ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay
University of Texas MD Anderson Cancer Center

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

The MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay will be performed at the University of Texas MD Anderson Cancer Center in Houston, Texas, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as per the UT MD Anderson Cancer Center Molecular Diagnostic Lab SARS-CoV-2 Real-Time RT-PCR Test Laboratory Instructions for Use that was reviewed by the FDA under this EUA.

INTENDED USE

The MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay is intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs) from individuals suspected of COVID-19 by their health care provider. Testing is limited to the Molecular Diagnostic Laboratory (MDL) at the University of Texas MD Anderson Cancer Center, 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 detection and 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. 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 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 management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

The MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay is a reverse transcriptase real-time polymerase chain reaction (rRT-PCR) assay for the qualitative detection of SARS-CoV-2 specific RNA. This test uses primer/probe sets developed by the CDC that target two viral gene targets in the Nucleocapsid gene of SARS-CoV-2, N1 and N2, and an internal control gene, RNase P (RP).

The test consists of three processes in a single assay: 1) reverse transcription of target RNA to cDNA, 2) PCR amplification of target and Internal Control DNA, and 3) simultaneous detection of PCR amplicons by fluorescent dye labelled probes.

Three pipelines (i.e., A, B, and C) can used with this assay (see **Table 1**). Pipelines A and B are fully automated, while pipeline C is performed manually, except for a semi-automated extraction step.

Table 1. Description of MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay Pipelines

Pipeline	Extraction Kit	Lysis Instrument	Extraction Instrument	Reagent Prep Instrument	Sample Addition Instrument	Real Time PCR Instrument[1]
A	Promega Maxwell HT Viral TNA Kit	Hamilton Star (SN#: D170)	Hamilton Starlet (SN#: C119)	Hamilton Star (SN#: D043)	Hamilton Star (SN#: D169)	QuantStudio 7 Flex System with Fast block (SN#: 278871404)
B	Promega Maxwell HT Viral TNA Kit	Hamilton Star (SN#: D170)	Hamilton Star (SN#: D167)	Hamilton Star (SN#: D262)	Hamilton Star (SN#: D133)	QuantStudio 7 Flex System with Fast block (SN#: 278871404)
C	Promega Maxwell RSC Viral TNA Kit	Manual	Maxwell RSC 48 (SN#: 20000175)	Manual (PCR Workstation)	Manual (PCR Workstation)	QuantStudio 7 Flex System with Fast block (SN#: 278871404)

[1] For each pipeline, the Applied Biosystems 7500 Fast Real-Time PCR Instrument (Software V1.4) or QuantStudio Dx Real-Time PCR Instrument with 96-well fast block (Software V1.3) can be used rather than the QuantStudio 7 Flex System with Fast block. These additional Real-Time PCR Instruments have been validated with the assay (see the "Performance Evaluation" Section of this document for more information).

INSTRUMENTS USED WITH TEST

The MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay is to be used with the following instrumentation:

- Specimen Lysis/RNA Extraction/Realtime PCR Reagent Preparation: Hamilton Robotics MicroLab Starlet, Hamilton Robotics MicroLab Star, or Promega Maxwell RSC 48.

- RT-PCR Platform: QuantStudio 7 Flex Fast Sequence Detection System (Software V1.3), Applied Biosystems 7500 Fast Real-Time PCR Instrument (Software V1.5.1), or QuantStudio Dx Real-Time PCR Instrument with 96-well fast block (Software V1.0.3).

REAGENTS AND MATERIALS

- 2019-nCoV CDC EUA Kit, 500 rxn
- 2019-nCoV_N_Positive Control (IDT)
- TaqPath 1-Step RT-qPCR Master Mix, CG (Applied Biosystems)
- Vortex mixer
- Microcentrifuge
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL)
- Multichannel micropipettes (5-50 µL)
- Racks for 1.5 mL microcentrifuge tubes
- Racks for PCR tubes
- 5 ml fluidX Tall LidLock (Thomas Scientific)
- -20°C cold blocks
- Molecular grade water, nuclease-free
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- 70% Ethanol (7:10 dilution of Laboratory grade 200 Proof Ethanol)
- Conflickt ready to use disinfectant spray
- DNAZap (Ambion) or equivalent
- RNAseAWAY (Fisher Scientific) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 96-well 0.2 mL PCR reaction plates (Applied Biosystems)

CONTROLS TO BE USED WITH THE MD ANDERSON HIGH-THROUGHPUT SARS-CoV-2 RT-PCR Assay

Table 2. Assay Controls Run with Each Test

Control Type	Purpose	Frequency of Testing
Positive Control	To monitor the integrity of the RT-PCR reagents and process	Once per RT-PCR run
No Template control	To monitor for reagent contamination and/or environmental contamination during extraction and RT-PCR	Once per RT-PCR run
Human Specimen (HSC) Extraction Control	Failure in lysis and extraction procedure, potential contamination during extraction	Once per batch of specimens
Human RP Internal Control (IC)	To monitor the integrity of nucleic acid extraction and RT-PCR for each specimen	Each patient specimen

External Positive Control

- A positive control is used to verify proper assay set-up and SARS-CoV-2 reagent integrity. The positive control is a DNA plasmid that contains the N gene of SARS-CoV-2 combined with DNAse I treated total RNA extracted from a human cell line. The plasmid control is commercially available from Integrated DNA Technologies (2019-nCoV_N_Positive Control, Catalogue #1006625). The human cell line is cultured and the RNA extracted at the Molecular

Diagnostic Laboratory at MD Anderson Cancer Center. A positive control is included in each RT-PCR run.

No Template Control

- A "no template" (negative) control (NTC) is needed to check for contamination of extraction and assay reagents. Nuclease free water is used in place of sample nucleic acid for this control. One NTC is included during the extraction phase and another is included at the RT-PCR setup phase of the protocol. These two NTCs are included on every RT-PCR plate.

Human Specimen Control (HSC) Extraction Control

- An HSC extraction control is included to (1) demonstrate detection of human RP mRNA, assuring RNA extraction and amplification and; (2) absence of N1 and N2 target sequences, assuring absence of cross-contamination. An HL-60 cell-line (ATCC, Catalogue # ATCC CCL-240) will serve as the HSC. The HSC control will be included with each batch.

Human Ribonuclease P (RP) Internal Control

- The human ribonuclease P (RP) internal control serves as an internal process control for nucleic acid extraction to ensure that clinical samples and controls contains sufficient and quality RNA to be used in the RT-PCR reactions.

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 (Refer to **Table 3** for a summary of expected control results).

1. ***COVID-19 RT-PCR Test controls – Positive, Negative, Extraction, and Internal*:**

   Controls should produce the results outlined in **Table 3**, below.

   Table 3. Expected Control Results for the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay

Control Type	N1	N2	RP (IC)	Expected Ct values
Positive Control	+	+	+	< 40 Ct
No Template Control	-	-	-	None detected
Human Specimen (HSC) Extraction Control	-	-	+	< 40 Ct
Human RP Internal Control	Any	Any	+	< 40 Ct

   If the Positive Control, No Template Control, or HSC Extraction Control do not perform as shown above, the run is considered invalid and all specimens should be re-tested. If all controls perform as expected except the human RP internal control, the sample yielding an invalid IC result should be repeated.

2. ***Examination and Interpretation of Patient Specimen Results***:
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 controls are not valid, the patient results cannot be interpreted. Please see **Table 4** for guidance on patient specimen result interpretation and reporting of results.

Table 4. Interpretation of Patient Results for the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay

N1	N2	RP (IC)	Status	Result	Action
+	+	+/-	Valid	SARS-CoV-2 Detected	Report results to healthcare provider and appropriate public health authorities.
If only one of the two targets is positive		+/-	Valid	Inconclusive	Repeat testing. If the same result is obtained, or both targets are positive, report as "SARS-CoV-2 Detected".
-	-	+	Valid	SARS-CoV-2 Not Detected	Report results to healthcare provider.
-	-	-	Invalid	Invalid	Re-extract the specimen and repeat testing.

PERFORMANCE EVALUATION

Analytical Sensitivity – Limit of Detection (LoD):

The limit of detection (LoD) was established for device pipelines A-C (see **Table 1**) by spiking an encapsulated synthetic RNA, AccuPlex SARS-CoV-2 (Product # 0505-0129, SeraCare) into a pool of negative nasopharyngeal (NP) specimens. The NP pool was shown to be negative for SARS-CoV-2 by testing with an EUA approved assay. The LoD was estimated for each pipeline by testing five concentrations of a dilution series (ranging from 100 copies/µL to 0.2 copies/µL) of the NP pool spiked with AccuPlex SARS-CoV-2 synthetic RNA on the QuantStudio 7 Flex System Real-Time PCR Instrument.

The initial LoD for the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay was determined to be 5 copies/µL, irrespective of the pipeline. The LoD was verified by testing 20 additional replicates consisting of pooled negative NP specimens spiked at 5, 1.67, and 1 copies/µL. The LoD was confirmed to be 5 copies/µL for pipeline A and B and 1.67 copies/µL for pipeline C. Confirmatory LoD Study results are illustrated in **Table 5**.

Table 5. LoD Confirmatory Results, Stratified by Pipeline

Concentration (copies/µL)	Pipeline A		Pipeline B		Pipeline C	
	#Positive/ # Tested	% Positive	#Positive/ # Tested	% Positive	#Positive/ # Tested	% Positive
5	20/20	100%	20/20	100%	20/20	100%
1.67	13/20	65%	8/20	40%	20/20	100%
1	N/A	N/A	N/A	N/A	16/20	80%

Note: Each pipeline was evaluated with the QuantStudio 7 Flex System Real-Time PCR Instrument

In addition, LoDs for alternative real-time PCR instruments were established using Device Pipeline A. The LoD was estimated for each pipeline by testing five concentrations of a modified dilution series (ranging from 12.5 copies/µL to 2.5 copies/µL) of the NP matrix pool spiked with AccuPlex SARS-CoV-2 synthetic RNA on the QuantStudio 7 Flex System Real-Time PCR Instrument, ABI7500 Fast and QuantStudio DX Real Time PCR System. The initial LoD was estimated to be 5 copies/µL, irrespective of the real-time PCR instrument. The LoD was confirmed by testing 20 replicates consisting of pooled NP specimens spiked at either 5 or 1.67 copies/µL. The LoD was confirmed to be 5 copies/µL, irrespective of the real-time PCR instrument. Confirmatory LoD Study results are illustrated in **Tables 6**.

Table 6. LoD Confirmatory Results for Device Pipeline A, Stratified by Real-Time PCR Instrument

Concentration (copies/µL)	QuantStudio 7		QuantStudio DX		ABI 7500 Fast	
	#Positive/ # Tested	% Positive	#Positive/ # Tested	% Positive	#Positive/ # Tested	% Positive
5	20/20	100%	20/20	100%	20/20	100%
1.67	13/20	65%	17/20	85%	15/20	75%

Analytical Sensitivity – Inclusivity:
The sequences for the N1 and N2 primers/probes used in this assay are identical to the primer/probe sequences used in the FDA emergency use authorized CDC 2019-Novel Coronavirus (2019-nCoV) Diagnostic Panel. CDC has provided a right of reference to their Inclusivity Study data, which is available at https://www.fda.gov/media/134922/download.

Analytical Specificity – Cross-Reactivity:
In-silico Cross-Reactivity Assessment
The sequences for the N1 and N2 primers/probes used in this assay are identical to the primer/probe sequences used in the FDA emergency use authorized CDC 2019-Novel Coronavirus (2019-nCoV) Diagnostic Panel. CDC has provided a right of reference to their Cross-Reactivity Study data, which is available at https://www.fda.gov/media/134922/download.

Cross-Reactivity Wet-Testing
To supplement the *in-silico* cross-reactivity analysis, 21 clinical samples harboring 9 distinct organisms (see **Table 7**) were evaluated with the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay. All samples were negative for SARS-CoV-2, as expected.

Table 7. Cross-Reactivity Wet-Testing Study Results

Organism	Strain/Genotype	SARS-CoV-2 – N1 Target (# Pos / # Tested)	SARS-CoV-2 - N2 Target (# Pos / # Tested)
Human Coronavirus	HKU1	0/3	0/3
Human Coronavirus	NL63	0/3	0/3
Human Coronavirus	OC43	0/3	0/3
Influenza A	2009 H1N1	0/3	0/3
Influenza B	N/A	0/3	0/3
Parainfluenza 2	N/A	0/1	0/1
Parainfluenza 3	N/A	0/1	0/1
Parainfluenza 4	N/A	0/1	0/1
RSV	N/A	0/3	0/3

Clinical Evaluation:

Performance of the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay was evaluated using residual nasopharyngeal (NP) specimens collected from individual patients. Forty-eight (48) nasopharyngeal swabs were collected at MD Anderson and tested in-house on the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay (Pipeline C) and an FDA Emergency Use Authorized (EUA) Assay. Twenty-six (26) specimens were positive by both assays while twenty-two specimens (22) were negative by both assays, for a positive percent agreement (PPA) and negative percent agreement (NPA) of 100%. Results are presented in **Table 8**.

In addition, eleven (11) residual nasopharyngeal specimens collected at MD Anderson were tested with the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay (Pipeline C) and an FDA EUA assay at the Houston Health Department Laboratory. Five (5) specimens were positive by both assays while six (6) were negative by both assays, yielding a positive percent agreement (PPA) and negative percent agreement (NPA) of 100%. Results are presented in **Table 8**.

Table 8. Performance of the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay Compared to Two Independent FDA Emergency Use Authorized (EUA) Assays

Nasopharyngeal Swabs		**FDA EUA Assay -1**		
		Positive	**Negative**	**Total**
MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay	**Positive**	26	0	26
	Negative	0	22	22
	Total	26	22	48
Positive Agreement		100% (26/26), 95% CI: (87.1, 100%)[1]		
Negative Agreement		100% (22/22), 95% CI: (85.1, 100%)		
Nasopharyngeal Swabs		**FDA EUA Assay -2**		
		Positive	**Negative**	**Total**
MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay	**Positive**	5	0	5
	Negative	0	6	6
	Total	5	6	11
Positive Agreement		100% (5/5), 95% CI: (56.6, 100%)		
Negative Agreement		100% (6/6), 95% CI: (61.0, 100%)		

[1] Two-sided 95% confidence intervals

In total, 36 NP specimens were shown to be positive and 28 NP specimens were shown to be negative by the MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay and an FDA emergency use authorized assay.

FDA SARS-CoV-2 Reference Panel Testing:

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method and instrument used were the Promega Maxwell RSC Viral TNA Kit using the Maxwell RSC 48 and the QuantStudio 7 Flex System with Fast block rtPCR instrument. The results are summarized in the following Table.

Table 9. Summary of LoD Confirmation Results Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not Detected

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- 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.