EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
MedArbor Diagnostics SARS-CoV-2 Assay
(MedArbor, LLC dba MedArbor Diagnostics)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

The MedArbor Diagnostics SARS-CoV-2 Assay will be performed at MedArbor, LLC dba MedArbor Diagnostics, located at 200 Rittenhouse Circle East Suite 5, Bristol, PA 19007, 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 described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The MedArbor Diagnostics SARS-CoV-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, and nasal aspirate specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to MedArbor, LLC dba MedArbor Diagnostics, located at 200 Rittenhouse Circle East Suite 5, Bristol, PA 19007, 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.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, and nasal aspirate 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 definitive cause of disease. Laboratories within the United States and its territories are required to report all test 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/or epidemiological information.

The MedArbor Diagnostics SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The MedArbor Diagnostics SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The MedArbor Diagnostics SARS-CoV-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimen types including nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal

swab, nasopharyngeal wash/aspirate, and nasal aspirate specimens from individuals suspected of COVID-19 by their healthcare provider.

Description of Test Steps:

Reagent preparation instructions are described in detail in the "MedArbor Diagnostics SARS-CoV-2 Assay Testing Procedure with LabTurbo AIO COVID-19 RNA Testing Kit" procedure, which includes the following assay test steps:

Reagent and Control preparation

Nucleic extraction reagents and controls, i.e., extraction controls (PCE and NCE) and assay controls (PC and NC), are prepared via laboratory Standard Operating Protocol (SOP) procedures.

Nucleic Acid Extraction

Nucleic acid extraction is automated and carried out on the Kingfisher extraction system with a validated laboratory protocol. Prior to performing extraction, the PCE and NCE are added to extraction plates along with patient samples.

Real time RT-PCR Reaction

RT-PCR reagents are prepared using master mix recipe. Samples and controls are added to 384-well plates manually following the addition or RT-PCR reagents to each well. Plates are then sealed, centrifuged for a minute, and loaded onto a QuantStudio5 system for completion of the RT-PCR reaction.

INSTRUMENTS AND REAGENTS USED WITH THE TEST ASSAY

Table 1: The MedArbor Diagnostics SARS-CoV-2 Assay is to be used with the following instrument, kits and reagents:

Table 1. MedArbor Diagnostics SARS-CoV-2 Assay Instrument, Kits and Reagents

Name	Vendor	Catalog ID
KingFisher Flex Purification System	ThermoFisher	24078830
QuantStudio 5 Real-Time PCR System	ThermoFisher	A28568
MagMax 96 Deep Well Plates	ThermoFisher	4388476
MicroAmp Clear Adhesive Film	ThermoFisher	4306311
MagMAX™ Viral/Pathogen Binding Beads	Applied Biosystems	A42362
MagMAX Viral/Pathogen Binding Solution	Applied Biosystems	A42359
MagMAX Viral/Pathogen Wash Solution	Applied Biosystems	A42360
MagMAX Viral/Pathogen Proteinase K	Applied Biosystems	A42363
MagMAX Viral/Pathogen Elution Buffer	Applied Biosystems	A42364

Name	Vendor	Catalog ID
LabTurbo AIO COVID-19 RNA Testing Kit	Taigen Bioscience Corporation	Acov11240
2019-nCoV-N-Positive control	Integrated DNA Technologies, Inc.	10006625
ATCC# VR-1986HK Heat Inactivated 2019 Novel Coronavirus	ATCC	VR-1986HK

CONTROLS

Controls are included on each 96 well plate. Each plate will contain (1) Positive Extraction Control (PCE) (2) Positive Control (PC), (3) Negative Extraction Controls (NEC), (4) Negative Control (NC), and (5) Endogenous Internal Control (IC).

The following further describes there controls:

a) An external negative extraction control (NCE) is needed to monitor cross-contamination and non-specific signal throughout the assay procedure, i.e., from nucleic extraction through real-time RT-PCR reaction. The NCE is used as one sample in each batch of testing.

b) An external positive extraction control (PCE) is needed to validate the correct reagents, nucleic acid extraction and real-time RT-PCR procedure and is used at 3x LoD (3750 copies/ml) as one sample in each batch of testing through nucleic acid extraction and real-time RT-PCR.

c) An endogenous internal control (IC) RNaseP (RP) gene is needed to validate the specimen quality, ensuring correct reagents and the procedure throughout nucleic extraction and real-time RT-PCR process steps and is used to validate the nasopharyngeal specimen quality and successful nucleic acid extraction and real-time RT-PCR of each specimen.

d) A “no template” (negative) control (NC) can be used to validate reactions from cross-contamination and non-specific signal in RT-qPCR procedure and is used once per real-time RT-PCR. Material: RNase-free water. The NC is not required if NCE is used for the batch; NC is used with NCE to ensure good performance of the extraction and PCR process.

e) A positive template control (PC) can be used to validate correct reagent in RT-qPCR procedure and is used once per real-time RT-PCR at 3x LoD. Material: SARS-CoV-2 inactivated virus reconstituted to 3750 copies/ml. The PC is not required if PCE is used for the batch.

Refer to **Table 2** for Expected Control Results for N1 Gene and **Table 3** for potential invalid control results and recommended actions (N1 gene). PC is used with PCE to ensure good performance of the extraction and PCR process.

Table 2. Expected Control Results for N1 Gene

NCE	PCE	NC	PC	Result	Interpretation	Action
-	+	-	+	Valid	Valid testing	Report Result

Table 3. Potential invalid control results and recommended actions (N1 gene)

NCE	PCE	NC	PC	Result	Interpretation	Action
+	+	-	+	Invalid	Possible contamination of extraction or NCE.	Repeat extraction and qPCR. Decontaminate the extraction system and replace reagents if necessary. Replace NCE if necessary.
-	-	-	+	Invalid	Possible extraction failure or compromised PCE.	Repeat extraction and qPCR. Check proper function of the extraction system and extraction kit. Change PCE if necessary.
-	+	+	+	Invalid	Possible contamination during PCR setup liquid-handling, qPCR, or contaminated NC.	Repeat PCR setup liquid-handling and qPCR. Change NC if necessary.
-	+	-	-	Invalid	Possible PC handling error or degradation.	Repeat PCR setup liquid-handling and qPCR. Change PC if necessary.
+	+	+	+	Invalid	Possible PCR reagent contamination or extraction contamination.	Repeat extraction and qPCR. Decontaminate the extraction system and replace reagents if necessary. Change PCR reagents if necessary.
-	-	-	-	Invalid	Possible extraction failure, PCR setup liquid-handling error or compromised PCR reagent.	Repeat extraction, PCR setup liquid-handling and qPCR. Replace extraction and PCR reagents if necessary.
+	+	-	+	Invalid	Possible contamination of extraction or NCE.	Repeat extraction and qPCR. Decontaminate the extraction system and replace reagents if necessary. Replace NCE if necessary.

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 1. Interpretations of patient sample results

N1 gene	RNaseP gene	Result	Interpretation	Report (Action)
+	+/-	Valid	SARS-CoV-2 RNA is detected.	Report result
-	+	Valid	SARS-CoV-2 RNA is not detected.	Report result
-	-	Invalid	Possible extraction failure or sample collection error on particular sample(s)	Repeat extraction and qPCR of the sample(s). If the repeat fails, recollect the sample.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity:

A preliminary LoD study for the MedArbor Diagnostics SARS-CoV-2 Assay was completed using quantified heat-inactivated SARS-CoV-2 virus, 2019-n-CoV/USA-WA1/2020 (ATCC, VR-1986HK) spiked into pooled negative matrix nasopharyngeal (NP) swab collected into VTM. A series of eight SARS-CoV-2 concentrations via serial dilutions were created and three

replicates of each concentration were evaluated. The preliminary LoD of SARS-CoV-2 detection from NP swab specimens was determined as 312.5 copies/ml.

Table 2. Preliminary LoD results

Concentration (cp/mL)	N1 Detected	RNaseP Detected	Interpretation
10000	3/3	3/3	Positive
5000	3/3	3/3	Positive
2500	3/3	3/3	Positive
1250	3/3	3/3	Positive
625	3/3	3/3	Positive
312.5	**3/3**	**3/3**	**Positive**
156.25	2/3	3/3	Negative
78.125	0/3	3/3	Negative

An LoD confirmation study of the MedArbor Diagnostics SARS-CoV-2 Assay was completed by running 20 replicate samples at 10000, 5000, 2500, 1250, 625, and 312.5 cp/ml. The extracted samples and controls were included in the evaluation per the assay SOP. Additionally, one PC of 10 copies/ul and one NC (5 µL each). The LoD was confirmed to be 1250 copies/ml.

Table 3. LoD Confirmation Results

Concentration (cp/mL)	N1 Detected
10000	100% (20/20)
5000	100% (20/20)
2500	100% (20/20)
1250	**100% (20/20)**
625	65% (13/20)
312.5	40% (8/20)

2) **Inclusivity (Analytical Reactivity):**

The SARS-CoV-2 Assay uses primer and probe sequences for N1 gene and RNaseP gene of LabTurbo AIO COVID-19 RNA Testing kit. *In silico* analysis was performed to determine reactivity of LabTurbo COVID-19 RNA Testing kit. A total of 2234 entries of SARS-CoV-2 genomes as of 1/10/2023 from GISAID as defined as complete were downloaded. Less than 2% of the genomes harbored more than a single mismatch or double mismatches to the N1 target sequences and are all on the 5' end.

3) **Cross-Reactivity (Analytical Specificity):**

Cross reactivity was evaluated via *in-silico* analysis for the N1 gene primer/probe sets to assess potential cross-reactivity with microorganisms commonly found in nasopharyngeal swab samples. The study revealed that the forward primer for the N1 gene had 80% homology with Influenza A, which could potentially impact the performance of the assay.

Table 4. *In silico* Cross-reactivity homology analysis for N1 gene primer/probe set

Substance	N gene Forward Primer	N gene Probe	N gene Reverse Primer
Human coronavirus 229E	50%	33%	45%

Substance	N gene Forward Primer	N gene Probe	N gene Reverse Primer
Human coronavirus OC43	50%	41%	41%
Human coronavirus HKU1	50%	41%	41%
Human coronavirus NL63	45%	41%	41%
SARS-coronavirus	40%	**91%**	**91%**
MERS-coronavirus	50%	37%	41%
Adenovirus type 1	45%	37%	37%
Adenovirus type 7	45%	37%	37%
Human Metapneumovirus	55%	37%	45%
Parainfluenza virus 1	No alignment*	No alignment*	No alignment*
Parainfluenza virus 2	No alignment*	No alignment*	No alignment*
Parainfluenza virus 3	No alignment*	No alignment*	No alignment*
Parainfluenza virus 4	40%	37%	37%
Influenza A	**80%**	58%	58%
Influenza B	50%	50%	45%
Enterovirus	65%	45%	50%
Respiratory syncytial virus type B	50%	37%	33%
Rhinovirus	70%	50%	45%
Chlamydia pneumoniae	60%	50%	50%
Haemophilus influenzae	65%	58%	50%
Legionella pneumophila	70%	54%	54%
Mycobacterium tuberculosis	60%	50%	50%
Streptococcus pneumoniae	65%	54%	66%
Streptococcus pyogenes	70%	54%	58%
Bordetella pertussis	65%	No alignment*	No alignment*
Mycoplasma pneumoniae	60%	54%	45%
Pneumocystis jirovecii (PJP)	60%	54%	54%
Candida albicans	70%	58%	79%
Pseudomonas aeruginosa	75%	62%	54%
Staphylococcus epidermidis	60%	50%	50%
Human coronavirus 229E	45%	54%	54%

* The target sequences were blasted against NCBI Database and no alignment results were found. No potential unintended cross reactivity is expected based on this *in silico* analysis.

4) Microbial Interference:

Influenza A was found to have greater than 80% homology with the assay's forward primer following *in silico* analysis. Additionally, SARS-coronavirus was found to have greater than 80% homology with the assay's reverse primer and probe. Therefore, a microbial interference study was performed to further evaluate potential interference with assay results. To evaluate microbial interference, samples in triplicate with and without SARS-CoV-2 spiked at 3X LoD were evaluated for detection of the N1 gene in the presence of influenza A or SARS-coronavirus at 1 x 10^6 copies/ml.

Table 8. Microbial Interference Study Results

Organism	with 3X LoD SARS-CoV-2		without 3X LoD SARS-CoV-2	
	N1 gene	RNaseP gene	N1 gene	RNaseP gene
Influenza A	3/3	3/3	0/3	3/3

Organism	with 3X LoD SARS-CoV-2		without 3X LoD SARS-CoV-2	
	N1 gene	RNaseP gene	N1 gene	RNaseP gene
(ATCC catalog VR-1894/lot 70055531; concentration is 2.1 x 10^8 CEID50/mL)				
SARS-coronavirus (ATCC catalog VR-1986HK/lot 70037781; concentration is 4.2 x 10^5 genome copies/µL)	3/3	3/3	0/3	3/3

5) Endogenous/Exogenous Interference Evaluation:

Interference with potentially interfering endogenous and exogenous substances commonly found in upper respiratory samples was evaluated to assess their impact on assay performance. Contrived specimens were prepared by spiking heat inactivated SARS-CoV-2 into pooled negative NP swab preservation medium at 3x LoD. The contrived specimens were tested with interfering substances at relevant concentrations in triplicate. Three (3) replicates of each potential interfering substance were evaluated in the presence and absence of virus. 100% of samples were detected in the presence of each potentially interfering substance.

Table 9. Microbial Interference Study Results for Replicates That Were Detected

Interference Substance	with SARS-CoV-2 virus		without SARS-CoV-2 virus	
	N1 gene	RNaseP gene	N1 gene	RNaseP gene
Afrin Original nasal (15% v/v)	3/3	3/3	0/3	3/3
Cepacol Lozenges (benzocaine/menthol) (3 mg/mL)	3/3	3/3	0/3	3/3
Chloroseptic Sore Throat spray (5% v/v)	3/3	3/3	0/3	3/3
Robitussin (5% v/v)	3/3	3/3	0/3	3/3
Mucin: bovine submaxillary gland, type I-S (2.5 mg/mL)	3/3	3/3	0/3	3/3
Tobacco (0.03 mg/mL)	3/3	3/3	0/3	3/3

6) Specimen Stability:

Specimen stability was evaluated to support claims for storage of nasopharyngeal samples in VTM at room temperature or at 4˚C for up to 144 hours (i.e., 6 days) after collection prior to testing with the MedArbor Diagnostics SARS-CoV-2 Assay.

7) Clinical Evaluation:

The clinical evaluation study was performed on 32 retrospective confirmed SARS-CoV-2 positive patient nasopharyngeal samples and 42 retrospective confirmed SARS-CoV-2 negative nasopharyngeal patient samples. Samples were tested by the MedArbor Diagnostics SARS-CoV-2 Assay testing method and the comparator test. Agreement between positive results (PPA) and negative results was 100% (**Table 10**).

Table 10. Clinical Evaluation Summary

		Comparator Test	
		Positive	**Negative**
MedArbor Diagnostics SARS-CoV-2 Assay	**Positive**	32	0
	Negative	0	42
PPA: 100% (89.28 – 100%) NPA: 100% (91.62 – 100%)			

Limitations

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.

WARNINGS

- For prescription use only.
- For in vitro diagnostic use.
- For use under Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratory;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product 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 declaration is terminated or authorization is revoked sooner.