ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY COVID-19 KEY ASSAY (MICROGEN DX, SOUTHWEST REGIONAL PCR LABORATORY LLC.)

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

The COVID-19 Key Assay will be performed at MicroGen DX, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE

The COVID-19 Key assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swabs, nasopharyngeal awash/aspirate or nasal aspirates as well as sputum specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Southwest Regional PCR Laboratory LLC. dba MicroGen DX located in Lubbock, TX, which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, certified high-complexity laboratory.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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 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 COVID-19 Key assay is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays. The COVID-19 Key assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The COVID-19 Key assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The oligonucleotide primers and probes in the COVID19 Key are designed to detect multiple target sequences of the 2019-nCoV virus nucleocapsid (N) gene (Table 1). The test includes the same N1 and N2 primer and probe sequences as the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel for the specific detection of the 2019-nCoV, and the N3 primer and probe sequences for the universal detection of SARS-like coronaviruses. Oligonucleotide primers and probe to detect the human RNase P gene (RP) in control samples and clinical specimens is also included.

RNA is isolated from respiratory specimens including nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab specimens as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum using the Zymo Viral RNA Mag Bead Kit (cat #R1041) performed on the Thermo Fisher Scientific King Fisher Flex II extraction platform (cat #5400610). Extracted RNA is reverse transcribed to cDNA and subsequently amplified using the Roche Lightcycler 480II with Lightcycler 480 software version 1.5.1 sp3. 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 (BHQ-1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

INSTRUMENTS USED WITH TEST

The COVID-19 Key assay is to be used with the Zymo Viral RNA Mag Bead Kit on the Thermo Fisher Scientific King Fisher Flex II extraction platform and the Roche Lightcycler 480II with Lightcycler 480 software version 1.5.1 sp3.

REAGENTS AND MATERIALS

Reagent Manufacturer and Description	Catalog #	Manufacturer
Zymo Viral RNA Mag Bead Kit	R1041	Zymo BioScience
King Fisher Flex I extraction platform	5400610	Thermo Fisher
QuantaBio Ultraplex 1-Step RT-PCR Mastermix	95168-500	Quanta BioScience
COVID-19_N1-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N1-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N1-P Probe (N1 probe)	10006606	Integrated DNA Technologies
COVID-19_N2-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N2-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N2-P Probe (N2 probe)	10006606	Integrated DNA Technologies
COVID-19_N3-F Primer (forward primer)	10006606	Integrated DNA Technologies
COVID-19_N3-R Primer (reverse primer)	10006606	Integrated DNA Technologies
COVID-19_N3-P Probe (N3 probe)	10006606	Integrated DNA Technologies
RP-F Primer (forward primer)	10006606	Integrated DNA Technologies
RP-R Primer (reverse primer)	10006606	Integrated DNA Technologies
RP-P Probe (RNase P probe)	10006606	Integrated DNA Technologies
COVID-19_N_Positive Control Hs_RPP30_Internal Extraction Control	10006626	Integrated DNA Technologies
2019-nCoV_N_Positive Control	10006625	Integrated DNA Technologies

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

1) A "no template" (negative) control (NTC) is needed to check for contamination of assay reagents. Molecular grade, nuclease-free water is used in place of sample nucleic acid for this control. The NTC is used on every assay plate and is only subjected to the reverse transcription and the qPCR steps.

2) A positive (2019-nCoV_N_Positive Control) control is needed to verify proper assay set-up and SARS-CoV-2 reagent integrity. The positive control is used on every assay plate with patient samples to ensure that the master mix was properly prepared and that primer/probe sets are detecting reactive template in the RT-PCR reaction. The positive control is commercially supplied from IDT (cat # 10006625) and is made of *in vitro* transcribed and purified viral RNA target that contains one copy each of N1, N2, and N3 as well as RP. The positive control is supplied in a working concentration of 200,000 copies/µL and is diluted 1:1000 for the COVID-19 Key assay.

3) A negative extraction control (molecular grade water) (NEC) is used to ensure all extraction reagents are free of contamination. A negative extraction control is included with every batch of extractions and is treated as a patient sample; however, the human specimen extraction control (Hs_RPP30) is not added. The purpose of the negative extraction control is to ensure the integrity of the extraction reagents and to determine post-analysis, if contamination has occurred. The NEC is subjected to extraction, reverse transcription, and the PCR process.

1) The human specimen extraction control (HSC) is a human RNA extract from non-infected samples that is purchased from IDT (cat #10006626). It serves both as a negative extraction control to monitor for any cross-contamination that occurs during the extraction process, as well as an extraction control to validate extraction reagents and successful RNA extraction. The HSC is used in each batch of extractions but is not spiked into every patient sample.

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 1 for a summary of control results).

1) <u>COVID-19 RT-PCR test Controls – Positive, Negative, and Internal</u>:

- All NTC reactions should be negative for all targets, meaning no amplification curves cross the PCR cycle threshold (Ct ≤ 35). If any of the N1, N2, N3, or RP NTC reactions exhibit positive fluorescence above the threshold (Ct ≤ 35), it is possible that contamination occurred, or that the assay was setup improperly. The

RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is positive, re-extract and re-test all samples.

- Positive control reactions for the N1, N2, N3, and RP assays should yield positive results with a Ct value Ct ≤ 35. Negative results with either N1, N2, N3, or RP primer/probe sets invalidates the run and suggests the assay may have been set up incorrectly, or the integrity of the primers/probes is compromised. The RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is negative for SARS-CoV-2 targets, re-extract and re-test all samples.

- The Human RNase P gene is used as a positive extraction control which verifies successful extraction. Hs_RPP30 is used in one well on the PCR plate and is not added to every patient sample prior to extraction. The assay relies on having adequate detection of RP from each clinical sample, rather than an additional spike in of RP for the test to be considered valid. Every patient sample should show amplification ≤ 35.00 Ct for RP; otherwise the result is inconclusive due to insufficient material. Negative results will be repeated one time using residual extracted nucleic acid. If results for Hs_RPP30 remain negative, the ordering physician will be instructed to collect a new sample from the patient.

- The negative extraction control (NEC) reactions should yield negative results with the N1, N2, and N3 assays, as well as the RP assay. If positive results occur in the N1, N2, or N3 reaction wells with the NEC control, contamination of nucleic acid extraction reagents or cross-contamination of samples may have occurred. The extraction run and the RT-PCR run are invalid and should be repeated using residual patient sample. If results for NEC remain positive, the ordering physician will be instructed to collect a new sample from the patient.

Table 1: Expected Results of Controls Used in the COVID-19 Key Assay

Control Type	Control Name	Used to Monitor	Expected Results and Ct Values							
			SARS-CoV-2 N1		SARS-CoV-2 N2		SARS-CoV-2 N3		RNase P (RP)	
			Call	Ct	Call	Ct	Call	Ct	Call	Ct
Negative	NTC	Amplification	Negative	ND*	Negative	ND	Negative	ND	Negative	ND
Positive	COVID-19_N_Positive Template Control (PTC)	Amplification	Positive	≤ 35	Positive	≤ 35	Positive	≤ 35	Positive	≤ 35
Extraction	Negative Extraction Control	Extraction/ Amplification	Negative	ND	Negative	ND	Negative	UND	Negative	ND
	Internal HS_RPP30 Control	Extraction/ Amplification	Negative	ND	Negative	ND	Negative	ND	Positive	≤ 35

*ND; Not Detected

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 the table below (Table 2) for guidance on interpretation and reporting of results.

Table 2: Interpretation of Patient Results Using the COVID-19 Key Assay

SARS-CoV-2 N1	SARS-CoV-2 N2	SARS-CoV-2 N3	RNase P	Interpretation	Report Result	Actions
+	+	+	+	SARS-CoV-2 **Detected**	**POSITIVE**	Reported to sender and appropriate public health authorities.
+	+	+	-	**Insufficient material**	**Inconclusive**	Repeat one time using residual extracted nucleic acid. If the results remain inconclusive, a recollect order will be issued to the physician.
If only one, or two of three targets are positive			+	**Inconclusive**	**Inconclusive**	All 3 N targets and RP must be reactive. Repeat one time using residual extracted nucleic acid. If the results remain inconclusive, a recollect order will be issued to the physician.
If only one, or two of three targets are positive			-	**Insufficient material**	**Inconclusive**	Repeat one time using residual extracted nucleic acid. If the results remain inconclusive, a recollect order will be issued to the physician.
-	-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE**	Reported to sender.
-	-	-	-	**Invalid test**	**INVALID**	Sample is repeated once using residual extracted nucleic acid. If the results for all four remain negative, a recollect order will be issued to the physician.

- If the SARS-CoV-2 N1, N2, and the N3 assays are positive (Ct value ≤ 35), and the RP result is positive (Ct value ≤ 35), the patient sample is reported as positive.
- If the SARS-CoV-2 N1, N2, and the N3 assays are positive (Ct value ≤ 35), and the RP result is negative (Ct Not Detected), this suggests that there is insufficient extracted material and the test result is considered inconclusive. The sample will be repeated one time using residual extracted nucleic acid. If the RNase P gene remains non-reactive at or before 35 cycles, the test will be invalidated, and a recollect order will be issued to the physician.
- If only one or two of the three N gene targets are positive (Ct ≤ 35) and the RNase P target is positive (Ct ≤ 35), an inconclusive result will be issued. The COVID-19 Key assay requires that all 3 N targets be reactive in order for a SARS-CoV-2 positive result to be reported. In this scenario, the patient sample will be re-tested using residual extracted nucleic acid. If the results remain inconclusive, a recollect order will be issued to the physician.
- If only one or two of the three N gene targets are positive (Ct ≤ 35) and the RNase P target is negative (Not Detected), this suggests that there is insufficient extracted material and the test result is considered inconclusive. The sample will

be repeated one time using residual extracted nucleic acid. If the RNase P gene remains non-reactive at or before 35 cycles, the test will be invalidated, and a recollect order will be issued to the physician.

- If all SARS-CoV-2 N1, N2, and N3 assays are negative (Ct Not Detected), and the RP result is positive (Ct ≤ 35), a result of not detected will be issued.
- If all four assay targets (N1, N2, N3, RP) are negative (Ct Not Detected), the result is invalid. The test is repeated using residual extracted nucleic acid. If the repeat result is invalid, a recollect order will be issued to the physician.

PERFORMANCE EVALUATION

1) *Analytical Sensitivity:*

Limit of Detection (LoD):

The LoD of the COVID-19 Key assay was determined using quantified viral SARS-CoV-2 genomic RNA isolated from a positive patient sample. A preliminary LoD was determined by testing six concentrations of a 10-fold dilution series (100,000 copies/mL - 1 copy/mL) of RNA spiked into pooled clinical negative, nasopharyngeal swab matrix and negative sputum matrix in triplicate. Spiked samples were tested with the COVID-19 Key assay following extraction using the Zymo Viral RNA Mag Bead Kit on the King Fisher Flex II extraction platform. Real-Time RT-PCR was performed using the QuantaBio Ultraplex 1-Step RT-PCR Mastermix on the Roche Lightcycler 480 II instrument.

The initial LoD determination of the COVID-19 Key assay was 100 copies/mL for both the sputum and nasopharyngeal swabs.

The LoD was verified by testing 20 additional extraction replicates consisting of pooled negative clinical nasopharyngeal swab matrix or pooled negative sputum matrix spiked at 5X LoD (500 copies/mL) and 2X LoD (200 copies/mL, respectively). Samples were spiked with extracted patient RNA prior to extraction with the Zymo Viral RNA Mag Bead Kit on the King Fisher Flex II extraction platform.

The results of the LoD confirmatory study are summarized below in Table 3.

Table 3: LoD Verification Study Results for Sputum and Nasopharyngeal Swab

Sputum							
Concentration (copies/mL)	**Concentration (copies/reaction)**	**Average Ct Values**			**SARS-CoV-2 N1, N2, N3 Detection Rate**		
		N1	**N2**	**N3**	**N1**	**N2**	**N3**
200 (2X LoD)	1000	32.65	34.69	34.75	20/20	20/20	20/20
Nasopharyngeal Swab							
Concentration (copies/mL)	**Concentration (copies/reaction)**	**Average Ct Values**			**SARS-CoV-2 N1, N2, N3 Detection Rate**		
		N1	**N2**	**N3**	**N1**	**N2**	**N3**
500 (5X LoD)	2500	31.66	32.69	32.68	20/20	20/20	20/20

2) ***Analytical Inclusivity/Specificity:***

The COVID Key assay utilizes identical oligonucleotide sequences for the N1, N2, and N3 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. The inclusivity and cross-reactivity of the CDC EUA assay has been previously evaluated and therefore, additional evaluation is not required. 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.

3) ***Clinical Evaluation:***

Performance of the COVID-19 Key assay was evaluated using individual negative clinical nasopharyngeal swab and sputum specimens spiked with SARS-CoV-2 positive patient RNA to create reactive specimens. Non-reactive specimens included 30 negative nasopharyngeal swab and sputum clinical matrix samples spiked with molecular grade water.

Of the 60 contrived positive clinical samples, 30 nasopharyngeal swabs specimens were prepared with concentrations of SARS-CoV-2 RNA at 5X the assay LoD (500 copies/mL). Thirty additional reactive sputum samples were prepared with concentrations of SARS-CoV-2 RNA at 2.5X the assay LoD.

Contrived samples were randomized and blinded, and RNA was extracted using the Viral RNA Mag Bead Kit on the King Fisher Flex II extraction platform

Table 4: Contrived Clinical Evaluation Summary Data

SARS-CoV-2 concentration (copies/mL)	Number of samples	Average Ct			Detection Rate		
		N1	N2	N3	N1	N2	N3
Nasopharyngeal Swab 5X LoD (500 copies/mL)	30	31.75	32.87	32.78	30/30	30/30	30/30
Sputum 2.5X LoD (250 copies/mL)	30	32.31	32.87	33.06	30/30	30/30	30/30
Nasopharyngeal Swab – Negative Clinical Matrix	30	ND*	ND	ND	0/30	0/30	0/30
Sputum – Negative Clinical Matrix	30	ND	ND	ND	0/30	0/30	0/30

*ND – Not Detected

The results at all tested levels for spiked positives in clinical negative nasopharyngeal swab and sputum matrix demonstrated 100% agreement and all negative samples were non-reactive.

Confirmatory testing for the first five positive and five negative clinical specimens evaluated by the COVID-19 Key assay was conducted at an alternate testing

laboratory. Results were 100% concordant with another EUA authorized assay performed at the alternate testing location.

4) *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 confirm the LoD. The results are summarized in the following Table.

Table 5: 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	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

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.
- The performance of the COVID-19 Key assay was evaluated using contrived nasopharyngeal swab and sputum samples.
- Anterior nasal swabs, mid-turbinate nasal swabs and oropharyngeal (throat) swabs, nasopharyngeal wash/aspirate, nasal washes and nasal aspirates are also considered acceptable specimen types for use with the COVID-19 Key Assay. Testing of anterior nasal and mid-turbinate nasal swabs (self-collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to the FDA FAQs on Diagnostic Testing for SARS-CoV-2 (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2) for additional information regarding acceptable specimen types for detection of SARS-CoV-2.

WARNINGS:

- This product has not been FDA cleared or approved by FDA, but has been authorized 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.