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 Tide Laboratories, LLC or other laboratories designated by Tide Laboratories, LLC that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.

INTENDED USE

The DTPM COVID-19 RT-PCR test is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, nasal swabs, and mid-turbinate swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Tide Laboratories, LLC or other laboratories designated by Tide Laboratories, LLC that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high-complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The 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. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

The DTPM COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of human SARS-CoV-2 RNA in oropharyngeal, nasopharyngeal, nasal, and mid-turbinate swab specimens. 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 to detect a human S9 ribosomal gene in a clinical sample. RNA isolated from swab 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 (FAM) to separate from the 3' nonfluorescent quencher (NFQ), generating a fluorescent signal. During the course of the PCR amplification, fluorescence generated by degradation of the target-specific probe is monitored by the QuantStudio instrument.

INSTRUMENTS USED WITH TEST

The DTPM COVID-19 RT-PCR test is to be used with the ThermoFisher QuantStudio 5 instrument with software version 1.5.1.

LIMITATIONS

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 positive results to the appropriate public health authorities.

REAGENTS AND MATERIALS

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

Reagent	Manufacturer	Catalogue #
RNeasy Mini Kit	QIAGEN	74106
TAQMAN FAST Virus 1-step Master Mix	Thermo Fisher	4444426C001
HyPure Molecular Biology Water	GE	SH30538.02
DTPM COVID-19 Forward Primer, Reverse Primer	Thermo Fisher	4304972
DTPM COVID-19 Probe	Thermo Fisher	4316032
Endogenous Control Forward Primer, Reverse Primer	Thermo Fisher	4304972
Endogenous Control Probe	Thermo Fisher	4316032
DTPM COVID-19 and Endogenous Positive Control	Thermo Fisher	817000DE
384 well Real-time PCR Plates	VWR	60941-078

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 SAR-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 extraction control is run 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 extraction control – Endogenous extraction control must exceed the cutoff (Ct<35) and be positive in all the clinical specimens. In the case of a negative extraction result, users are instructed to repeat sample preparation and analysis.

   Results from the Positive control, Endogenous extraction 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. If deviations exceed this limit, the assay results will be rejected and the instrument and assay will be run using new QC materials. All Corrective Action Reports (CARs) are tracked by the laboratory and records are maintained for a minimum of 2 years.

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 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 (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 DTPM COVID-19 RT-PCR test was determined using quantified whole viral SARS-related coronavirus 2 (USA-WA1/2020) RNA obtained from BEI Resources (NR-52285). A preliminary LoD was determined by testing serial dilutions of RNA (1,345 - 3 genomic copies/µL) spiked into pooled nasopharyngeal specimens in pooled NP swab matrix in quadruplicate. Spiked samples were tested with the DTPM COVID-19 RT-PCR test following extraction with the Qiagen RNeasy Kit (Table 3).

Table 3. Initial LoD Determination

SARS-CoV-2 Concentration (Genomic Copies/µL)	SARS-CoV-2 Concentration (Genomic Copies/Reaction)	CT	Percent Reactive
1345	4035	26.92	100% (4/4)
		26.99	
		26.89	
		26.93	
673	2019	28.06	100% (4/4)
		28.17	
		28.15	
		28.11	
337	1011	29.29	100% (4/4)

SARS-CoV-2 Concentration (Genomic Copies/µL)	SARS-CoV-2 Concentration (Genomic Copies/Reaction)	CT	Percent Reactive
		29.17	
		28.93	
		29.08	
169	507	30.56	100% (4/4)
		30.01	
		29.97	
		30.10	
85	255	31.80	100% (4/4)
		31.71	
		31.29	
		30.62	
43	129	32.02	100% (4/4)
		32.14	
		32.02	
		31.99	
22	66	33.14	100% (4/4)
		32.87	
		32.60	
		33.20	
11	33	33.96	75% (3/4)
		34.35	
		35.55	
		33.97	
6	18	33.48	75% (3/4)
		34.02	
		33.98	
		35.44	
3	9	34.52	50% (2/4)
		35.45	
		35.45	
		33.95	

The lowest concentration of SARS-CoV-2 RNA that yielded a detection rate ≥95% was 22 genomic copies/µL (66 genomic copies/reaction).

The LoD was verified by testing 20 additional extraction replicates consisting of pooled nasopharyngeal specimens in NP swab matrix spiked at the preliminary LoD concentration of 22 copies/µL. All samples were spiked with RNA prior to extraction with the Qiagen RNeasy Kit. Results are summarized in Table 4 below.

Table 4. LoD Confirmation Study

Concentration (Copies/µL)	Mean CT (SD)	Percent Reactive
22	32.75 (0.5)	100% (20/20)

Inclusivity:

The sponsor's *in silico* BLAST analysis was performed on 4/15/2020 and comparison to sequences in the GISAID database was performed on 3/20/2020. The DTPM COVID-19 RT-PCR test was designed from the alignment of all available sequence data contained in GenBank and GISAID databases (using ClustalW progressive alignment). The specific DTPM COVID-19 RT-PCR test targets a 57 base pair conserved region of the nucleocapsid phosphoprotein (N gene). *In silico* analysis of the forward and reverse primers demonstrated 100% sequence identity with all 493 SARS-CoV-2 sequences contained in GenBank (as of 4/15/2020). Analysis with 341 SARS-CoV-2 sequences downloaded from the GISAID database on (3/20/2020) also demonstrated 100% sequence identity. Collectively, these analyses demonstrate that 100% of the reported SAR-CoV-2 sequences will be detectable with the DTPM COVID-19 RT-PCR test.

2) Analytical Specificity:

The DTPM COVID-19 RT-PCR assay was exposed to concentrations of pathogen in excess of 10^6 CFU/mL or 10^5 PFU/mL. In order to establish specificity of the DTPM COVID-19 assay, a laboratory cross-reactivity study was performed using other respiratory microorganism species. The DTPM COVID-19 assay was tested in triplicate using the following list of respiratory microbial flora or pathogens (Table 5). The high concentration bacteria or virus did not demonstrate any observed response consistent with specific detection of SARS-CoV-2.

Table 5. Cross-reactivity Study

Pathogen	Reactivity
Adenovirus	0/3
B. pertussis	0/3
Bocavirus	0/3
C. pneumoniae	0/3
Coronavirus 229E	0/3
Coronavirus HKU-1	0/3
Coronavirus NL63	0/3
Coronavirus OC43	0/3
Enterovirus	0/3
H. influenzae	0/3
Influenza A	0/3
Influenza B	0/3

Pathogen	Reactivity
K. pneumoniae	0/3
Legionella	0/3
M. catarrhalis	0/3
M. pneumoniae	0/3
Metapneumovirus A	0/3
Parechovirus	0/3
Parainfluenza virus 1	0/3
Parainfluenza virus 2	0/3
Parainfluenza virus 3	0/3
Parainfluenza virus 4	0/3
Rhinovirus	0/3
Respiratory syncytial virus A	0/3
S. aureus	0/3
S. pneumoniae	0/3
Salmonella enterica Typhimurium	0/3

In addition to the wet testing above, each component of the assay (Forward, Reverse and Probe sequences) was subjected to an *in silico* analysis to demonstrate specificity of the assay against multiple whole genome sequences for additional organisms not included in wet testing as detailed in Table 6 below.

Table 6. Cross-reactivity organisms evaluated *in silico*

Pathogen
SARS-CoV-1
MERS
Mycobacterium tuberculosis
Streptococcus pyogenes
Pneumocystis jirovecii
Candida albicans
Pseudomonas aeruginosa
Staphylococcus epidermis
Streptococcus salivarius

The DTPM primer and probe sequences were compared to the complete genome for the Toronto strain of SARS-CoV-1 (AY274119) The forward primer of the DTPM assay showed 90% homology to the SARS-CoV-1 sequences. The reverse primer showed 89% homology to the SARS-CoV-1 sequences. The probe showed 87.5% homology to the SARS-CoV-1 sequences. Further testing may be necessary to differentiate between SARS-CoV-1 or other Sarbecoviruses currently unknown to infect humans. No significant homology was observed for any of the other organisms analyzed.

3.) <u>Clinical Evaluation:</u>

Performance of the DTPM COVID-19 RT-PCR test was evaluated using individual nasopharyngeal (NP) swab specimens spiked with whole viral SARS-CoV-2 RNA (USA—WA1/2020). Prepared samples were randomized and blinded, and RNA was extracted using the Qiagen RNeasy Kit. In total, 30 contrived positive clinical matrix samples and 30 negative clinical matrix samples were tested.

Of the 30 contrived positive clinical samples, 24 were prepared with concentrations of SARS-CoV-2 RNA at the assay LoD (22 copies/µL). Half of the remaining six samples contained RNA at concentrations equivalent to 4X the assay LoD (88 copies/µL), while the other half contained RNA at concentrations equivalent to 32X the assay LoD (704 copies/µL). Data are shown in Table 7 below.

Table 7. Clinical Performance of the DTPM COVID-19 RT-PCR Test with Contrived NP Swab Specimens

SARS-CoV-2 Concentration (Copies/µL)	Percent Positive
1X LoD (22)	100% (24/24)
4X LoD (88)	100% (3/3)
32X LoD (704)	100% (3/3)
Negative (0)	0% (0/30)
Positive Percent Agreement (at 1X LoD)	100% (24/24) 95% CI: 86.2-100%
Positive Percent Agreement (at >1X LoD)	100% (6/6) 95% CI: 61.0-100%
Negative Percent Agreement	100% (30/30) 95% CI: 88.6-100%

Additionally, 43 clinical NP swab samples were tested by the DTPM COVID-19 RT-PCR test and an FDA EUA assay. The data is presented in Table 8 below. Two of the specimens that were negative by the DTPM COVID-19 RT-PCR test were inconclusive when evaluated by the FDA EUA assay. These samples were not retested and were excluded from analysis.

Table 8. Clinical Performance of the DTPM COVID-19 RT-PCR Test with Clinical NP Swab Specimens

		FDA EUA Assay	
		Positive	**Negative**
DTPM COVID-19 Test	**Positive**	23	0
	Negative	0	18

Positive Percent Agreement: 100% (23/23) 95% CI: 85.7-100%
Negative Percent Agreement: 100% (18/18) 95% CI: 82.4-100%

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized 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, to perform high complexity tests;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.