EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
MiraDx SARS-CoV-2 RT-PCR assay
(MiraDx)

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

(The MiraDx SARS-CoV-2 RT-PCR assay will be performed at the MiraDx laboratory, located at 11600 Wilshire Blvd., Suite 410, Los Angeles, CA 90025, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets the 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 MiraDx SARS-CoV-2 RT-PCR assay is a real-time reverse transcription polymerase chain reaction (rRT- PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to MiraDx located at 11600 Wilshire Blvd., Suite 410, Los Angeles, CA 90025 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 identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory samples 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 MiraDx SARS-CoV-2 RT-PCR assay 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 MiraDx 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 MiraDx SARS-CoV-2 RT-PCR assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test which includes two primer and probe sets designed to detect RNA from the SARS-CoV-2 nucleocapsid (N) gene in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) from individuals suspected of COVID-19 by their healthcare provider. In addition, an additional primer/probe set is used to detect the human RNase P gene (RP) in relevant control samples and clinical specimens and serves as an extraction, reverse transcription, and PCR amplification positive control for each well.

RNA isolated from respiratory specimens is reverse transcribed to cDNA and subsequently amplified using the Applied Biosystems QuantStudio 6 Flex PCR Instrument. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (FAM) to separate from the quencher dye (3IABkFQ), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the thermocycler.

INSTRUMENTS USED WITH THE TEST

Automated Liquid Handling (Extraction and PCR Set Up)
Manufacturer: Hamilton
Model(s): Microlab STAR
Catalog #: 173027
Software Name and Version: Hamilton ML_STAR version 4.5.0.5217

Polymerase Chain Reaction
Manufacturer: Applied Biosystems
Model(s): QuantStudio 6 Flex
Catalog #: 4485694
Software Name and Version: QuantStudio Software Version 1.3

REAGENTS AND MATERIALS

The following reagents and materials are required to run the test in addition to general lab consumables for the extraction and PCR:

1. Sterile flocked swab and generally recommended media such as VTM, ITM, Liquid Amies, Phosphate buffered saline (PBS) or normal saline solution for nasopharyngeal swab collection. Note: Samples are handled and stored following the CDC guidelines (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html) for collection by a health care provider.
2. DNA Genotek ORAcollect RNA collection device (Catalog# OR-100) for oropharyngeal swab collection. Note: Samples are handled and stored following the

CDC guidelines (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html) for collection by a health care provider

3. Omega Bio-Tek Mag-Bind Viral DNA/RNA kit (Catalogue #M6246-03) for RNA extraction
4. Applied Biosystems TaqPath 1-Step RT-qPCR Master Mix (ThermoFisher; Catalog# A15300)
5. Primer/probe sequences for N1 and N2 targets of SARS-CoV-2 and human RNase P (IDT; Catalog#10006606)
6. Control materials listed below. Refer to "Control Materials To Be Used" for additional details.
    a. RNase Free water
    b. Twist Bioscience Catalog #102019 Twist Synthetic SARS-CoV-2 RNA Control 1 MT007544.1
    c. Twist Bioscience Catalog #102024 Twist Synthetic SARS-CoV-2 RNA Control 2 MN908947.3
    d. IDT 2019-nCoV_N_Positive Control; Catalog #10006625
    e. IDT Hs_RPP30 Positive Control; Catalog #10006626

CONTROL MATERIALS TO BE USED

MiraDx runs two paired sets of controls (Extraction and PCR) as well as an internal RP control in tandem with patient samples during each run.

The Extraction Control pair includes a 1) Negative control (RNase free water) and a 2) Positive control consisting of synthetic RNA for two strains of SARS-COV-2 (Twist Bioscience Catalog #102019 Twist Synthetic SARS-CoV-2 RNA Control 1 MT007544.1; Twist Bioscience Catalog #102024 Twist Synthetic SARS-CoV-2 RNA Control 2 MN908947.3) spiked into negative sample matrix. The controls are used at a concentration of 10 copies/µL (2.5x LOD). This enables an extraction control for SARS-CoV-2 as well as an internal control for RP.

The PCR Control Pair includes a 1) Negative Control (NTC): A "no template" (negative) control with PCR Grade (RNase free) water and 2) Positive template controls. One positive template control (plasmid DNA) uses the N1 and N2 targets within the SARS-CoV-2 nucleocapsid gene (IDT; Catalog #10006625). For PCR, the template concentration is 10 copies/µL (2.5x LOD). The second positive template control for PCR is for RP, and is detected by adding Hs_RPP30 (IDT; Catalog #10006626). The Hs_RPP30 Control contains a portion of the RPP30 gene, a single copy gene present in the human genome. The positive template control is used at a concentration of 10 copies/µL.

INTERPRETATION OF RESULTS

The following control results must be obtained for each extracted sample and its PCR run in order for the sample run to be deemed valid. If the controls are not valid, the patient results cannot be interpreted, are not valid, and the sample run must be repeated.

Table 1: Expected Performance of Controls

	N1	N2	RP	Interpretation
Extraction: Positive Control for SARS-CoV-2 and internal RP (Twist synthetic SARS-CoV-2 RNA)	Detected (CT≤40)	Detected (CT≤40)	Detected (CT≤40)	Valid
PCR: Positive Control (IDT 2019-nCoV_N plasmid DNA)	Detected (CT≤40)	Detected (CT≤40)	Not Detected (CT≥45)	Valid
PCR: Positive Control (IDT Hs_RPP30)	Not Detected (CT≥45)	Not Detected (CT≥45)	Detected (CT≤40)	Valid
PCR and Extraction: Negative Control (NTC)	Not Detected (CT≥45)	Not Detected (CT≥45)	Not Detected (CT≥45)	Valid

Table 2: Interpretation of Sample Results

N1 Result	N2 Result	RP Result	Interpretation	Action
DETECTED (CT≤40)	DETECTED (CT≤40)	ANY RESULT	SARS-CoV-2 DETECTED	Release results to sender and report to Public Health Authorities.
DETECTED (CT≤40)	NOT DETECTED (CT≥45)	ANY RESULT		
NOT DETECTED (CT≥45)	DETECTED (CT≤40)	ANY RESULT		
DETECTED (CT≤40)	INDETERMINATE (40<CT<45)	ANY RESULT		
INDETERMINATE (40<CT<45)	DETECTED (CT≤40)	ANY RESULT		
INDETERMINATE (40<CT<45)	INDETERMINATE (40<CT<45)	DETECTED (CT≤40)	INDETERMINATE FOR SARS-CoV-2	Release results to sender and report to Public Health Authorities. Recommend recollection in 2-3 days.
INDETERMINATE (40<CT<45)	NOT DETECTED (CT≥45)	DETECTED (CT≤40)		
NOT DETECTED (CT≥45)	INDETERMINATE (40<CT<45)	DETECTED (CT≤40)		
NOT DETECTED (CT≥45)	NOT DETECTED (CT≥45)	DETECTED (CT≤40)	SARS-CoV-2 NOT DETECTED	Release results to sender and report to Public Health Authorities.
ANY RESULT	ANY RESULT	NOT DETECTED (CT≥45)	INVALID SPECIMEN	Repeat testing from elution, if sample fails again, re-extract. If re-extraction fails, request a repeat sample.

For N1, N2 and RP result, "DETECTED" refers to Ct ≤ 40. For N1 and N2, "INDETERMINATE" refers to late or mild amplification with 40 < Ct < 45.

Ct Value	Interpretation
Ct ≤ 40	Detected
40 < Ct < 45	Indeterminate
Ct ≥ 45 or "Undetermined"	Not Detected

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) -Analytical Sensitivity

The LoD is defined from this study as the lowest concentration at which 44/46 replicates (at least 95%) are positive. The samples for the preliminary LoD were prepared by spiking synthetic RNA of two strains of SARS-COV-2 (102019: Twist Synthetic SARS-CoV-2 RNA Control 1 MT007544.1; 102024: Twist Synthetic SARS-CoV-2 RNA Control 2 MN908947.3) into pooled oropharyngeal swab specimens previously tested negative for SARS-CoV-2 by the MiraDx SARS-CoV-2 RT PCR assay. The preliminary LoD was identified by testing dilutions of 1000, 100, 10, 8, 6, 4, 2, and 1 copies/µL with 20 replicates per dilution. The spiked samples were extracted with the Hamilton instrument using the Omega Bio-Tek Mag-Bind Viral DNA/RNA Kit and were amplified/detected with the Applied Biosystems Quantstudio 6 Flex PCR instrument. The preliminary LoD determined that the MiraDx SARS-CoV-2 RT-PCR assay could detect samples with a concentration of the SATS-CoV-2 between 4 and 6 copies/µL. A confirmatory LoD followed the initial preliminary LoD study by spiking synthetic RNA for two strains of SARS COV-2 (102019: Twist Synthetic SARS-CoV-2 RNA Control 1 MT007544.1; 102024: Twist Synthetic SARS-CoV-2 RNA Control 2 MN908947.3) at 4 copies/uL into individual oropharyngeal swab specimens previously tested negative for SARS-CoV-2 by the MiraDx SARS-CoV-2 RT-PCR assay. Each synthetic RNA strain (Strain 1 and Strain 2) and target (N1 and N2) were tested with 46 replicates. See **Tables 3, 4, 5 and 6** below for the summary results.

Table 3: Preliminary LOD Results – Strain 1 N1 and N2 in pooled oropharyngeal swab specimens

Target Level	Valid tested replicates	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
1000 cp/µL	20	20	19.36	100%	20	21.02	100%
100 cp/µL	20	20	20.51	100%	20	21.75	100%
10 cp/µL	20	20	24.60	100%	20	25.88	100%
8 cp/µL	20	20	29.07	100%	20	29.41	100%
6 cp/µL	20	20	30.43	100%	20	32.90	100%
4 cp/µL	20	19	31.17	95%	18	36.33	90%
2 cp/µL	20	19	38.04	95%	18	37.65	90%
1 cp/µL	20	16	38.33	80%	15	38.55	75%

Table 4: Preliminary LOD Results – Strain 2 N1 and N2 in pooled oropharyngeal swab specimens

Target Level	Valid tested replicates	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
1000 cp/µL	20	20	18.83	100%	20	20.40	100%
100 cp/µL	20	20	21.64	100%	20	22.23	100%
10 cp/µL	20	20	26.11	100%	20	26.76	100%
8 cp/µL	20	20	30.48	100%	20	31.95	100%
6 cp/µL	20	20	32.39	100%	19	34.53	95%
4 cp/µL	20	19	36.12	95%	19	37.30	95%
2 cp/µL	20	17	37.19	85%	18	38.14	90%
1 cp/µL	20	16	38.86	80%	16	38.58	80%

Table 5: Confirmatory LOD Results – Strain 1 N1 and N2 in pooled oropharyngeal swab specimens

Target Level	Valid tested replicates	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
4 cp/µL	46	46	28.98	100%	45	32.76	97.8%

Table 6: Confirmatory LOD Results – Strain 2 N1 and N2 in pooled oropharyngeal swab specimens

Target Level	Valid tested replicates	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
4 cp/µL	46	46	29.17	100%	44	33.01	95.7

An additional LoD evaluation was conducted to determine whether the LoD of the MiraDx SARS-CoV-2 RT-PCR assay was similar in nasopharyngeal swab specimens as compared to oropharyngeal swab specimens. Samples were prepared by spiking synthetic RNA for two strains of SARS-COV-2 (102019: Twist Synthetic SARS-CoV-2 RNA Control 1 MT007544.1; 102024: Twist Synthetic SARS-CoV-2 RNA Control 2 MN908947.3) into pooled nasopharyngeal swab specimens collected in normal saline solution. The nasopharyngeal swab specimens had previously tested negative for SARS-CoV-2 by the MiraDx SARS-CoV-2 RT PCR assay. The LoD was evaluated by testing dilutions of 12, 10, 8 and 4 copies/µL with 10 replicates at dilutions 12 and 8 copies/µL and 5 replicates at dilutions 8 and 4 copies/µL. The spiked samples were extracted with the Hamilton instrument using the Omega Bio-Tek Mag-Bind Viral DNA/RNA Kit and were amplified/detected with the Applied Biosystems Quantstudio 6 Flex PCR instrument. The LoD results with nasopharyngeal swab specimens demonstrated that the LoD of the MiraDx SARS-CoV-2 RT-PCR assay was comparable to LoD results with oropharyngeal swab specimens. See Table 7 below for the summary results.

Table 7: LOD Results – Strain 1 and 2; N1 and N2 in pooled nasopharyngeal swab specimens

Target Level	Valid tested replicates	SARS-CoV-2 N1 Positive			SARS-CoV-2 N2 Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
12 cp/µL	10	10	25.26	100%	10	27.90	100%
10 cp/µL	10	10	25.57	100%	10	28.39	100%
8 cp/µL	5	5	25.71	100%	5	28.60	100%
4 cp/µL	5	5	27.44	100%	5	30.10	100%

2) Inclusivity (analytical sensitivity)

The sequences for the N1 and N2 primers and probes used in the MiraDx SARS-CoV-2 RT-PCR assay are identical to the primer and probe sequences used in the FDA authorized CDC test (EUA200001). According to the latest IFU (CDC-006-00019, Revision: 05; Effective 7/13/2020) for the CDC EUA assay, an *in silico* analysis of the CDC primers and probe sequences was done on June 20th, 2020 and therefore, additional evaluation is not required. The CDC has granted a right of reference to the performance data contained in their EUA to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

3) Cross-reactivity (Analytical Specificity)

The MiraDx SARS-CoV-2 RT-PCR assay utilizes the identical oligonucleotide sequences as those used in the FDA authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (EUA200001). The CDC has granted a right of reference to the performance data contained in the CDC's EUA request (FDA submission number EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

4) Clinical Evaluation

A total of 69 clinical oropharyngeal and nasopharyngeal specimens (32 positive and 37 negative for SARS-CoV-2) were tested with the MiraDx SARS-CoV-2 RT-PCR assay and compared to the results obtained by 2 different FDA-authorized tests. The results are summarized in Table 8. Samples were extracted with the Hamilton Microlab STAR instrument using the Omega Bio-Tek Mag-Bind Viral DNA/RNA Kit and the reverse transcription RT-PCR was performed using Applied Biosystems Quantstudio 6 Flex PCR instrument.

Table 8: Clinical performance of the MiraDx SARS-CoV-2 RT-PCR Assay in oropharyngeal and nasopharyngeal swab specimens

		FDA EUA Tests		
		Positive Patient Specimen	Negative Patient Specimen	**Total**
MiraDx SARS-CoV-2 RT-PCR assay	Positive Patient Specimen	**31**	**0**	**31**
	Negative Patient Specimen	**1**	**37**	**38**
	Total (69)	**32**	**37**	

Results:

The positive and negative percent agreements between the MiraDx SARS-CoV-2 RT-PCR assay and FDA EUA tests are:

PPA = 100% * 31/(31 + 1) = 96.9%. (95% C.I. = 84.3% - 99.5%)

NPA = 100% * 37/(37 + 0) = 100%. (95% C.I. = 90.6% - 100%)

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 used was the Omega Mag- Bind Viral DNA/RNA Kit on the Hamilton MicroStar Robotic System. Amplification was carried out on the ThermoFisher/ABI QuantStudio 6 Flex. The results are summarized in the following Table.

Table 9: Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	**Specimen Type**	**Product LoD**	**Cross-Reactivity**
SARS-CoV-2	Nasopharyngeal Swab	$0.6x10^3$ 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 MiraDx laboratory, located at 11600 Wilshire Blvd., Suite 410, Los Angeles, CA 90025;
- 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* 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 authorization is terminated or revoked sooner.

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to MiraDx laboratory 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.
- The MiraDx SARS-CoV-2 RT-PCR assay was established using nasopharyngeal and oropharyngeal swab specimens. Nasal and mid-turbinate swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens are also considered acceptable specimen types for use with the MiraDx SARS-CoV-2 RT-PCR assay but performance has not been established.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- False negative results can arise from:
  - Specimen collection conducted prior to symptom onset.
  - Failure to follow the authorized assay procedures.
  - Failure to use authorized extraction kit and platform.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay.
- A false positive result may arise from cross contamination during specimen handling or preparation, or between patient samples.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.