MassARRAY® SARS-CoV-2 Panel

INSTRUCTIONS FOR USE

Multiplex RT-PCR/MALDI-TOF test intended for the qualitative detection of nucleic acid from SARS-CoV-2

REF 13279F, 13278D, 13281D

IFU-CUS-001 R06

11/20/20

IVD

For *in vitro* diagnostic use. Rx only.
For Emergency Use Authorization Only.

Agena® BIOSCIENCE

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[0819]

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[0818]

REF	13279F	Σ	960 tests
REF	13281D	Σ	3840 tests
REF	13278D	Σ	768 tests

Revision History

Revision Number	Date	Changes Made
R06	11/20/20	• Update to section 7.8, Carry-over and cross contamination study.
R05	11/10/20	• Added MassARRAY System with Chip Prep Module (CPM) 384. • Updated limit of detection study section (page 27) and carry-over and cross contamination study section (page 37) to include MassARRAY System with CPM 384 data.

R04	10/26/20	• Changed panel name to MassARRAY SARS-CoV-2 Panel.
		• Removed CE mark and EU and Australia representatives (now separate IFUs).
		• Added Emergency Use Authorization language.
		• Removed MassARRAY Analyzer/Nanodispenser RS1000 System and MassARRAY System with Chip Prep Module 384.
		• Updated intended use statement and sample types.
		• Added information on allowable freeze-thaw cycles for panel kit (page 3).
		• Added clarification to Table 1.6: Controls.
		• Updated the Warnings and Precaution section and the Limitations section.
		• Changed RNA extraction method to Mag-Bind/KingFisher method (page 9).
		• Updated thermocyling conditions (Table 3.6, Table 3.8, Table 3.10).
		• Updated concentration of SARS-CoV-2 RNA to be used in preparing positive control samples (page 11).
		• Updated to SARS-CoV-2 Report v1.3 software:
		  • *Inconclusive* result now reported when number of SARS-CoV-2 targets detected is 1.
		  • A negative control with a result of *Inconclusive* will fail QC.
		  • Wording of QC messages edited for clarity.
		• Added detail to Table 6.2: Expected Results for MassARRAY SARS-CoV-2 Panel Patient Specimens.
		• Updated : Performance Characteristics with new study results.

R03	7/9/20	• Changed amount of MS2 Control added in RNA extraction protocol to 1 µL. • Updated report software to SARS-CoV-2 Report v1.1 (name change only). • Added CE mark and EU and Australian representatives. • Updated the Intended Use statement (page 1).
R02	6/17/20	Correction to panel name and FDA statement.
R01	6/14/20	Initial release.

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Contents

Chapter 1

MassARRAY® SARS-CoV-2 Panel Product Information

1.1 Intended use

The MassARRAY SARS-CoV-2 Panel, for use on the MassARRAY System, is a reverse transcription (RT-PCR)/MALDI-TOF test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (nasopharyngeal swab, oropharyngeal swab, nasal and mid-turbinate swabs, and nasal and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited 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.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The MassARRAY SARS-CoV-2 Panel is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of reverse transcriptase-PCR and *in vitro* diagnostic procedures. The MassARRAY SARS-CoV-2 Panel is only for use under the Food and Drug Administration's Emergency Use Authorization.

1.2 Workflow/Test principle

After collection of upper respiratory (nasopharyngeal swab, oropharyngeal swab, nasal and mid-turbinate swabs, and nasal and nasopharyngeal aspirate) or bronchoalveolar lavage (BAL) specimens in viral transport media (VTM) or universal transport media (UTM), RNA extraction is performed using the ThermoFisher KingFisher Flex Purification System with the Omega Bio-Tek Mag-Bind Viral RNA Xpress Kit. RT-PCR with iPLEX® Pro chemistry then reverse transcribes and amplifies target regions of interest. After the inactivation of unincorporated dNTPs, a sequence-specific primer extension step is performed, in which a single nucleotide is added to the probe, using the supplied Extend primers and iPLEX Pro reagents.

The extension products (analyte are desalted, transferred to a SpectroCHIP® Array (a silicon chip with pre-spotted matrix crystal and then loaded into the MassARRAY Analyzer (a MALDI-TOF mass spectrometer. The analyte/matrix co-crystals are irradiated by a laser, inducing their desorption and ionization. The positively charged molecules accelerate into a flight tube towards a detector. Separation occurs by time-of-flight, which is proportional to the mass of the individual molecules. After data processing, a spectrum

is produced with relative intensity on the y-axis and mass/charge on the x-axis. Data acquired by the MassARRAY Analyzer is processed by MassARRAY Typer software, and then the MassARRAY SARS-CoV-2 Report software.

Table 1.1 Workflow

Step	See...
1. Software Setup	Chapter 2
• Import the assay design file. *Only required before the first time you run the panel.* • Create a virtual plate.	
2. Assay Protocol	
RNA extraction RT-PCR amplification SAP reaction iPLEX Pro extension reaction Water addition	
3. Data Acquisition on the MassARRAY System	
MassARRAY System with Chip Prep Module 96	
MassARRAY System with Chip Prep Module 384	
4. Generate Results Reports	

1.3 Product description

The panel consists of 5 assays targeting the genome of SARS-CoV-2: three probe the viral nucleocapsid (*N*) gene and two probe *ORF1* and *ORF1ab*. The panel also contains an MS2 phage assay that monitors RNA extraction.

Table 1.2 MassARRAY SARS-CoV-2 Panel Content

Assay Name	Region
SC2-N1	Nucleocapsid
SC2-N2	Nucleocapsid
SC2-N3	Nucleocapsid
SC2-ORF1	ORF1
SC2-ORF1ab	ORF1ab
MS2 Phage	-

1.4 Materials provided

The following items (Table 1.3) are provided in the MassARRAY SARS-CoV-2 Panel Kit. Upon receipt, store the items as described.

Table 1.3 MassARRAY SARS-CoV-2 Panel Kit Contents

Materials Provided	Shipping Condition	Storage Temperature	Storage Location (see Table 3.1)
MassARRAY SARS-CoV-2 Panel • MassARRAY SARS-CoV-2 Panel PCR Primers • MassARRAY SARS-CoV-2 Panel Extend Primers • UNG (heat labile) • MMLV Enzyme • RNase Inhibitor • MS2 Control	Dry Ice	-25°C to - 10°C	Lab Area 2
PCR Reagent Set with dUTP • $MgCl_2$, 25 mM • 10X PCR Buffer • dUTP/dNTP Mix • PCR Enzyme	Dry Ice	-25°C to - 10°C	Lab Area 2
iPLEX® Pro Reagent Set • 3-Point Calibrant • iPLEX Termination Mix • iPLEX Buffer Plus, GPR • iPLEX Pro Enzyme • SAP Buffer • Shrimp Alkaline Phosphatase (SAP)	Dry Ice	-25°C to - 10°C	Lab Area 2
SpectroCHIP CPM Arrays	Ambient Temperature	15°C to 25°C	Lab Area 3

The MassARRAY SARS-CoV-2 Panel Kit can be used up to ten times, as long as components are maintained on ice or a cold block during access and returned to storage conditions after use.

Table 1.4 MassARRAY SARS-CoV-2 Panel Kits

Part #	Number of tests	Type of SpectroCHIP Array
REF 13279F	Σ 960 tests	SpectroCHIP CPM-96, 10 x 96
REF 13281D	Σ 3840 tests	SpectroCHIP CPM-384, 10 x 384
REF 13278D	Σ 768 tests	SpectroCHIP CPM-384, 2 x 384

1.5 Materials required but not provided

Table 1.5 Materials Required but not Provided

Item	Source/Specification
Instruments and Equipment	
MassARRAY System with: • Typer software v5.0.1 or greater • RT-Workstation v4.1 or greater • Chip Prep Controller v2.2 or greater	Agena Bioscience; MassARRAY System with Chip Prep Module (CPM) 96 or Agena Bioscience; MassARRAY System with Chip Prep Module (CPM) 384
Plate centrifuge	MLS; Max. RCF with plate rotor: 3,486 x g
Vortex	MLS; Variable speed, suitable for tubes and plates
Mini tube centrifuge	MLS; Recommend additional rotor option for 2 x 8-tube PCR strips
Refrigerated tube centrifuge	MLS; Capacity up to 12,000 ng
Thermocycler	MLS; With appropriate plate block; max ramp rate 4°C/second
PCR workstation with UV irradiation	MLS; UV light with timer; dual UV bulb preferred
Additional Software	
MassARRAY SARS-CoV-2 Report v1.4 or greater	Agena's customer support portal
RNA Extraction Materials	
Mag-Bind® Viral RNA Xpress Kit	Omega Bio-Tek, #M6219-2304
KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head	ThermoFisher, #5400630
KingFisher 96 deep-well plate	ThermoFisher, #95040450
KingFisher 96 standard plate	ThermoFisher, #97002540
KingFisher 96 tip comb for deep-well magnets plate	ThermoFisher, #97002534
KingFisher tip comb presenting plate	ThermoFisher, #267600
Ethanol	MLS; Absolute (200 proof), molecular biology grade
Nuclease-free water	MLS; molecular biology-grade
Isopropanol, molecular biology grade	Sigma Aldrich (#I9516) or equivalent
Additional Reagents	
Positive SARS-CoV-2 RNA Control	Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 (#102019)
HPLC-grade water	MLS; Residue after evaporation ≤2ppm; Sterile, nuclease- and DEPC-free; >18.2 MΩ
DNA AWAY™	MLS
Type 1 water or deionized water	MLS; NCCLS, CAP or ATSM; >18.2 MΩ
Clean Resin	Agena Bioscience, #08060 [40g], #08040 [28g]

Item	Source/Specification
Labware	
Electronic multichannel pipettes and filtered tips (Optional; can use manual multichannel pipettes)	MLS; 8- or 12-channel electronic adjustable tip spacing pipette; 0.5 µL -12.5 µL
Manual multichannel pipettes and filtered tips	MLS; 8- or 12-channel pipette 0.5 µL - 20 µL 20 µL - 200 µL
Single channel pipettes and filtered tips	MLS; 0.1 µL - 2 µL 0.5 µL - 10 µL 10 µL - 100 µL 100 µL - 1000 µL
Microtubes	MLS; RNase-, DNase-, human DNA-, and PCR inhibitor-free Volume: 1.5 mL, 5 mL
PCR strip tubes (optional)	MLS; 8- or 12-well strips with caps; volume: 0.2 mL
Tube racks	MLS
Sealing roller tool and paddle	MLS
Disposable pipetting reservoirs	MLS; DNase- and RNase-free; volume: 25 mL and 50 mL; sterile
Clear adhesive plate seals	MLS; Strong adhesive, -20ºC to 120ºC
MassARRAY System with Chip Prep Module 96	
96-well semi-skirted, colored microtiter plates OR 96-well non-skirted microtiter plates	ThermoFisher MicroAmp® EnduraPlate™ #4483343 or equivalent.; Working vol.: 0.2 mL; Max well volume: 0.25 mL Thermo Scientific™ PCR Plate #AB0600L or equivalent; Working volume: 0.2 mL; Max fill volume: 0.3 mL
Isopropanol	MLS
MassARRAY System with Chip Prep Module 384	
384-well microtiter plates	Thermo Scientific™ 384-Well Full Skirted PCR Plate #TF-0384 or equivalent; Full skirted: Working volume: 25 µL, Max volume: 40 µL
Isopropanol	MLS

"MLS" indicates that the item is available from major laboratory suppliers.

1.6 Samples and controls

Patient samples must be collected according to appropriate clinical guidelines. Positive and negative test controls must be included and examined prior to interpretation of patient test results. Include the following controls on each plate:

Table 1.6 Controls

Control Type	Description	Purpose	Frequency of Testing
Positive Control*	Twist Bioscience Synthetic SARS-CoV-2 RNA Control 1 #102019	Used to monitor RT-PCR reaction set up and reagent integrity.	Each run, starting at RT-PCR.
Negative Control**	Nuclease-free water	Used to monitor contamination during RNA extraction and RT-PCR reaction.	Each run, starting at extraction.
Extraction Control	MS2 Phage RNA	Use to monitor RNA extraction.	Every sample.

*PC must be “detected” with all 5 targets in order to pass QC
**NC must be “not detected” by all 5 targets in order to pass QC.

1.7 Symbols

Table 1.7 Symbols

	Catalog number		Temperature limits
	Batch code		Manufacturer
	Use-by date		For *in vitro* diagnostic use
	Quantity		Contains sufficient for <n> tests

Chapter 2

Software Setup

2.1 Import the assay design file

Note: The assay design file only needs to be imported once, prior to running the panel for the first time.

1. Locate the MassARRAY SARS-CoV-2 assay design file (*MassARRAY SARS-CoV-2_ADF.tsv*), at C/MassARRAY/Typer/bin/Reports/MassARRAY SARS-CoV-2 Report.
2. Open MassARRAY Typer Assay Editor.
3. Create a new assay project in the Database Browser by right clicking the root node and selecting **Project Administrator**.
4. Add a new Assay Project with an appropriate name. The new Assay Project will appear in the database browser. The MassARRAY SARS-CoV-2 assay design file will be stored in this project.
5. Right-click on the newly created Assay Project and select **Import Assay Group in Designer format...**
6. Remove the checkmarks next to **Design Summary** and **SNP Group**. Make sure that there is a checkmark next to **Assay Group**.
7. Click the **Browse** button next to Assay Group. In the Open window, select **All Files** in the **Files of type:** field.
8. Navigate to the folder containing the MassARRAY SARS-CoV-2 assay design file (*MassARRAY SARS-CoV-2_ADF.tsv)*, select it, and click **Open**.
9. Click the **Import** button to import the file.

2.2 Create a virtual plate

Note: Plate names and sample names should all be unique.

Create a sample group

Note: Each plate contains one positive control sample and one negative control sample. In order for the software to recognize the controls, “PC” must be the prefix for positive control samples, and “NC” must be the prefix for negative control samples (e.g., *PC_1*, *NC_1*).

1. Create a text file (.txt) of sample descriptions in a spreadsheet application such as Microsoft® Excel. The file must contain the sample ID in column A, and may contain a description in column B.
2. Create a sample customer and sample project in the database, if you have not already done so.
   a. Open the MassARRAY Typer Plate Editor software and click on the Sample tab.
   b. To create a sample customer, right-click the root-node and select **Add New Sample Customer**. Enter a sample customer ID and additional optional information and click **OK**. The new sample customer will appear in the Sample tab.
   c. To create a sample project, right-click the sample customer that the sample project will be under and select **Add New Sample Project**. Enter a sample

project ID and optional additional information and click **OK**. The new sample project will appear in the Sample tab.

3. Create a sample group.
   a. Right-click on the sample project that you want to add the sample group to and select **Add New Sample Group**.
   b. Enter a sample group ID and click the folder button in the toolbar to browse to the location of your sample group text file, and click **Open**, then click **OK**.

Create a Plate

1. Create a customer and project in the database, if you have not already done so.
   a. In the MassARRAY Typer Plate Editor software click on the Plate tab.
   b. To create a customer, right-click the root-node and select **New Customer**. Enter a customer ID and additional optional information and click **OK**. The new customer will appear in the Plate tab.
   c. To create a project, right-click the customer that the project will be under and select **New Project**. Enter a project ID and optional additional information and click **OK**. The new project will appear in the Plate tab.
2. Create a plate.
   a. Right-click on the project that you want to add the plate to and select **New Plate**.
   b. Enter a unique plate ID and select the plate type (96- or 384-well) and click **OK**. The new plate will appear in the Plate tab and a plate layout will be created automatically, based on the plate type specified.

Apply Assays to the Plate

1. In the Plate tab, select the plate that was just created.
2. Select the Assay tab and locate the MassARRAY SARS-CoV-2 assay design file you imported earlier (*MassARRAY SARS-CoV-2_ADF.tsv*).
3. In the plate layout, select the wells of interest.
4. In the Assay tab, right-click the plex or assay that you want to assign to the selected wells and select **Add plex** or **Add assay**.

Apply Samples to the Plate

1. Click on the Sample tab.
2. In the plate layout, select the wells of interest.
3. Right-click the sample or sample group that you want to assign to the selected wells and select **Apply Samples from Group** or **Add Sample**.
4. Select **File** > **Save** from the toolbar.

Chapter 3

Assay Protocol

3.1 Lab areas and plate layout

The laboratory space should include three separate (non-contiguous) work areas to prevent contamination of PCR products. Table 3.1 shows the activities that are conducted in each area.

Table 3.1 Lab Area Activities

Lab Area	Activities
1	Isolation and preparation of RNA.
2	Pre-PCR preparation, including preparation of the RT-PCR cocktail, addition of the RT-PCR cocktail and RNA to the reaction plate, and preparation of the SAP and extension cocktails.
3	Positive control sample preparation. Thermocycling the reaction plate after addition of RT-PCR cocktail and RNA; addition of the SAP cocktail to the reaction plate and thermocycling; addition of the extension reaction cocktail to the reaction plate and thermocycling; desalting; nanodispensing; and data acquisition.

Include one positive control and one negative control on each plate.

3.2 Sample collection, handling, and storage

Upper respiratory (nasopharyngeal swab, oropharyngeal swab, nasal and mid-turbinate swabs, and nasal and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens must be collected and stored in VTM or UTM. Samples must be collected, transported at stored using appropriate procedures and conditions as outlined in CDC guidelines (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html). Store specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

3.3 RNA extraction

IMPORTANT

Perform this procedure in **Lab Area 1**.

Preparation before each extraction

1. Thaw MS2 Control, carrier RNA, and samples on ice.
2. Shake the tubes containing the sample at room temperature for 30 minutes.
3. Use 200 µL as the input sample in Step 4 below.
4. Use 200 µL of nuclease-free water as the negative control sample input in Step 4 below.

Reagent and sample preparation

1. Determine the number of required reactions based on the number of patient samples to be processed, plus one negative control per extraction set.
2. Prepare fresh lysis master mix by adding 1 µL of carrier RNA and 5 µL of MS2 to 240 µL of TNA lysis buffer, per sample.
   Lysis master mix can be made in bulk and distributed to individual samples. It is recommended to use a 10% overage for 96 or more samples, and 15% for less than 96 samples.
3. Ensure Mag-Bind Particles RQ are completely resuspended in solution before adding to master mix by vortexing and mixing thoroughly.
   a. Prepare fresh binding master mix by adding 5 µL of Mag-Bind Particles RQ to 280 µL of 100% Isopropanol per sample. Binding master mix can be made in bulk and distributed to individual samples. It is recommended to use a 10% overage for 96 or more samples, and 15% for less than 96 samples.
4. Transfer 200 µL of sample to each well of a KingFisher 96 deep-well plate.
5. Add 246 µL lysis master mix to each sample. Mix samples by pipetting up and down 10 times.
6. Add 285 µL binding master mix to each sample. Mix samples by pipetting up and down 10 times.
7. Prepare other reagent plates according to Table 3.2. The plate position dictates deck positions of each plate on the KingFisher Flex instrument. Load plates into deck positions according to Table 3.2.

Table 3.2 Plate Layout

Plate Layout Position	Plate Type	Content	Volume (µL)
1	KingFisher 96 deep-well	Lysate (lysis master mix, sample, binding master mix)	731
2	KingFisher 96 deep-well	RMP Wash Buffer	350
3	KingFisher 96 deep-well	80% Ethanol	350
4	KingFisher 96 deep-well	80% Ethanol	350
5	KingFisher 96 standard or KingFisher 96 deep-well	Nuclease-Free Water	50
6	KingFisher 96 standard or KingFisher 96 tip comb presenting	96 tip comb for deep-well magnets	n/a
7	n/a	n/a	n/a
8	n/a	n/a	n/a

KingFisher Flex operation

1. Refer to KingFisher Flex User Manual for general instructions on using the KingFisher Flex.
2. Turn on the KingFisher Flex.
3. Load Bindit 4.0 software.
4. Select **Open** and load program (**Omega_M6219_50Elution_KFF.bdz**) on instrument. Load plates onto instrument when prompted.
   - The protocol is stored on C:\Users\Public\Documents\Protocols.
   - Scripts can be obtained from Omega Bio-Tek at https://www.omegabiotek.com/.

- 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 ($1x10^6$ 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/µL)	Amount of SARS-CoV-2 RNA Control transferred (µL)	Source	Nuclease-free water	Final Volume (µL)
Dilutions	**1**	10,000	10	Stock	990	1,000
	2	1,000	10	Step 1	90	100
	3	100	10	Step 2	90	100
	4	50	50	Step 3	50	100

Label with correct copies/µL and store each dilution in aliquots at -80°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 (µL)
10X PCR Buffer	0.500
$MgCl_2$, 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
MassARRAY SARS-CoV-2 PCR Primer	0.500
RT-PCR Cocktail Final Volume	**2.000**
Sample RNA/Positive Control/Negative Control	3.000
RT-PCR Reaction Final Volume	**5.000**

3. Pulse vortex the tube briefly 3 times and briefly centrifuge.
4. Dispense 2.0 µL RT-PCR cocktail into each well of a new microtiter plate.
5. Add 3.0 µL 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 µL	2.0 µL	2.0 µL
Purified sample RNA (from RNA Extraction section above)	3.0 µL	--	--
Positive control (from Positive Control Preparation section above)	--	3.0 µL	--
Purified negative control (from RNA Extraction section above)	--	--	3.0 µL
Total volume	**5.0 µL**	**5.0 µL**	**5.0 µL**

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
SAP Cocktail Final Volume	**2.00**

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
1	Dephosphorylation	37°C	10 minutes	1 cycle
2	Enzyme inactivation	85°C	5 minutes	1 cycle
3	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 overage 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
MassARRAY SARS-CoV-2 Extend Primer	0.94
Extension Cocktail Final Volume	**2.00**

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 HPLC-grade water to each well of the reaction plate.
   a. For 96-well plates, add 41 µL.
   b. For 384-well plates, add 16 µL.
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 (), 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.

3.9 Data acquisition

Follow the data acquisition instructions in the chapter for the MassARRAY System you are using.

Table 3.11 Data Acquisition Instructions

System	Instructions
MassARRAY System with Chip Prep Module 96	Chapter 4
MassARRAY System with Chip Prep Module 384	Chapter 5

Chapter 4

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 µL 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 beveled 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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Chapter 5

Data Acquisition on the MassARRAY System with Chip Prep Module 384

5.1 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 384 to 384** 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.

5.2 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 µL 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 beveled 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 384-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.

5.3 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 as shown in Table 5.1.

Table 5.1 Experiment Setup Settings

	Value
Use Autotune	Selected
Start Dispense Condition	Default (350)
Resin Volume	10
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 5.2.

Table 5.2 Analyzer Setup Settings

	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-384

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.

5.4 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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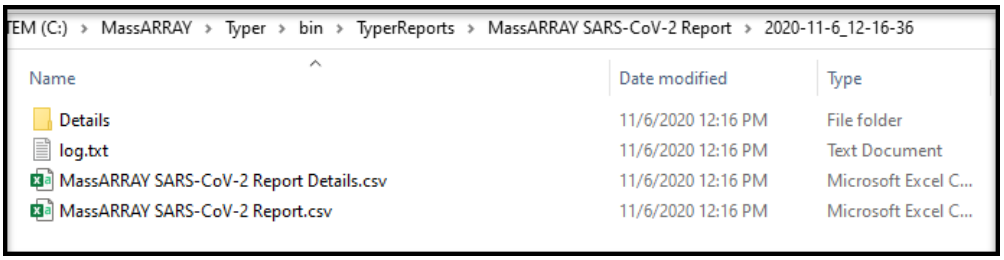
6.1 Analyze the data

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 > MassARRAY SARS-CoV-2 Report-v1** in the MassARRAY Typer Analyzer menu bar.

When the report is complete, the *MassARRAY 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/MassARRAY SARS-CoV-2 Report folder.



6.2 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 6.1.

Table 6.1 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 ≥2 assays.
**PC will pass QC only if it is Detected by all targets
***NC will pass QC only it is Not Detected by all targets

Examination and interpretation of patient specimen results

Interpretation of the results is performed by the MassARRAY 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 6.2.

Table 6.2 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 **Detected**	Report results
Any 1 (+)					(+)	PASS	MS2 is detected, and only 1 SARS-CoV-2 assay detected, result is Inconclusive	SARS-CoV-2 **Inconclusive**	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 **NotDetected**	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 **Detected**	Report results.
Any 1 (+)					(-)	WARNING*	MS2 is not detected, and only 1SARS-CoV-2assaydetected, result is Inconclusive	SARS-CoV-2 **Inconclusive**	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-2assaysdetected, result is Invalid	**Invalid**	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

7.1 Limit of detection (LoD)

This LoD study established the lowest SARS-CoV-2 viral concentration (copies of SARS-CoV-2 RNA per uL 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 both the MassARRAY System with Chip Prep Module (CPM) 96 and the MassARRAY System with Chip Prep Module (CPM) 384 as described in Chapters 2-5. A two-phase approach was used to determine the LoD.

On the MassARRAY System with CPM 96

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/µL, 0.16 copies/µL, 0.31 copies/µL, 0.63 copies/µL, 1.25 copies/µL, 2.5 copies/µL, 5 copies/µL and 10 copies /µL. The preliminary LoD was determined at 0.31 copies/µL. See Table 7.1.

Table 7.1 Preliminary LoD Determination Study Results, CPM 96 Phase 1

SARS-CoV-2 copies/µL	# 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/µL with 20 out of 20 replicates detected. See Table 7.2.

Table 7.2 LoD Determination Study Results, CPM 96 Phase 2

SARS-CoV-2 copies/µL	# 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%

On the MassARRAY System with CPM 384

Phase One

Preliminary LoD determination was performed testing 5 replicates of 2-fold dilution of heat inactivated SARS-CoV-2 virus (BEI Resources, part number NR52286) at 0.313 copies/µL, 0.625 copies/µL, 1.25 copies/µL, 2.5 copies/µL, 3.75 copies/µL, and 5 copies/µL). The preliminary LoD was determined at 1.25 copies/µL. See Table 7.3.

Table 7.3 Preliminary LoD Determination Study Results, CPM 384 Phase 1

SARS-CoV-2 copies/µL	# Detected/Total Replicates	% Positive
0	0/20	0
0.313	0/5	0
0.625	2/5	40
1.250	5/5	100
2.500	5/5	100
3.750	5/5	100
5.000	5/5	100

Phase Two

NP specimen LoD was confirmed at 2.5 copies/µL with 20 out of 20 replicates detected. See Table 7.4.

Table 7.4 LoD Determination Study Results, CPM 384 Phase 2

SARS-CoV-2 copies/µL	# Detected/Total Replicates	% Positive
0	0/20	0
1.250	18/20	90
1.875	18/20	90
2.500	20/20	100

7.2 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 7.5 showed 100% sequence homology except for the forward PCR primer for the SC2_N2 assay (see Table 7.5).

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 7.7), 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 underling mutations.

Table 7.5 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. [=(0.86x0.32)+(1x0.68)]

7.3 Cross-reactivity

In-silico cross-reactivity was carried out with NCBI nucleotide BLAST with a list of FDA-recommended cross-reactivity micro-organisms (Table 7.6). 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 7.6) are listed in Table 7.7.

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 reverser 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 7.6 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
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius

Table 7.7 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 7.6)
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-primemismatch)	GGGGAACTTCTCCTGCTAGAAT	97.3**	91	SARS-coronavirus
	SC2_N2_PCR2	64	CAGACATTTTGCTCTCAAGCTG	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	CAATAGTCTGAACAACTGGTGT	100	50	Staphylococcus phage tp310-3
	SC2_ORF1_PROBE	56	GGTTCAACCTCAATTAG	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	ATCAACTCCGCGAACCC	100	88	SARS-coronavirus
MS2_Ctrl	MS2_Ctrl_PCR1	65	TTGACAATCTCTTCGCCCTG	-	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. [=(0.86x0.32)+(1x0.68)]

7.4 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.

7.5 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). 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.

7.6 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[1] 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 (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. You, authorized distributors, 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.

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

7.7 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 7.8 provides a summary of the clinical evaluation results.

Table 7.8 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[1]	60	63
	Inconclusive	3[2]	0	3
	Total	63	60	123
Positive Percent Agreement: 95% (57/60) 95% CI: 86.3% to 98.3%				
Negative Percent Agreement: 100% (60/60) 95% CI: 94.0% to 100%				

[1]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.

[2]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.

7.8 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 ($1x10^5$ 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 two full 384-well PCR plates 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, with the exception of one negative sample which was detected by the ORF1 assay and resulted in an inconclusive result. 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 7.9 and Table 7.10 present a summary of the results.

Table 7.9 Summary of Carry-over/Cross Contamination Study, 96-well

Sample Group	N	Detected	NotDetected	Inconclusive[1]	Invalid[2]
Negative	94	0 (0%)	94 (100%)	0 (0%)	0 (0%)
High Positive ($1x10^5$ copies/mL)	94	94 (100%)	0 (0%)	0 (0%)	0 (0%)

[1]When MS2 is detected or not detected, and only one SARS-CoV-2 assay is detected, the result is inconclusive.

[2]When MS2 is not detected and zero SARS-CoV-2 assays are detected, the result is invalid.

Table 7.10 Summary of Carry-over/Cross Contamination Study, 384-well

Sample Group	N	Detected	NotDetected	Inconclusive[1]	Invalid[2]
Negative	382	0 (0%)	381 (99.7%)	1 (0.3%)	0 (0%)
High Positive ($1x10^5$ copies/mL)	382	382 (100%)	0 (0%)	0 (0%)	0 (0%)

[1]When MS2 is detected or not detected, and only one SARS-CoV-2 assay is detected, the result is inconclusive.

[2]When MS2 is not detected and zero SARS-CoV-2 assays are detected, the result is invalid.

Chapter 8

Support

8.1 Customer support

Please contact your local Agena Bioscience office for customer support.

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[0620]

8.2 Related documentation

Table 8.1 Related User Documentation

Document	Document #
MassARRAY System with Chip Prep Module 96 User Guide	USG-CUS-069
MassARRAY System with Chip Prep Module 384 User Guide	USG-CUS-097
MassARRAY Typer User Guide	USG-CUS-027