EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS-COV-2 MASSARRAY TEST
(NATIONAL JEWISH HEALTH
ADVANCED DIAGNOSTICS LABORATORY)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The SARS-CoV-2 MassArray Test will be performed at National Jewish Health Advanced Diagnostics Laboratory, located at 1400 Jackson St., Denver, CO 80206, which is certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests as per the laboratory procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 MassArray Test is a RT-PCR and MALDI-TOF mass spectrometry assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 RNA in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swabs and nasal/nasopharyngeal aspirate and wash specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the National Jewish Health Advanced Diagnostics Laboratory, located at 1400 Jackson St., Denver, CO 80206, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage 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 infective 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 SARS-CoV-2 MassArray Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of RT-PCR and MALDI-TOF mass

spectrometry and *in vitro* diagnostic procedures. The SARS-CoV-2 MassArray Test is only for use under the Food and Drug Administration's Emergency Use Authorization

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS-CoV-2 MassArray Test uses the Agena SARS-CoV-2 Panel Set kit for amplification and detection of SARS-CoV-2 RNA. The Agena assay is a real-time reverse transcription polymerase chain reaction assay that utilizes a RT-PCR to reverse transcribe viral RNA into cDNA and amplify the nucleic acid material in the same reaction from three regions of the SARS-CoV-2 single stranded RNA genome: N gene, ORF1 gene, and ORF1ab gene.

Nucleic acids are first isolated and purified from upper respiratory specimens using the MagMAX Viral/Pathogen Nucleic Acid Isolation kit on the KingFisher Flex Purification System or the Mag-Bind Viral DNA/RNA kit. The purified nucleic acid is then reverse transcribed into cDNA and subsequently amplified using a multi-step process in the Thermo Fisher Veriti thermal cycler. Reverse transcription PCR is performed using TaqPath One-step Master Mix or One-step Multiplex Master Mix with 2.5 µl of the extracted samples

The first step is the amplification of the genomic DNA using the five sets of SARS-CoV-2 PCR primers. Then, short amplicon primer (SAP) treatments are performed to dephosphorylate any remaining free deoxynucleotides that can prevent interference with extension. Finally, the primers are extended by one of the terminator nucleotides -A, T, C, and G- in the extension step, which produces allele-specific extension products of different masses.

These products are ionized and analyzed using a MALDI-TOF mass spectrometer where they are separated based on their mass-to-charge ratio through a drift tube. Using the arrival time of the individual ionized DNA analytes, the MassARRAY System determines the mass and displays a mass spectrum identifying the different genetic targets.

INSTRUMENTS USED WITH TEST

The SARS-CoV-2 MassArray Test is to be used with the following instruments:

- KingFisher Flex Purification System (Thermo Fisher, Firmware Versions 1.00.23 and 1.01.0) Catalog#5400630
- MagMAX Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher, A48310)
- Hamilton StarLet Liquid Handling System (Method Editor and Run Control version 4.5.0.7977)
- Omega Bio-Tek Mag-Bind Viral DNA/RNA Kit, Catalog # M6246-03
- Veriti 96 Thermal Cycler (Thermo Fisher, firmware version 2.0.4), Catalog#4375786
- MassARRAY System with SpectroACQUIRE software v4.3.145 and v.5.0 and TyperAnalyzer software v.4.1.83 and v5.0.1. (Agena Bioscience, Catalog#10445)

REAGENTS AND MATERIALS

The SARS-CoV-2 MassArray Test has been validated using only the components referenced in this submission.

Table 1: Reagents Used for the SARS-CoV-2 MassArray Test

Reagent	Specification	Manufacturer	Catalog #
SARS-CoV- 2 genomic RNA	5.5 x 10^7 genome equivalents/mL	BEI Resource	NR-52285, Lot #70033700
iPLEX Pro Reagent and SpectroCHIP kit	96x10	Agena	10160F
Clean Resin	28g	Agena	08040
Nuclease-free Water	Non-DEPC-Treated	Life Technologies or equivalent	AM9938 or equivalent
Primers	25 nM or 250 nM	Integrated DNA Technologies or equivalent	N/A
Master Mix	TaqPath 1-Step Multiplex Master Mix	Thermo Fisher	A28521, A28522
	TaqPath 1-Step Master Mix	Thermo Fisher	A28523,A28525
MagMAX Viral/Pathogen Nucleic Acid Isolation kit (A48310)	MagMAX Viral/Pathogen Wash Solution	Thermo Fisher	A42359
	MagMA Viral/Pathogen Wash Solution	Thermo Fisher	A42360
	MagMAX Viral/Pathogen Binding Beads (Cat. No. A42362)	Thermo Fisher	A42362
	MagMAX Viral/Pathogen Proteinase K	Thermo Fisher	A42363
	MagMAX Viral/Pathogen Elution Buffer	Thermo Fisher	A42364
KingFisher Deepwell 96 Plate	96-well plate	Thermo Fisher	95040450
Mag-Bind Viral DNA/RNA Kit	DNA/RNA it	Omega Bio-Tek	M6246-03

CONTROLS TO BE USED WITH THE SARS-COV-2 MASSARRAY TEST

- Negative Extraction Control (NEC): a previously characterized negative patient sample. It serves both as a negative extraction control to monitor for any cross contamination that occurs during the extraction process, as well as an extraction control to validate extraction reagents and successful RNA extraction. It will be performed per extraction run (Kingfisher Flex).
- SARS-CoV-2 MassArray Test: one positive and one no-template control (NTC) on every plate.
    - Positive Template Control: A positive template control is needed to verify that the assay run is performing as intended and is used on every assay plate starting at master mix addition. The positive control (extracted RNA from known positive clinical samples) will be added when loading RNA into plates.
    - No-Template Control: A no-template control is needed to eliminate the possibility of sample contamination on the assay run and is used on run started from RNA extraction plate. This control is molecular grade, nuclease-free water.
- Internal Control: an internal control which targets human RNase P (Hs_RPP30) will be used for every sample. It serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing, and also as a reverse transcription and amplification control for RT-PCR.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Table 2: Interpretation of Results for Quality Controls

Control	Control Abbreviation	Used to Monitor	N1	N2	N3	ORF1	ORF1ab	RNase P
No template control	NTC	Reagent contamination	(-)	(-)	(-)	(-)	(-)	(-)
Negative extraction control	NEC	Cross contamination, extraction efficiency	(-)	(-)	(-)	(-)	(-)	(+)
Positive template control	PTC	rRT-PCR efficiency	(+)	(+)	(+)	(+)	(+)	(+)/(-)

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the positive and negative controls are not valid, the patient results cannot be interpreted.

Table 3: Interpretation of Clinical Samples and Result Reporting

N1	N2	N3	ORF1	ORF1ab	RP	Result Interpretation	Report
Any 2 or More Targets Detected					+/-	RNase P is detected or not detected, but two or more SARS-CoV-2 targets are detected; test is valid; SARS-CoV-2 is detected	Detected
0 Targets Detected					+	The test is valid, and SARS-CoV-2 is not detected	Not Detected
Any 1 Detected					+	The test is valid and only 1 SARS-CoV-2 gene was detected; test is valid; the test is Indeterminate. Repeat once. If results are the same, result as Indeterminate	Indeterminate
Any 1 Detected					-	RNase P is not detected, and only one SARS-CoV-2 target is detected; test is valid; the test is Indeterminate. Repeat once. If results are the same, result as Indeterminate	Indeterminate

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- Use of this assay is limited to personnel who are trained in the procedure of RT-PCR and mass spectrometry. Failure to follow these instructions may result in erroneous results.
- The SARS-CoV-2 MassArray Test can be used with the specimen types listed in the Intended Use statement. Other specimen types have not been evaluated and should not be used with this assay.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - 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
- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling
- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.
- Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.
- Laboratories are required to report all results to the appropriate public health authorities.

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

The Limit of Detection (LoD) was determined for the SARS-CoV-2 MassArray Test. The Limit of Detection is the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all replicates test positive.

KingFisher Flex Purification System using MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

An initial LoD study was performed to determine the LoD of each target assay in the SARS-CoV-2 MassArray Test with SARS-CoV- 2 RNA (NR-52285, BEI Resource) that was spiked in SARS-CoV-2 confirmed-negative nasopharyngeal swab specimens at concentrations ranging from 0.34 to 110 copies/µL. Nucleic acid was extracted from the contrived samples using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler with the TaqPath 1-step Multiplex Master Mix and MALDI-TOF was performed on the Agena MassARRAY System and tested in triplicate. The preliminary LoD results are summarized in the Table below:

Table 4: LoD Determination

Concentration copies/µL	Results: Detected/Total Samples	By Targets: Detected Sample/Total Samples				
		SC2_N1	SC2_N2	SC2_N3	SC2_ORF1	SC2_ ORF1ab
110	3/3	3/3	3/3	3/3	3/3	3/3
55	3/3	3/3	3/3	3/3	3/3	3/3
27.5	3/3	3/3	3/3	3/3	3/3	3/3
13.75	3/3	3/3	3/3	3/3	3/3	3/3
11	3/3	3/3	3/3	3/3	3/3	3/3
6.8	3/3	3/3	3/3	3/3	3/3	3/3
5.5	3/3	3/3	3/3	3/3	3/3	0/3
3.4	3/3	3/3	3/3	3/3	3/3	3/3
2.75	3/3	3/3	1/3	3/3	3/3	0/3
1.38	3/3	2/3	0/3	3/3	3/3	0/3
0.69	3/3	0/3	0/3	3/3	3/3	0/3
0.34	1/3	0/3	0/3	3/3	1/3	0/3

The LoD was confirmed by spiking 24 replicates of the three lowest concentrations (0.69, 1.38 and 2.75 copies/µL) of SARS-CoV-2 into nasopharyngeal swab matrix previously confirmed to be negative for SARS-CoV-2. Nucleic acid was extracted from the contrived samples using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF was performed on the Agena MassARRAY System and tested in triplicate. The LoD results are summarized in the Table below:

Table 5: LoD Confirmation Study Summary

Concentration copies/µL	Detected/ total samples	Detected %	By Targets: Detected Sample/Total Samples				
			SC2_N1	SC2_N2	SC2_N3	SC2_ORF1	SC2_ORF1ab
2.75	23/24	95.8%	23/24*	9/24	24/24	23/24*	2/24
1.38	23/23	100%	19/23	4/23	23/23	23/23	1/23
0.69	24/24	100%	3/24	1/24	24/24	24/24	0/23

* partial evaporation was observed from one sample that might lead to lower extension**.**

The LoD of the SARS-CoV-2 MassArray Test with the KingFisher Flex Purification System using MagMAX Viral/Pathogen Nucleic Acid Isolation Kit was confirmed to be 0.69 copies/µL based on a positivity rate of ≥95% for 24/24 replicates.

Hamilton StarLet liquid handling system and Omega Viral DNA/RNA kit

An initial LoD study was performed to determine the LoD of each target assay in the SARS-CoV-2 MassArray Test SARS-CoV- 2 RNA (NR-52285, BEI Resource) that was spiked in SARS-CoV-2 confirmed-negative nasopharyngeal swab specimens at concentrations ranging from 0.69, 1.375, 2.75 and 5.5 copies/µL. Nucleic acid was extracted from the contrived samples using the Hamilton StarLet liquid handling system and Omega Viral DNA/RNA kit, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF was performed on the Agena MassARRAY System and tested in triplicate. The preliminary LoD results are summarized in the Table below:

Table 6: LOD Determination for the Hamilton StarLet Liquid Handling System and Omega Viral DNA/RNA Kit

Concentration copies/µL	Result	SC2_N1	SC2_N2	SC2_N3	SC2_ORF1	SC2_ORF1ab
5.5	3/3	3/3	1/3	3/3	3/3	0/3
2.75	3/3	0/3	0/3	3/3	3/3	0/3
1.375	3/3	1/3	0/3	3/3	3/3	0/3
0.6875	0/3	0/3	0/3	0/3	0/3	0/3

The LoD was determined as shown below in Table 7 as 2.75 copies/µL for the Hamilton StarLet Liquid Handling System and Omega Viral DNA/RNA Kit. It is the lowest concentration that > 95% of replicates are positive. NOTE: the LoD for the KingFisher-MassArray is 0.7 copies/µL compared to 2.75 copies with Hamilton-MassArray. This is likely due to the difference in input extraction volume from 400 µL (KingFisher) to 200 µL (Hamilton).

Table 7: LoD Confirmation for the Hamilton StarLet Liquid Handling System and Omega Viral DNA/RNA Kit

Concentration copies/µL	Result	%	SC2_N1	SC2_N2	SC2_N3	SC2_ORF1	SC2_ORF1ab
2.75	23/24	95.83%	14/24	14/24	23/24	16/24	0/20
1.375	8/24	33.33%	1/24	10/24	11/24	6/24	0/20

TaqPath 1-step Master Mix

An equivalency study was performed to assess the SARS-CoV-2 MassArray Test performance for use with the TaqPath 1-Step Multiplex Master Mix compared to the TaqPath 1-Step Master Mix. SARS-CoV-2 MassArray Test with SARS-CoV- 2 RNA (NR-52285, BEI Resource) was spiked in SARS-CoV-2 confirmed-negative nasopharyngeal swab specimens at concentrations ranging from 2.75, 1.38 and 0.69 copies/µL. Nucleic acid was extracted from the contrived samples using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler with the TaqPath 1-step Master Mix and MALDI-TOF was performed on the Agena MassARRAY System and tested with 24 replicates. The results are summarized in the Table below:

Table 8: LoD Confirmation for the TaqPath 1-step Master Mix

Concentration copies/µL	Detected/ total samples	Detected %	BY TARGETS: DETECTED /TOTAL SAMPLES				
			SC2_N1	SC2_N2	SC2_N3	SC2_ORF1	SC2_ORF1ab
2.75	24/24	100%	23/24	16/24	24/24	23/24	4/24
1.38	24/24	100%	21/24	8/24	24/24	24/24	0/24
0.69	24/24	100%	21/24	9/24	24/24	24/24	0/24

Study results demonstrated that the SARS-CoV-2 MassArray generated comparable results when performed with the TaqPath 1-step Master Mix.

2) Analytical Inclusivity:

The SARS-CoV-2 MassArray Test has been designed using publicly available SARS-CoV-2 viral RNA sequences for the detection of SARS-CoV-2 strains or isolates. 165 NCBI and target sequences were retrieved and aligned to identify conserved regions and specific regions of the SARS-CoV-2 genome, where primers were designed for the assay. Alignments were performed with the designed oligonucleotide primer sequences of SARS-

CoV-2 MassArray Test with 2,661 SARS-CoV-2 sequences publicly available in Genbank as of August18, 2020 to demonstrate the estimated inclusivity of the SARS-CoV-2 MassArray Test. All the alignments exhibited 100% identity to the available SARS-CoV-2 sequences except for the forward PCR primer for the SC2_N2 assay. The N2 assay exhibited >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 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.

3) Cross-Reactivity:

In-silico Analysis:

In-silico analysis for the N1, N2, N3, ORF1, and ORF1ab primer/probe set of the SARS-CoV-2 MassArray Test was conducted to assess cross-reactivity against sequences of pathogens potentially present in respiratory specimens and/or with genetic similarities to SARS-CoV-2 according to the Recommended List of Organisms to be analyzed *in-silico* or by Direct wet lab Testing.

The following is an analysis of the cross-reactivity results for the SARS-CoV-2 MassArray Test that exhibit > 80% homology in at least one of their components:

Table 9: *In-silico* Analysis of Cross-Reactivity of the SARS-CoV-2 MassArray Test

Assay Name	Assay Primer	Highest % Other Species Homology	Highest Other Species
N1	SC2_N1_For	82	SARS-coronavirus
	SC2_N1_Rev	75	SARS-coronavirus
	SC2_N1_Probe	94	SARS-coronavirus
N2	SC2_N2_For	91	SARS-coronavirus
	SC2_N2_Rev	68	SARS-coronavirus
	SC2_N2_Probe	55	Human Coronavirus NL63
N3	SC2_N3_For	60	MERS-coronavirus
	SC2_N3_Rev	60	MERS- coronavirus
	SC2_N3_Probe	57	Human coronavirus HKU1
ORF1	SC2_ORF1_For	55	Enterovirus F strain BEV-261
	SC2_ORF1_Rev	50	*Staphylococcus* phage tp310-3
	SC2_ORF1_Probe	76	Possum enterovirus W1
ORF1ab	SC2_ORF1ab_For	76	SARS-coronavirus

Assay Name	Assay Primer	Highest % Other Species Homology	Highest Other Species
	SC2_ORF1ab_Rev	68	Enterovirus SEV-gx
	SC2_ORF1ab_Probe	88	SARS-coronavirus

Results of *in silico* analysis demonstrates that there is significant homology between the N1, N2 and ORFlab assay to SARS-coronavirus. All other homologies were not significant for the pair of primers and probes in order to predict a false positive result *in silico*.

4) Clinical Evaluation

Clinical Evaluation of the SARS-CoV-2MassArray Test using the KingFisher Flex Purification System using MagMAX Viral/Pathogen Nucleic Acid Isolation Kit:

A clinical study was performed to evaluate the performance of the SARS-CoV-2 MassArray Test. Results obtained from a total of 247 clinical nasopharyngeal swab specimens (208 negatives and 39 positives for SARS-CoV-2) tested with the FDA-authorized RT-PCR Assays were compared to results obtained with the SARS-CoV-2 MassArray Test. Samples were extracted using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF was performed on the Agena MassARRAY System. The results are summarized in the Tables below.

Table 10: Clinical Evaluation of the SARS-CoV-2 MassArray Test using the KingFisher Flex Purification System using MagMAX Viral/Pathogen Nucleic Acid Isolation Kit

SARS-CoV-2 MassArray Test	FDA EUA RT-PCR Assay		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	39	1	40	PPA 100% (39/39)	91-100%
Not Detected	0	207	207	NPA 99.5% (207/208)	97.3-99.9%
Total	39/39	207/208	247		

Clinical Evaluation of the SARS-CoV-2MassArray Test using the Hamilton StarLet liquid handling system and Omega Viral DNA/RNA kit:

A clinical study was performed to assess the performance of the Hamilton StarLet liquid handling system and Omega Viral DNA/RNA kit for use with the SARS-CoV-2 MassArray Test. Results obtained from a total of 106 clinical nasopharyngeal swab specimens (74 negatives and 32 positives for SARS-CoV-2) tested with FDA-authorized RT-PCR Assays

were compared to results obtained with the SARS-CoV-2 MassArray Test. Samples were extracted using the Hamilton StarLet liquid handling system and Omega Viral DNA/RNA kit, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF was performed on the Agena MassARRAY System. The results are summarized in the Tables below.

Table 11: Clinical Evaluation of the SARS-CoV-2 MassArray Test

SARS-CoV-2 MassArray Test	FDA EUA RT-PCR Assay		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	32	0	32	PPA 100% (32/32)	89.3-100%
Not Detected	0	74	74	NPA 100% (74/74)	95.1-100%
Total	32	74	106		

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by National Jewish Health Advanced Diagnostics Laboratory located at 1400 Jackson St., Denver, CO 80206.
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests 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.