EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
DTPM COVID-19 RT-PCR TEST (TIDE LABORATORIES)

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

The DTPM COVID-19 RT-PCR Test will be performed at laboratories designated by Tide Laboratories that includes Tide Laboratories, LLC, 913 Airport Rd., Fort Payne, AL 35968 that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the requirements to perform high complexity tests, as described in the Laboratory Instructions for Use that was reviewed by the FDA under this EUA.

INTENDED USE

The DTPM COVID-19 RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, and nasal washes collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories designated by the Tide Laboratories, LLC including Tide Laboratories, LLC, 913 Airport Rd., Fort Payne, AL 35968 that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the 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 specimen 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 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 epidemiological information.

The DTPM COVID-19 RT-PCR test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The DTPM COVID-19 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION TEST PRINICIPLE

The DTPM COVID-19 RT-PCR test is a reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of human SARS-CoV-2 RNA in nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, and nasal washes. The test utilizes one primer and probe set to detect a conserved region in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set (Figure 1) to detect a human S9 ribosomal gene in a clinical sample. RNA isolated from specimens is reverse transcribed to cDNA and subsequently amplified using a ThermoFisher QuantStudio 5 instrument with software version 1.5.1. During the amplification process, the probe anneals to a specific target sequence between the forward and reverse primers. The 5' exonuclease activity of Taq polymerase degrades the bound probe during the extension phase of the PCR cycle, which causes the 5' labeled reporter dye to separate from the 3' nonfluorescent quencher (NFQ), generating a fluorescent signal. During PCR amplification, fluorescence generated by degradation of the target-specific probe is monitored by the QuantStudio 5 RT-PCR instrument.

Figure 1: DTPM COVID-19 RT-PCR test multiplex assay and second reverse primer

DTPM COVID-19 RT-PCR test	Final Assay Concentration (nM)
SARS-CoV-2 Fwd	300
SARS-CoV-2 Rev	500
SARS-CoV-2 v2.0 Om Rev	500
SARS-CoV-2 Probe	350
Endogenous Control Fwd	300
Endogenous Control Rev	600
Endogenous Control Probe	350

INSTRUMENTS USED WITH TEST:

1. The DTPM COVID-19 RT-PCR Test is to be used with the ThermoFisher QuantStudio 5 instrument with software version 1.5.1. Optional automated extraction may be performed using ThermoFisher KingFisher Flex Catalog # 5400630

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.

- Specimens 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 specimens must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- The DTPM COVID-19 RT-PCR test can be used only with the specimens listed in the Intended Use statement. Other specimen types have not been evaluated and should not be tested with this assay.
- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the DTPM COVID-19 RT-PCR test. SARS-CoV is not known to be currently circulating in the human population, and therefore is highly unlikely to be present in patient specimens.
- The instrument and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in the laboratory SOP.
- False-negative results may arise from:
    - Improper specimen 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
- As with any molecular test, mutations within the target regions of DTPM COVID-19 RT-PCR test could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision. Follow up testing should be performed according to the current CDC recommendations.
- Detection of SARS-CoV-2 RNA indicates presence of viral RNA, however this does not confirm that SARS-CoV-2 is the causative agent of clinical symptoms. 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.

REAGENTS AND MATERIALS

Table 1. Reagents and Materials Used with the DTPM COVID-19 RT-PCR test

Biologicals (Storage Conditions)		
ThermoFisher Invitrogen GeneArt Gene Synthesis Positive control plasmid constructs (-20º C – 8º C)	ThermoFisher	Cat# 817000DE
ThermoFisher Sequence Detection Primers (-20º C – 8º C)	ThermoFisher	Cat# 4304972
ThermoFisher Custom *Taq*Man® Probes (-20º C – 8º C)	ThermoFisher	Cat# 4316032
ThermoFisher Fast Virus 1-Step MM (-20º C – 8º C)	ThermoFisher	Cat# 4444426C001
Kits		
Disposable Sampling Tube – MTM(15º C – 25º C)	DTPM	Cat# DTPM-MTM
IndiMag Pathogen Kit (15º C – 25º C)	Indical Biosciences	Cat# SP947457
QIAamp Viral RNA Kit (15º C – 25º C)	Qiagen	Cat# 52906
QIAamp 96 Viral RNA Kit (15º C – 25º C)	Qiagen	Cat# 52962
MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (15º C – 25º C)	Applied Biosystems	Cat# A48383
Chemicals		
Molecular Grade Water (0º C – 40º C)	Fisher	Cat# SH30538.02
Ethanol 200 proof (100%) (0º C – 40º C)	Fisher	Cat# 04-355-450
Isopropyl Alcohol (0º C – 40º C)	Fisher	Cat# MPX18341
Phosphate Buffered Saline (PBS) (15º C – 30º C)	Fisher	Cat# 70011044
RNAse Away	Fisher	Cat# 21-402-178
Disposables		
1.5 ml Microcentrifuge Tubes	VWR	Cat# 1002-726
2.0 ml Eppendorf Tubes	Eppendorf	Cat# 022363352
2.0 ml Screw Cap Microcentrifuge Tube	VWR	Cat# 16466-058
Pipette Tips – Low Retention		
10 µL Neptune BT10	VWR	Cat# 89140-160
20 µL Neptune BT20	VWR	Cat# 89140-932
100 µL Neptune BT100	VWR	Cat# 89140-162
200 µL Neptune BT200	VWR	Cat# 89140-936
1000 µL Neptune BT1000.96	VWR	Cat# 89217-468
384 Well PCR Plates	VWR	Cat# 60941-078
2.2 mL 96-well Deep Well Plates	VWR	Cat# 76329-996
0.5 mL 96-well Elution Plate	VWR	Cat# 76210-518
Optical Adhesive PCR Film	ThermoFisher	Cat# 4360954

Kim-Wipe™ tissues	VWR	Cat# 470224-038
Nitrile gloves	VWR	Cat# 40101-348
Pipettes		
Research® plus Adjustable Volume Pipettes	Eppendorf	
0.5 – 10 µL, 10 – 100 µL, 100 – 1000 µL	2 – 20 µL, 20 – 200 µL, 100 – 1000 µL	Cat# 2231000222
MultiChannel Pipette	Eppendorf	Cat# 2231000224
Small Lab Equipment		
Vortex-Genie® 1 Touch Mixer	VWR	Cat# 14216-184
Centrifuge (Microfuge mySPIN™6)	ThermoFisher	Cat# 75004061
96-well microtube racks	VWR	Cat# 21150-234
King Fisher Flex	ThermoFisher	Cat# 5400610

CONTROLS TO BE USED WITH THE DTPM COVID-19 RT-PCR ASSAY

a) A no template control (NTC) is needed to confirm the absence of template in the reagents being used for patient analysis. It will be performed with each plate of patient specimens tested. This control consists of molecular grade, nuclease-free water.

b) A positive control is needed to assess the stability of the entire analytical system and assure proper operation and stability of the equipment and chemistry used for all assays run on that thermal cycler. It will be performed with each patient sample plate. This control consists of a 2359 base pair recombinant plasmid that contains a 70 base pair inset corresponding to the conserved region of the SARS-CoV-2 genome targeted by the assay.

c) An endogenous extraction control is needed to verify that patient sample is not degraded and was extracted correctly. This control also ensures that the RT enzyme is functioning properly and that PCR amplification occurs properly. An endogenous (internal) control is performed for each patient sample tested. This control targets an expressed human S9 ribosomal gene.

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.

1) ***DTPM COVID-19 RT-PCR TEST – Positive, Negative, and Extraction controls:***
NTC – Assay results should demonstrate no amplification.

Positive control – The assay positive controls contain material near the assay LoD. Result must have Ct value ≤35.

Endogenous (internal) control – Endogenous (internal) control must exceed the cutoff (Ct≤35) and be positive in the clinical specimen. In the case of a negative endogenous (internal) control result, users are instructed to repeat sample preparation and analysis.

Results from the Positive control, Endogenous (internal) control, and NTC will be monitored through continual charting. The Ct values of the controls should be within ±20% of the mean value of existing results.

2) Examination and Interpretation of Patient Specimen Results:

The DTPM COVID-19 RT-PCR test result interpretation algorithm is described below.

Table 2. DTPM COVID-19 RT-PCR Test Results Interpretation

SARS-CoV-2 N Gene	S9 Ribosomal (Internal) Control	Result Interpretation	Report
+ (Ct ≤35)	+ (Ct≤35)	SARS-CoV-2 Detected*	Reactive (Positive/Detected)
- (Ct>35)	+ (Ct ≤35)	SARS-CoV-2 Not Detected	Non-reactive (Negative/Not Detected)
Any Result	- (Ct>35)	Invalid Result	Invalid. Repeat sample preparation and analysis. If second test yields an invalid result, report Invalid.

* Additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecoviruses currently unknown to infect humans, for epidemiological purposes or clinical management.

PERFORMANCE EVALUATION

1) Analytical Sensitivity

Limit of Detection (LoD):

The LoD of the multiplex DTPM COVID-19 RT-PCR test was determined using quantified Genomic RNA from SARS-CoV-2, Isolate USA-WA1/2020 (NR-52285) as well as SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated (NR-52286) both obtained from BEI Resources. A preliminary LoD was determined by testing serial dilutions of SARS-CoV-2 RNA (500 – 0.2 genomic copies/µL) spiked into pooled negative nasopharyngeal swab clinical matrix collected in molecular transport media (MTM) in triplicate. Samples were prepared using the QIAamp Viral RNA Kit and manual extraction method in accordance with the standard operating procedure for this method.

The LoD was verified by testing 20 additional extraction replicates consisting of pooled negative nasopharyngeal swab clinical matrix collected in molecular transport media (MTM) spiked at the preliminary LoD concentration of 1 copies/µL (NR-52286) prior to nucleic acid extraction. Results for LoD confirmation (NR-52286) yielded 20/20 detected extraction replicates and observed mean Ct 32.43 and standard deviation 0.75. Results for LoD confirmation (NR-52285) yielded 20/20 detected extraction replicates and observed mean Ct 31.24 and standard deviation 1.36. The results form LoD study are summarized in Table 3.

Table 3. Summary of LoD Determination

Sample ID	Sample Matrix	Spiking concentration (copies/µL)	Mean Ct Target 1 N-Gene	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated							
NR-52286 500	NPS	500	24.09	0.19	26.61	0.15	3/3 100%
NR-52286 125	NPS	125	26.43	0.32	26.93	0.07	3/3 100%
NR-52286 25	NPS	25	28.55	0.07	26.91	0.05	3/3 100%
NR-52286 5	NPS	5	31.16	0.70	26.94	0.09	3/3 100%
NR-52286 1	NPS	1	33.33	1.02	26.99	0.06	3/3 100%
NR-52286 0.2	NPS	0.2	37.99	3.48	27.12	0.02	1/3 33%
LoD Confirmation Replicates							
NR-52286 1	NPS	1	32.43	0.75	26.57	0.49	20/20 100%
Genomic RNA from SARS-CoV-2, Isolate USA-WA1/2020							
NR-52285 500	NPS	500	20.51	3.64	26.84	0.10	3/3 100%
NR-52285 125	NPS	125	23.80	0.68	26.77	0.24	3/3 100%
NR-52285 25	NPS	25	27.76	0.20	27.17	0.11	3/3 100%
NR-52285 5	NPS	5	30.14	0.11	27.33	0.15	3/3 100%
NR-52285 1	NPS	1	32.22	0.34	27.21	0.14	3/3 100%
NR-52285 0.2	NPS	0.2	34.47	0.83	27.37	0.22	2/3 66%
LoD Confirmation Replicates							
NR-52285 1	NPS	1	31.24	1.36	26.65	0.61	20/20 100%

To improve the inclusivity of the multiplex DTPM COVID-19 RT-PCR test and reduce the risk of false-negative results for the detection of SARS-CoV-2, B.1.1.529 (Omicron) variant, a second reverse primer was introduced into the device design which compensates for the nucleocapsid 9 nucleotide deletion: N E31del, N R32del, N S33del. To demonstrate the efficacy of this change and further, that no adverse impact is observed, a new LoD bridging study was performed in parallel using negative NPS clinical matrix and spiked heat-inactivated virus (BEI-52286). Results from the parallel study and confirmation of the LoD suggest equivalent performance of the assay with the inclusion of the second reverse primer and are presented in Table 4.

Table 4. results for DTPM COVID-19 RT-PCR test (MP) - Including New Omicron Primer and DTPM COVID-19 RT-PCR TEST

DTPM COVID-19 RT-PCR test (MP) - Including New Omicron Primer						
Sample Matrix	**Spiking concentration Copies/µL**	Mean Ct Target 1 N-Gene	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
NPS	500	24.01	0.16	26.82	0.13	5/5 100%
NPS	125	26.10	0.12	26.91	0.07	5/5 100%
NPS	25	28.56	0.56	27.04	0.37	5/5 100%
NPS	5	30.74	0.67	26.94	0.27	5/5 100%
NPS	1	33.23	0.29	26.68	0.30	5/5 100%
NPS	0.2	36.18	2.21	27.46	0.63	2/5 40%
NPS	1	33.57	0.77	27.63	0.13	20/20 100%
DTPM COVID-19 RT-PCR Test						
Sample Matrix	**Spiking concentration Copies/µL**	Mean Ct Target 1 N-Gene	SD Ct Target 1 N-Gene	Mean Ct IC S9 ribosomal gene	SD Ct S9 ribosomal gene	Reactivity
NPS	500	24.23	0.20	26.89	0.25	5/5 100%
NPS	125	26.24	0.21	26.83	0.21	5/5 100%
NPS	25	28.81	0.42	27.05	0.17	5/5 100%
NPS	5	30.75	0.56	26.79	0.32	5/5 100%
NPS	1	33.82	0.66	26.95	0.11	5/5 100%
NPS	0.2	37.56	2.91	27.85	0.23	1/5 20%

Inclusivity:

The multiplex DTPM COVID-19 RT-PCR test is designed to detect up to an expressed 81 base pair (bp) region of the nucleocapsid phosphoprotein (N gene) in the SARS-CoV-2 genome. The translated amplicon spans up to amino acids 16 to 42.

In silico inclusivity was evaluated on December 14, 2021 using the basic local alignment search tool (BLAST) which compares primary biological sequence information to the International Nucleotide Sequence Database Collaboration [comprised of the DNA data Bank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at the National Center for Biological Information (NCBI)] as well as the NCBI SARS-CoV-2 reference sequence database from GenBank.

Each oligonucleotide was entered as an independent query sequence. Sequence results were evaluated for the 276,121 target sequences which maintained full coverage of all three

oligonucleotide-binding regions (Fwd, Rev primers, and Probe). Results demonstrated >99.5% sequence homology for the sequences evaluated (see Table 5 below).

Table 5. *In silico* analysis Summary

	N Gene SARS-CoV-2 Fwd Primer (5'-3')	N Gene SARS-CoV-2 Rev Primer (5'-3')	N Gene Sars-CoV-2 v2.0 Om Rev	N Gene SARS-CoV-2 Probe (5'-3')
Oligonucleotide length (bp)	20	19	19	16
Total strains Evaluated	276,121	276,121	276,121	276,121
100% match	>99.5 %	>99.9 %	100%	100%

*mismatches < 0.0007 % of 276,121 sequences examined.

In silico analysis was also performed against current variants of concern (VOC) and variants being monitored (VBM) as defined by the Centers for Disease Control and Prevention (CDC).

1.) Variants of Concern (VOC):

As of December 14, 2021, Delta (B.1.617.2, AY.1, AY.2, AY.3) and Omicron (B.1.1.529) variants circulating in the United States are classified as a variant of concern. A database was constructed for the VOC and mismatches were evaluated for each component of the multiplex DTPM COVID-19 RT-PCR test (Fwd, Rev primers, and Probe) by multiple sequence alignment using ClustalW2. The *in silico* analysis demonstrated 100% sequence homology for the delta variant and omicron variant (using second reverse primer) examined in the binding region of the primers and probe. This indicates the VOC examined would be detected by the multiplex DTPM COVID-19 RT-PCR test.

		Sars-CoV-2 Fwd			Sars-CoV-2 Rev		Sars-CoV-2 v2.0 Om Rev		SARS-COV-2 Probe	
Variant of Concern (VOC)[1]	Total VOI Examined	Mismatches	Homology		Mismatches	Homology	Mismatches	Homology	Mismatches	Homology
Delta (B.1.617.2, AY.1, AY.2, AY.3)	200	None	100%		None	100%	None	100%	None	100%
Omicron (B.1.1.529)	200	None	100%		9 Deletions	47%	None	100%	None	100%

[1]Sequences downloaded from GenBank and GISAID databases

2.) Variants Being Monitored (VBM):

Currently there are no SARS-CoV-2 mutations indicated as a variant of interest. However, the following SARS-CoV-2 variants are indicated as a variant being monitored per the

current CDC classifications: Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2). The *in silico* analysis demonstrated 100% sequence homology for each variant examined in the binding region of the primers and probe. This indicates the VBM examined would be detected by the multiplex DTPM COVID-19 RT-PCR test.

In silico molecular analysis demonstrates that the region in the N gene used to design the multiplex DTPM COVID-19 RT-PCR test is highly conserved. Combined mismatches for the Fwd, Rev primers, and Probe remain below 0.05% of total sequences examined. Further, current VOC and VBM contain base pair substitutions that are not located within the conserved binding region of the primers or probe used in the multiplex DTPM COVID-19 RT-PCR test. Taken collectively, these analyses demonstrate that all the SARS-CoV-2 variants examined are expected to be detected by the multiplex DTPM COVID-19 RT-PCR test.

		Sars-CoV-2 Fwd			Sars-CoV-2 Rev		Sars-CoV-2 v2.0 Om Rev		SARS-COV-2 Probe	
Variant Being Monitored (VBM)[1]	Total VBM Examined	Mismatches	Homology		Mismatches	Homology	Mismatches	Homology	Mismatches	Homology
Alpha (B.1.1.7, Q.x lineages)	20	None	100%		None	100%	None	100%	None	100%
Beta (B.1.351, B.1.351.2, B.1.351.3)	9	None	100%		None	100%	None	100%	None	100%
Gamma (P.1, P.1.1, P.1.2)	13	None	100%		None	100%	None	100%	None	100%
Epsilon (B.1.427, B.1.429)	13	None	100%		None	100%	None	100%	None	100%
Eta (B.1.525)	9	None	100%		None	100%	None	100%	None	100%
Iota (B.1.526)	20	None	100%		None	100%	None	100%	None	100%
Kappa (B.1.617.1)	20	None	100%		None	100%	None	100%	None	100%
1.617.3	13	None	100%		None	100%	None	100%	None	100%
Mu (B.1.621, B.1.621.1)	20	None	100%		None	100%	None	100%	None	100%
Zeta (P.2)	20	None	100%		None	100%	None	100%	None	100%

[1]Sequences downloaded from GenBank and GISAID databases

2) ***Analytical Specificity:***

In silico analysis was performed to evaluate the potential for cross-reaction for the assay with the inclusion of the second reverse primer. No homology was observed for the organisms indicated in Table 6 below.

Table 6. Cross-reactivity Analysis

		***In Silico* Sequence Homology (%)[2]**			
Pathogen	**Accession #[1]**	**SARS-CoV-2 Fwd**	**SARS-CoV-2 Rev**	**SARS-CoV-2 v2.0 Om Rev**	**SARS-CoV-2 Probe**
Adenovirus	AC_000019.1	None	None	None	None
B. pertussis	NC_018518	None	None	None	None
Bocavirus	NC_012729.2	None	None	None	None

C. pneumoniae	NC_000922.1	None	None	None	None
Coronavirus 229E	NC_002645.1	None	None	None	None
Coronavirus HKU-1	NC_006577.2	None	None	None	None
Coronavirus NL63	NC_005831.2	None	None	None	None
Coronavirus OC43	NC_006213.1	None	None	None	None
Enterovirus	NC_038308.1	None	None	None	None
H. influenzae	NZ_CP031681.1	None	None	None	None
Influenza A	NC_026426.1	None	None	None	None
Influenza B	NC_002204.1	None	None	None	None
K. pneumoniae	NZ_KE504629.1	None	None	None	None
Legionella	NC_009494.2	None	None	None	None
M. catarrhalis	CP018059.1	None	None	None	None
M. pneumoniae	CP039761.1	None	None	None	None
Metapneumovirus A	NC_39199.1	None	None	None	None
Parechovirus	NC_001897.1	None	None	None	None
Parainfluenza virus 1	NC_003461.1	None	None	None	None
Parainfluenza virus 2	NC_003443	None	None	None	None
Parainfluenza virus 3	NC_001796.2	None	None	None	None
Parainfluenza virus 4	NC_021928.1	None	None	None	None

Rhinovirus	NC_038311.1	None	None	None	None
Respiratory syncytial virus A	NC_038235.1	None	None	None	None
S. aureus	CP018629.1	None	None	None	None
S. pneumoniae	NZ_CP053210	None	None	None	None
Salmonella enterica Typhimurium	CP074092.1	None	None	None	None

[1] RefSeq defined by NCBI as comprehensive, integrated, non-redundant, curated anchor sequence.
[2] Sequence homology <80% identified as None.

In addition each component of the assay (Forward, Reverses, and Probe sequences) was subjected to an *in silico* analysis to demonstrate specificity of the assay components against multiple whole genome sequences for additional organisms as detailed in Table 7 below.

Table 7. Cross-reactivity organisms evaluated *in silico*

	In Silico Sequence Homology (%)[2]				
Pathogen	**Accession #[1]**	**Sars-CoV-2 Fwd**	**Sars-CoV-2 Rev**	**Sars-CoV-2 v2.0 Om Rev**	**Sars-CoV-2 Probe**
SARS-CoV-1	NC_004718.3	90	89.5	74	87.5
MERS	NC_019843.3	None	None	None	None
Mycobacterium tuberculosis	NZ_CP0477163.1	None	None	None	None
Streptococcus pyogenes	CP041615.1	None	None	None	None
Pneumocystis jirovecii	NC_020331.1	None	None	None	None
Candida albicans	NC_032092.1	None	None	None	None
Pseudomonas aeruginosa	CP022001	None	None	None	None
Staphylococcus epidermis	NZ_CP035288	None	None	None	None
Streptococcus salivarius	NZ_CP066093.1	None	None	None	None
[1] RefSeq defined by NCBI as comprehensive, integrated, non-redundant, curated anchor sequences.					
[2] Sequence homology <80% identified as None					

The DTPM primer and probe sequences showed homology to SARS-CoV. Further testing may be necessary to differentiate between SARS-CoV-1 or other Sarbecoviruses currently unknown to infect humans.

3.) Clinical Evaluation:

Performance of the multiplex DTPM COVID-19 RT-PCR test was evaluated using 60 individual, nasopharyngeal (NP) swab retrospective specimen collected from individuals suspected of COVID-19 by a healthcare professional.

A population of archived samples were first evaluated using an FDA authorized molecular comparator assay. From this population, 30 negative and 30 positive samples were selected for comparison against the candidate test. Samples were selected which represented "detected" results across the viral load range, including "weak positive" challenging samples to represent at least 20% of the detected population as determined by the comparator assay.

Each comparator sample underwent comparator testing per authorized IFU. Each of the selected samples were subsequently tested for detection of SARS-CoV-2 RNA using Qiagen Viral RNA manual extraction and multiplex DTPM COVID-19 RT-PCR test workflow. Positive Percent Agreement was observed to be 96.7% (29/30). Negative Percent Agreement was observed to be 100% (30/30).

Table 8. Clinical Validation Summary of Results

	FDA authorized molecular comparator assay POSITIVE	**FDA authorized molecular comparator assay NEGATIVE**
DTPM COVID-19 RT-PCR TEST POSITIVE	29	0
DTPM COVID-19 RT-PCR TEST NEGATIVE	1	30

PPA: 96.7% (29/30), 95% CI: 83.3% - 99.4%
NPA: 100% (30/30), 95% CI: 88.7% - 100%

An additional clinical evaluation was performed to demonstrate the efficacy of the multiplex DTPM COVID-19 RT-PCR test including the second reverse primer. Residual frozen sample extracts from the previous clinical evaluation were thawed and analyzed in parallel using the multiplex DTPM COVID-19 RT-PCR test and the multiplex DTPM COVID-19 RT-PCR test containing the additional reverse primer. Comparative results for each sample are presented in Table 9.

Table 9. Concordance between re-tested frozen extract tested on DTPM COVID-19 RT-PCR test compared to assay including new Omicron primer

	DTPM COVID-19 RT-PCR test (MP) POSITIVE	**DTPM COVID-19 RT-PCR test (MP) NEGATIVE**

DTPM COVID-19 RT-PCR test (MP) – Including New Omicron Primer POSITIVE	27	1[b]
DTPM COVID-19 RT-PCR test (MP) – Including New Omicron Primer NEGATIVE	1[a]	31

[a)] This sample was positive by the DTPM COVID-19 RT-PCR test when tested in the original clinical validation and was positive by the comparator assay
[b)] This sample was positive by the DTPM COVID-19 RT-PCR test when tested in the original clinical validation and was positive by the comparator assay

PPA: 96.4 %, 95% CI: 82.3% - 99.4%
NPA: 96.9%. 95% CI: 84.3% - 99.5%

Specificity to SARS-CoV-2 B.1.1.529 [Omicron] variant

To demonstrate empirically the multiplex DTPM COVID-19 RT-PCR test reactivity against the omicron variant, a synthetic construct was synthesized and analyzed in parallel. *In silico* analysis suggested the design of the single reverse primer would not bind to the B.1.1.529 9nt deletion mutation in the nucleocapsid protein. To mitigate the risk of false-negative results, the addition of the second reverse primer was introduced into the device design which compensates for the 9nt deletion. Parallel analysis using the omicron variant positive control as the representative template demonstrates the failure of the existing reverse primer to bind, as anticipated. However, the addition of the second reverse primer is demonstrated to be an effective resolution to the non-reactivity observed in the single reverse design. Serial dilution samples were prepared in molecular grade water and exposed to both assay configurations. Results from the comparison are present in the following table.

DTPM COVID-19 RT-PCR test (MP) - Including New Omicron Primer			
Sample ID	**Spiking concentration Copies/µL**	**Mean Ct Target 1 N-Gene**	**Reactivity**
SARS-CoV-2, Omicron Positive Control Plasmid with 9nt deletion			
1 x10^3	1 x10^3	29.41	3/3 100%
1 x10^2	1 x10^2	32.34	3/3 100%
1 x10^1	1 x10^1	33.86	3/3 100%
NTC	0	Undetermined	Not Detected
DTPM COVID-19 RT-PCR test (MP) - Current Device			
SARS-CoV-2, Omicron Positive Control Plasmid with 9nt deletion			

1 x10^3	1 x10^3	Undetermined	0/3 0%
1 x10^2	1 x10^2	Undetermined	0/3 0%
1 x10^1	1 x10^1	Undetermined	0/3 0%
NTC	0	Undetermined	Not Detected

Bridge Data to support Alternative Nucleic Acid Extraction Kits for detection of SARS-CoV-2 RNA.

Additional nucleic acid purification methods were evaluated to establish efficacy for use with the multiplex DTPM COVID-19 RT-PCR test. Contrived samples were prepared using SARS-CoV-2, Isolate USA-WA1/2020, Heat Inactivated (NR-52286) obtained from BEI Resources spiked into pooled negative nasopharyngeal swab clinical matrix collected in molecular transport media (MTM). A series of five samples per concentration were prepared at 0.3x; 1x; and 3x LoD based upon the limit of detection established using the Qiagen QIAamp Viral RNA (manual) extraction kit. Results for each extraction material are outlined in the Table 10 below.

Table 10. Alternative Nucleic Acid Extraction Bridge Study Data

				DTPM COVID-19 RT-PCR test (MP)				
Sample ID	**Sample Matrix**	**Spiking concentration (copies/µL)**	**Replicate Number**	**Ct Target 1 N-Gene**	**Result Target 1 (based on cutoff)**	**Ct IC S9 ribosomal gene**	**Result IC (based on cutoff)**	**Final Result**
Indical Indimag Extraction Kit using King Fisher Flex								
Indi Mag 3x	NPS	3	1	33.02	Detected	28.92	Detected	Detected
Indi Mag 3x	NPS	3	2	32.79	Detected	28.93	Detected	Detected
Indi Mag 3x	NPS	3	3	32.73	Detected	29.14	Detected	Detected
Indi Mag 3x	NPS	3	4	32.44	Detected	28.92	Detected	Detected
Indi Mag 3x	NPS	3	5	31.97	Detected	28.85	Detected	Detected
Indi Mag 1x	NPS	1	1	Undetermined	Detected	28.45	Detected	Not Detected
Indi Mag 1x	NPS	1	2	34.29	Detected	28.60	Detected	Detected
Indi Mag 1x	NPS	1	3	33.74	Detected	28.44	Detected	Detected
Indi Mag 1x	NPS	1	4	34.12	Detected	28.42	Detected	Detected
Indi Mag 1x	NPS	1	5	33.24	Detected	28.28	Detected	Detected
Indi Mag 0.3x	NPS	0.3	1	Undetermined	Not Detected	28.31	Detected	Not Detected
Indi Mag 0.3x	NPS	0.3	2	Undetermined	Not Detected	27.96	Detected	Not Detected
Indi Mag 0.3x	NPS	0.3	3	33.21	Detected	28.09	Detected	Detected
Indi Mag 0.3x	NPS	0.3	4	Undetermined	Not Detected	28.03	Detected	Not Detected

Indi Mag 0.3x	NPS	0.3	5	Undetermined	Not Detected	28.06	Detected	Not Detected
Omega Mag-Bind Viral DNA/RNA 96 kit using King Fisher Flex								
Omega 3x	NPS	3	1	31.83	Detected	28.61	Detected	Detected
Omega 3x	NPS	3	2	32.10	Detected	28.39	Detected	Detected
Omega 3x	NPS	3	3	31.92	Detected	28.36	Detected	Detected
Omega 3x	NPS	3	4	31.62	Detected	28.38	Detected	Detected
Omega 3x	NPS	3	5	32.26	Detected	28.42	Detected	Detected
Omega 1x	NPS	1	1	32.76	Detected	28.38	Detected	Detected
Omega 1x	NPS	1	2	33.27	Detected	28.35	Detected	Detected
Omega 1x	NPS	1	3	33.82	Detected	28.41	Detected	Detected
Omega 1x	NPS	1	4	33.61	Detected	28.58	Detected	Detected
Omega 1x	NPS	1	5	33.57	Detected	28.37	Detected	Detected
Omega 0.3x	NPS	0.3	1	Undetermined	Not Detected	28.83	Detected	Not Detected
Omega 0.3x	NPS	0.3	2	Undetermined	Not Detected	28.90	Detected	Not Detected
Omega 0.3x	NPS	0.3	3	Undetermined	Not Detected	28.47	Detected	Not Detected
Omega 0.3x	NPS	0.3	4	Undetermined	Not Detected	28.92	Detected	Not Detected
Omega 0.3x	NPS	0.3	5	Undetermined	Not Detected	28.90	Detected	Not Detected
Qiagen QiaAmp96 Extraction Kit								
QiaAmp 96 52286 3x	NPS	3	1	28.76	Detected	26.73	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	2	25.65	Detected	26.59	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	3	30.61	Detected	27.36	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	4	30.76	Detected	26.26	Detected	Detected
QiaAmp 96 52286 3x	NPS	3	5	25.28	Detected	26.26	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	1	32.53	Detected	27.42	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	2	34.45	Detected	27.38	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	3	31.53	Detected	27.19	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	4	33.66	Detected	26.30	Detected	Detected
QiaAmp 96 52286 1x	NPS	1	5	32.95	Detected	27.15	Detected	Detected
QiaAmp 96 52286 0.3x	NPS	0.3	1	34.66	Detected	26.92	Detected	Detected
QiaAmp 96 52286 0.3x	NPS	0.3	2	35.12	Not Detected	27.38	Detected	Not Detected

QiaAmp 96 52286 0.3x	NPS	0.3	3	34.53	Detected	27.11	Detected	Detected
QiaAmp 96 52286 0.3x	NPS	0.3	4	Undetermined	Not Detected	27.66	Detected	Not Detected
QiaAmp 96 52286 0.3x	NPS	0.3	5	19.30	Detected	27.63	Detected	Detected
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit using King Fisher Flex								
MagMax 3x	NPS	3	1	31.11	Detected	26.68	Detected	Detected
MagMax 3x	NPS	3	2	31.88	Detected	26.59	Detected	Detected
MagMax 3x	NPS	3	3	30.76	Detected	26.63	Detected	Detected
MagMax 3x	NPS	3	4	30.68	Detected	26.40	Detected	Detected
MagMax 3x	NPS	3	5	31.36	Detected	26.50	Detected	Detected
MagMax 1x	NPS	1	1	32.42	Detected	25.76	Detected	Detected
MagMax 1x	NPS	1	2	33.17	Detected	25.62	Detected	Detected
MagMax 1x	NPS	1	3	32.75	Detected	25.96	Detected	Detected
MagMax 1x	NPS	1	4	32.16	Detected	25.63	Detected	Detected
MagMax 1x	NPS	1	5	33.28	Detected	25.65	Detected	Detected
MagMax 0.3x	NPS	0.3	1	Undetermined	Not Detected	26.41	Detected	Not Detected
MagMax 0.3x	NPS	0.3	2	34.20	Detected	26.16	Detected	Detected
MagMax 0.3x	NPS	0.3	3	36.16	Not Detected	25.56	Detected	Not Detected
MagMax 0.3x	NPS	0.3	4	Undetermined	Not Detected	25.20	Detected	Not Detected
MagMax 0.3x	NPS	0.3	5	33.71	Detected	25.48	Detected	Detected

Warnings:

- For in vitro diagnostic use;
- For prescription use 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 authorized laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests;
- 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.