

Assessment of clinical specimen test results must be performed after the positive and negative controls (included on each plate) have been examined and determined to be valid and acceptable (i.e., QCStatus = PASS). If the controls are not valid, the patient results cannot be interpreted. The results from testing of patient samples are interpreted according to the criteria described in [Table 5.2](#).

**Table 5.2 Expected Results for MassARRAY SARS-CoV-2 Panel Patient Specimens**

N1	N2	N3	ORF1	ORF1 ab	MS2	QC Status	Result Interpretation	Report	Action
Any 2 or more (+)					(+)	PASS	MS2 is detected, and 2 or more SARS-CoV-2 assays detected, result is Detected	SARS-CoV-2 <b>Detected</b>	Report results
Any 1 (+)					(+)	PASS	MS2 is detected, and only 1 SARS-CoV-2 assay detected, result is Inconclusive	SARS-CoV-2 <b>Inconclusive</b>	Repeat test on original sample. If the repeat result remains inconclusive, report results. Additional confirmation testing should be conducted if clinically indicated.
None (+)					(+)	PASS	MS2 is detected, and 0 SARS-CoV-2 assays detected, result is Not Detected	SARS-CoV-2 <b>NotDetected</b>	Report results. Consider testing for other viruses.
Any 2 or more (+)					(-)	WARNING*	MS2 is not detected, and 2 or more SARS-CoV-2 assays detected, result is Detected	SARS-CoV-2 <b>Detected</b>	Report results.
Any 1 (+)					(-)	WARNING*	MS2 is not detected, and only 1 SARS-CoV-2 assay detected, result is Inconclusive	SARS-CoV-2 <b>Inconclusive</b>	Repeat test on original sample. If the repeat result remains inconclusive, report results. Additional confirmation testing should be conducted if clinically indicated.
None (+)					(-)	FAIL	MS2 is not detected, and 0 SARS-CoV-2 assays detected, result is Invalid	<b>Invalid</b>	Repeat test on original sample. If the repeat result remains invalid, consider collecting a new specimen.

\*Refer to Action column for operator response when QC status is *WARNING*.

## Performance Characteristics

### 6.1 Limit of detection (LoD)

This LoD study established the lowest SARS-CoV-2 viral concentration (copies of SARS-CoV-2 RNA per  $\mu\text{L}$  of clinical matrix) that can be detected by the MassARRAY SARS-CoV-2 Panel at least 95% of the time. Negative screened NP pooled clinical matrix in VTM by the MassARRAY SARS-CoV-2 Panel test was used for analytical sensitivity studies. The negative screen NP pool clinical matrix in VTM was spiked with heat inactivated SARS-CoV-2 virus (BEI Resources, part number NR52286) at specified concentrations and processed through the MassARRAY SARS-CoV-2 Panel workflow on the MassARRAY System with Chip Prep Module 96 as described in Chapters 2-5. A two-phase approach was used to determine the LoD.

#### Phase One

Preliminary LoD determination was performed testing 3 replicates of 2-fold dilution of heat inactivated SARS-CoV-2 virus (BEI Resources, part number NR52286) at 0.08 copies/ $\mu\text{L}$ , 0.16 copies/ $\mu\text{L}$ , 0.31 copies/ $\mu\text{L}$ , 0.63 copies/ $\mu\text{L}$ , 1.25 copies/ $\mu\text{L}$ , 2.5 copies/ $\mu\text{L}$ , 5 copies/ $\mu\text{L}$  and 10 copies / $\mu\text{L}$ . The preliminary LoD was determined at 0.31 copies/ $\mu\text{L}$ . See [Table 6.1](#).

**Table 6.1 Preliminary LoD Determination Study Results, Phase 1**

SARS-CoV-2 copies/ $\mu\text{L}$	# Detected/Total Replicates	% Positive
0	0/20	0%
0.08	1/3	33%
0.16	0/3	0%
0.31	3/3	100%
0.63	3/3	100%
1.25	3/3	100%
2.50	3/3	100%
5.00	3/3	100%
10.00	3/3	100%

#### Phase Two

NP specimen LoD was confirmed at 2.5 copies/ $\mu\text{L}$  with 20 out of 20 replicates detected. See [Table 6.2](#).

**Table 6.2 LoD Determination Study Results, Phase 2**

SARS-CoV-2 copies/ $\mu\text{L}$	# Detected/Total Replicates	% Positive
0	0/20	0%
0.31	7/20	35%
0.63	12/20	60%
1.25	18/20	90%
2.50	20/20	100%

## 6.2 Inclusivity

Homology verification (inclusivity) was carried out using the sequence data from 18,772 complete SARS-CoV-2 genomes (NCBI 6OCT2020). A multiple sequence alignment (MAFFT version 7) was carried out to generate the consensus sequence, which was used to determine conserved regions of the virus. All assay components listed in [Table 6.3](#) showed 100% sequence homology except for the forward PCR primer for the SC2\_N2 assay (see [Table 6.3](#)).

According to the in-silico analysis (6OCT2020), 32% of the SARS-CoV-2 sequences have a novel multi-nucleotide polymorphism that overlaps with the first three 5' end nucleotides of the SC2\_N2 forward PCR primer (AAC vs. GGG at the 5' end of the 22 total nucleotides). This results in the 86% PCR primer homology (3-nucleotide mismatch in a 22-nucleotide primer) for the 32% of SARS-CoV-2 population with the AAC sequence, resulting in a 95.5% average homology for the SC2\_N2 forward PCR primer to all available SARS-CoV-2 sequences as of October 6, 2020 (18,772). The mismatch will not affect the test performance because of the following:

- The mismatch is located at the 5' end of the PCR primer and will be tolerated by the PCR enzyme.
- The annealing temperature of the primer with the mismatch is 59 °C ([Table 6.5](#)), which is very close to the optimal 60 °C.
- The mismatch is localized to the codon substitution and we do not anticipate it to affect other nucleotides in the primer.
- The panel contains five SARS-CoV-2 target assays to remain robust in cases of underlying mutations.

**Table 6.3 MassARRAY SARS-CoV-2 Panel In-Silico Inclusivity Analysis Results**

Primer*/Probe	Minimum % Identity	Maximum Mismatches	Total Alignments	Number of Alignments with Mismatches	% Alignments with 100% Identity	Mismatch Accession Numbers
SC2_N1_PCR1	100	0	18,772	0	100	
SC2_N1_PCR2	100	0	18,772	0	100	
SC2_N1_PROBE	100	0	18,772	0	100	
SC2_N2_PCR1	95.5**	3/22	18,772	5,978	68	Codon 50, ORF14, positions 28,881-28,883
SC2_N2_PCR2	100	0	18,772	0	100	
SC2_N2_PROBE	100	0	18,772	0	100	
SC2_N3_PCR1	100	0	18,772	0	100	
SC2_N3_PCR2	100	0	18,772	0	100	
SC2_N3_PROBE	100	0	18,772	0	100	
SC2_ORF1_PCR1	100	0	18,772	0	100	
SC2_ORF1_PCR2	100	0	18,772	0	100	
SC2_ORF1_PROBE	100	0	18,772	0	100	
SC2_ORF1ab_PCR1	100	0	18,772	0	100	
SC2_ORF1ab_PCR2	100	0	18,772	0	100	
SC2_ORF1ab_PROBE	100	0	18,772	0	100	

\*PCR1 is forward primer and PCR2 is reverse primer.

\*\*95.5% average sequence homology as of 6OCT2020 as discussed above.  $[(0.86 \times 0.32) + (1 \times 0.68)]$

### 6.3 Cross-reactivity

In-silico cross-reactivity was carried out with NCBI nucleotide BLAST with a list of FDA-recommended cross-reactivity micro-organisms (Table 6.4). The analysis was carried out on 6OCT2020 versions of the microbial NCBI database with identical results. The in-silico homology to cross-reactivity micro-organisms is the primer-length adjusted BLAST homology. The highest non-CoV-2 homology and the specie (from the FDA list of cross-reactive microorganisms, Table 6.4) are listed in Table 6.5.

Components of three assays (forward primer and probe for N1 assay, forward primer for N2 assay, and probe for ORF1ab assay) exhibit greater than the 80% homology to a listed evaluation species. However, the risk of non-specific amplification and an erroneous detection of non-SARS-CoV-2 RNA is very small in all three cases. Agena iPLEX technology is a two-step process where an initial PCR step is followed by a probe extension step. Therefore, the assay PCR primers are used to initiate cDNA syntheses and amplify the template library while the probes are used to interrogate the template via a single-base extension. The RT-PCR and the extend steps instill target specificity and remove the deleterious PCR-probe primer interaction.

Following is an analysis of the cross-reactivity results for the three assays that exhibit >80% homology in at least one of their components:

- SC2\_N1 assay. Two SC2\_N1 assay components (forward primer and probe) exhibit >80% *in silico* homology to a listed organism (SARS-coronavirus). However, the cross-reactive assay components are not the PCR primers, but a combination of one PCR primer and the probe. The forward primer showed 82% homology while the reverse primer showed 75% homology and the probe showed 94% homology to SARS-coronavirus. The SC2\_N1 reverse primer shows low homology, therefore the risk of non-specific PCR amplification of SARS-coronavirus is low. This is confirmed by the NCBI Primer BLAST. Furthermore, reverse transcription of RNA into DNA requires high reverse PCR primer homology. Low homology of the reverse primer further reduces non-specific reverse-transcription and subsequent amplification.
- SC2\_N2 assay. One SC2\_N2 assay component (forward primer) exhibits >80% homology to a listed organism (SARS-coronavirus). The forward PCR primer showed 91% homology while the reverse primer showed 68% homology and the probe showed 55% homology to SARS-coronavirus. The SC2\_N2 reverse primer and probe show low homology, therefore the risk of non-specific PCR amplification and probe extension of SARS-coronavirus is low. This is confirmed by the NCBI Primer BLAST. Furthermore, reverse transcription of RNA into DNA requires high reverse PCR primer homology. Low homology of the reverse primer further reduces non-specific reverse-transcription and subsequent amplification.
- -SC2\_ORF1ab assay. One SC2\_ORF1ab assay component (probe) exhibits >80% homology to a listed organism (SARS-coronavirus). The probe showed 88% homology while the forward primer showed 76% and the reverse primer showed 37% homology to SARS-coronavirus. The SC2\_ORF1ab forward and reverse primers show low homology, therefore risk of non-specific PCR amplification of SARS-coronavirus is low. This is confirmed by the NCBI Primer BLAST. Furthermore, reverse transcription of RNA into DNA requires high reverse PCR primer homology. Low homology of the reverse primer further reduces non-specific reverse-transcription and subsequent amplification.

**Table 6.4 List of Organisms Analyzed *in Silico* Cross-Reactivity**

Other high priority pathogens from the same genetic family	High priority organisms likely present in a respiratory specimen
Human coronavirus 229E	Adenovirus (e.g., C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g., EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	<i>Chlamydia pneumoniae</i>
	<i>Haemophilus influenzae</i>
	<i>Legionella pneumophila</i>
	<i>Mycobacterium tuberculosis</i>
	<i>Streptococcus pneumoniae</i>
	<i>Streptococcus pyogenes</i>
	<i>Bordetella pertussis</i>
	<i>Mycoplasma pneumoniae</i>
	<i>Pneumocystis jirovecii</i> (PJP)
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract
	<i>Candida albicans</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Staphylococcus epidermidis</i>
<i>Streptococcus salivarius</i>	



Table 6.5 MassARRAY SARS-CoV-2 Panel *in Silico* Cross-Reactivity

Assay Name	Assay Primer*	Annealing Temperature (°C)	Sequence	SARS-CoV-2 Homology %	Highest Other Specie Homology %	Highest Other Specie from FDA List (Table 6.4)
SC2_N1	SC2_N1_PCR1	65	TGAGGAAGTTGTAGCACGATTG	100	82	SARS-coronavirus
	SC2_N1_PCR2	65	AGACGGCATCATATGGGTTG	100	75	SARS-coronavirus
	SC2_N1_PROBE	59	GTGCCAATGTGATCTTT	100	94	SARS-coronavirus
SC2_N2	SC2_N2_PCR1	66 (59 with 3-base 5-prime mismatch)	GGGGAAGTTCTCTGCTAGAAT	97.3**	91	SARS-coronavirus
	SC2_N2_PCR2	64	CAGACATTTTGTCTCAAGCTG	100	68	SARS-coronavirus
	SC2_N2_PROBE	69	GCAAAGCAAGAGCAGCATCACC	100	55	Human Coronavirus NL63
SC2_N3	SC2_N3_PCR1	64	GTGGATGAGGCTGGTTCTAA	100	60	MERS-coronavirus
	SC2_N3_PCR2	59	ACTACAAGACTACCCAATTT	100	60	MERS-coronavirus
	SC2_N3_PROBE	57	GAAACTGTATAATTACCGATA	100	57	Human coronavirus HKU1
SC2_ORF1	SC2_ORF1_PCR1	63	AACTGTTGGTCAACAAGACG	100	55	Enterovirus F strain BEV-261
	SC2_ORF1_PCR2	63	CAATAGTCTGAACAAGTGGTGT	100	50	Staphylococcus phage tp310-3
	SC2_ORF1_PROBE	56	GGTCAACCTCAATTAG	100	76	Possum enterovirus W1
SC2_ORF1ab	SC2_ORF1ab_PCR1	63	CCCTGTGGGTTTTACACTTAA	100	76	SARS-coronavirus
	SC2_ORF1ab_PCR2	65	ACGATTGTGCATCAGCTGA	100	68	Enterovirus SEV-gx
	SC2_ORF1ab_PROBE	65	ATCAACTCCGGAACCC	100	88	SARS-coronavirus
MS2_Ctrl	MS2_Ctrl_PCR1	65	TTGACAATCTTTCCGCCCTG	-	55	Enterovirus AN12 genomic RNA
	MS2_Ctrl_PCR2	64	CTGACATACCTCCGACAACT	-	50	SARS-CoV-2
	MS2_Ctrl_PROBE	70	CCCAACCCCGTAGCCGATT	-	58	Human rhinovirus NAT001 polyprotein gene

\*PCR1 is forward primer and PCR2 is reverse primer.

\*\*95.5 average sequence homology as of 6OCT2020 as discussed in section 6.2: Inclusivity.  
 $[(0.86 \times 0.32) + (1 \times 0.68)]$

## 6.4 Warnings and precautions

- For *In Vitro* Diagnostic Use.
- For use under Emergency Use Authorization (EUA) Only.
- For prescription use only.
- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an EUA for use by authorized laboratories.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564 (b) (1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Care must be taken to avoid mislabeling/misidentifying samples.
- Reduced sample input can adversely affect ability to detect SARS-CoV-2 virus.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Positive results are indicative of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- For use only with personnel trained in techniques of reverse transcriptase-PCR and *in vitro* diagnostic procedures.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with COVID-19 is suspected, specimens should be collected with appropriate infection control precautions.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases.
- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling samples. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Dispose of waste in compliance with local, state, and federal regulations.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- Fresh clean gloves must be worn in each area and must be changed before leaving that area.
- Do not pipette by mouth.

- Safety Data Sheets (SDS) are available on the Agena customer support portal, or by contacting Customer Support.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted.
- Do not use the kit or any kit components past the expiration date indicated on the kit carton label.
- In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
- Reagents must be stored as specified in Table 1.3 or by their manufacturer.
- Ensure all equipment utilized is calibrated and maintained according to manufacturer instructions.

## 6.5 Limitations

- This device may not be able to differentiate newly emerging SARS-CoV-2 subtypes.
- Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Samples (nasopharyngeal swab, oropharyngeal swab, nasal and mid-turbinate swabs, nasal and nasopharyngeal aspirate, and bronchoalveolar lavage) must be collected, transported, and stored using appropriate procedures and conditions, as outlined in CDC guidelines ([https:// www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html](https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html)). Improper collection, transport, or storage of samples may hinder the ability of the assay to detect the target sequences.
- The performance of the MassARRAY SARS-CoV-2 Panel was established using nasopharyngeal swabs (NP) samples. Oropharyngeal swabs, nasal and mid-turbinate swabs, nasal and nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) samples are also considered acceptable sample types for use with the MassARRAY SARS-CoV-2 Panel, but performance has not been established.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- This test is a qualitative test and does not provide the quantitative value of detected organisms present.
- There is a risk of false positive values resulting from:
  - Cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
  - Cross-contamination during sample handling or preparation.
  - Cross-contamination between patient samples.
  - Sample mix up.
  - RNA contamination during product handling.

- There is a risk of false negative values due to:
  - The presence of sequence variants in the pathogen targets of the assay, procedural errors, amplification inhibitors in samples, or inadequate numbers of organisms for amplification.
  - Improper sample collection.
  - Sample mix up.
  - Degradation of the SARS-CoV-2 RNA during shipping/storage.
  - Sample collection does not collect SARS-CoV-2 RNA.
  - Using unauthorized extraction or assay reagents.
  - The presence of RT-PCR inhibitors.
  - Mutation in the SARS-CoV-2 virus.
  - Failure to follow instructions for use.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
- This test cannot rule out infections caused by other viral or bacterial pathogens not present on this panel.
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- This device has been evaluated for use with human sample material only.
- The performance of this device has not been evaluated for patients without signs and symptoms of infection.
- The performance of this device has not been evaluated for monitoring treatment of infection.
- Based on the in-silico cross-reactivity analysis, other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with MassARRAY SARS-CoV-2 Panel. Other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 are not known to be currently circulating in the human population, and therefore are highly unlikely to be present in patient specimens.

## 6.6 Conditions of authorization for laboratory

The MassARRAY SARS-CoV-2 Panel Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling, are available on the FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>.

However, to assist clinical laboratories using the MassARRAY SARS-CoV-2 Panel (“your product” in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories<sup>1</sup> using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- B. Authorized laboratories using your product will use your product as outlined in the *MassARRAY SARS-CoV-2 Panel Instructions for Use* (IFU-CUS-001). Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Agena Bioscience, Inc. (1-858-882-2800; helpdesk@AgenaBio.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- F. All laboratory personnel using your product must be appropriately trained in molecular and mass spectrometry techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- G. Agena Bioscience, Inc. and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

<sup>1</sup> The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”

## 6.7 Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the MassARRAY SARS-CoV-2 Panel for testing nasopharyngeal (NP) swab specimens in VTM from patients suspected of COVID-19 by their health providers concurrently with another Real-Time RT-PCR kit authorized by the FDA.

A total of 63 positive and 60 negative specimens were tested. The samples were randomized, blinded, and tested with the MassARRAY SARS-CoV-2 Panel as described in Chapters 2-5.

All negative samples were tested negative. Of the positive samples, 56 tested positive, 3 tested negative, and 4 tested Inconclusive. Of the 4 Inconclusive samples, 1 was retested and found to be positive; 3 were unable to be retested due to insufficient sample volume and were excluded from the analyses. [Table 6.6](#) provides a summary of the clinical evaluation results.

**Table 6.6 Performance of the MassARRAY SARS-CoV-2 Panel Against EUA Comparator Test**

		Comparator Test EUA SARS-CoV-2 Assay		
		Detected	Not Detected	Total
MassARRAY SARS-CoV-2 Panel	Detected	57	0	57
	Not Detected	3 <sup>1</sup>	60	63
	Inconclusive	3 <sup>2</sup>	0	3
	Total	63	60	123
<b>Positive Percent Agreement: 95% (57/60) 95% CI: 86.3% to 98.3%</b>				
<b>Negative Percent Agreement: 100% (60/60) 95% CI: 94.0% to 100%</b>				

<sup>1</sup>Three (3) samples were not detected with the MassARRAY SARS-CoV-2 Panel and were detected by the comparator SARS-CoV-2 assay. These 3 false negative samples had low viral load with high Ct values with the comparator SARS-CoV-2 assay.

<sup>2</sup>Three (3) samples had inconclusive results and were not retested due to insufficient sample volume. They were excluded from the statistical analysis. These 3 samples had low viral load with high Ct values with the comparator SARS-CoV-2 assay. Agena Bioscience will further evaluate the performance of the MassARRAY SARS-CoV-2 Panel in a post-authorization study.

## 6.8 Carry-over and cross contamination study

A study was performed to assess the carry-over/cross-contamination of the MassARRAY SARS-CoV-2 Panel. The study was performed by testing an alternating series (i.e., checkboard pattern) of high positive ( $1 \times 10^5$  copies/mL of heat inactivated SARS-CoV-2 virus, BEI Resources part number NR52286 spiked in negative NP samples in VTM) and negative samples (negative screened NP pooled clinical matrix in VTM by the MassARRAY SARS-CoV-2 Panel test) on two full 96-well PCR plates and tested with the MassARRAY SARS-CoV-2 Panel as described in Chapters 2-5.

No false positive results were observed during testing of high positive samples alternating with negative samples. Results demonstrate that recommended sample handling and testing protocols are effective in preventing false positive results due to carryover or cross-contamination between samples. [Table 6.7](#) presents a summary of the results.

**Table 6.7 Summary of Carry-over/Cross Contamination Study**

Sample Group	N	Detected %	NotDetected %	Invalid %
Negative	94	0 (0%)	94 (100%)	0 (0%)
High Positive ( $1 \times 10^5$ copies/mL)	94	94 (100%)	0 (0%)	0 (0%)

## 7.1 Customer support

Please contact your local Agena Bioscience office for customer support.

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[0620]

## 7.2 Related documentation

**Table 7.1 Related User Documentation**

Document	Document #
<i>MassARRAY System with Chip Prep Module 96 User Guide</i>	USG-CUS-069
<i>MassARRAY Typer User Guide</i>	USG-CUS-027