EMERGENCY USE AUTHORIZATION (EUA) TEST SUMMARY FOR THE

Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay

For In vitro Diagnostic Use

Rx Only

For use under Emergency Use Authorization (EUA) only

The Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay which is an LDT that will be performed at the Scope Molecular Laboratory located at 101A S Center St, Sublimity, OR 97385 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.

INTENDED USE

The Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay is intended for the *in vitro* qualitative detection of RNA from SARS-CoV-2 in individual human anterior nasal swab samples collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals, including individuals without symptoms or other reasons to suspect COVID-19, when tested at least once per week.

Testing is limited to Scope Molecular Laboratory located at 101A S Center St, Sublimity OR 97385, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high-complexity testing.

The Scope Molecular Laboratory SARS nCoV-2019 Multiplexed 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 Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 nucleic acid is generally detectable in anterior nasal swab 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.

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.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

For serial testing programs, additional confirmatory testing for negative results may be necessary, if there is a high likelihood of COVID-19, such as an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Additional confirmatory testing for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposure to COVID-19 or residing in communities with low prevalence of infection.

1) ***Special Conditions for Use Statements:***
   For prescription use only
   For *in vitro* diagnostic use
   For Emergency Use Authorization (EUA) only

This test is authorized under the Umbrella EUA for SARS-CoV-2 Molecular Diagnostic Tests for Serial Testing (https://www.fda.gov/media/154111/download) for use in Scope Molecular Laboratory, that is certified under CLIA and meets requirements to perform high complexity tests, in which it was developed for qualitative detection of RNA from SARS-CoV-2 in individual human anterior nasal swab samples collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals, including individuals without symptoms or other reasons to suspect COVID-19, when tested at least once per week using the test procedures validated in accordance with the requirements of the Umbrella EUA for SARS-CoV-2 Molecular Diagnostic Tests for Serial Testing.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay is a reverse transcription polymerase chain reaction (RT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from SARS-CoV-2 in anterior nasal swab specimens that were collected from individuals, including individuals without symptoms or other reasons to suspect COVID-19.

Test Procedure: Nucleic acids are isolated and purified from anterior nasal swabs using a nucleic acid extraction system. The purified nucleic acid is reverse transcribed into cDNA and amplified in one step by combining purified nucleic acid with the RT-qPCR master mix and qPCR primer & probes. In the process, the probe anneals to a specific N1 and N2 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 probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle for each target for 45 cycles using Fam to detect the N1 gene, VIC to detect the N2 gene, and Rox to detect RNASE P.

INSTRUMENTS USED WITH TEST

Instruments

The Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay test is to be used with the Kingfisher Flex and the ABI QuantStudio Flex 12K or the BioRad CFX Opus96.

Reagents

The primary reagents used in Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay:

2x RT-qPCR OneStep Amplification Kit (catalog # RTQAK), containing 2x InhibiTaq Master Mix and Direct RT Mix. Primers and probes were ordered from LGC Biosearch Technologies or Integrated DNA Technologies and custom premixed by Empirical into a 20x working concentration (catalog #CNRDK).

Sample Type	Expiration
Swab Media	5 days or 120 hours

Swab Media	Top Color	Volume	Media Color
Hardy Diagnostics	Blue	3 mL	Pink
Resolution Biomedical	White	5 mL	Clear
Pacific Biosupply	Red	3 mL	Clear

CONTROL MATERIAL(s) TO BE USED WITH SCOPE MOLECULAR LABORATORY SARS nCoV-2019 MULTIPLEXED ASSAY:

Controls that are used with the test include:

a) A **negative extraction control (NEC)** is needed to determine that the extraction of patient samples was completed successfully and is multiplexed in with every sample's results. This is nuclease free water that is used in place of a patient sample. Amplification of this extraction control from any of the three targets tested indicates contamination of the extraction step and all samples are re-extracted.

b) A **positive template control (PTC)** is needed to determine the viability of the run to show potential positive patients. A positive plasmid control provided by the manufacturer is used at approximately 2X LOD and is included at the assay step.

c) A **"no template" (negative) control (NTC)** is needed to detect contamination in the assay plating process. A NTC is included in the PCR plating step and analysis. Amplification of this NTC from any of the three targets tested indicates contamination on the PCR plate and the plate is rerun after the extraction step.

INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Appropriate control interpretation criteria and result interpretation criteria are described here.

1. **Examination and Interpretation of Control Results**
   The following result evaluation rules apply to the controls of every nCoV-2019 plate run:

- **Negative Extraction Control (NEC)**
  - Expected result: No amplification of any target
  - If amplification is <40 for any target, all samples must be re-extracted.

- **Positive Template Control (PTC)**
  - Expected Result: Amplification of RNase P, N1, and N2
  - If no amplification, or amplification is > 40, the entire plate must be re-plated

- **No Template Control (NTC)**
  - Expected result: No amplification of any target
  - If amplification is <40 for any target, all elution plates must be re-plated

2. **Examination and Interpretation of Patient Sample Results:**

   Assessment of clinical specimen test results must be performed after the controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

   Every patient's curve resulting from the qPCR process is checked by a member of the molecular staff. Samples that are <40 Ct for N1 and N2 are considered positive. Samples that have a Ct above 40 for N1 and N2 are considered negative. Samples that do not show amplification of N1 or N2 are considered negative. Curves that are not exponential or potential false positive results are re-extracted and rerun. Samples that do not show amplification for RNaseP are re-extracted and rerun. See the below chart for the result evaluation rule.

N1	N2	RP	Result Interpretation
+	+	+/-	Positive Sars-Cov-2
Only 1 of 2 targets positive		+/-	Inconclusive*
-	-	+	Not Detected
-	-	-	Invalid**

* Inconclusive results should be repeated from extraction. If the result is still inconclusive the second time a new specimen should be collected if available. If it is not possible to collect a new specimen, it should be reported to the healthcare provider as inconclusive.

** Invalid results should be repeated from extraction. If the result is still invalid the second time a new specimen should be collected if available. If it is not possible to collect a new specimen, it should be reported to the healthcare provider as invalid.

PERFORMANCE EVALUATION

1) ***Limit of Detection (LoD) - Analytical Sensitivity:***

The LoD for the Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay was evaluated and verified using IDT 2019-nCoV_N_Positive Control (catalog # 100006625) containing a plasmid with the complete nucleocapsid gene from 2019-nCoV per validation required by Appendix A of the Umbrella EUA for SARS-CoV-2 Molecular Diagnostic Tests for Serial Testing. Nucleic acid was extracted from the swabs using Applied Biosystems MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit and the reverse transcription RT-PCR was performed using the QuantStudio 12K Real-time PCR Thermocycler and the BioRad CFX96 Opus. Preliminary and Confirmation LoD results are included in the tables below.

QuantStudio Preliminary LOD

Copies/uL	N1 (FAM)			N2 (HEX)		
	1	2	3	1	2	3
20	30.3	30.78	30.64	29.48	30.63	30.76
10	31.4	31.97	32.24	30.87	31.42	31.7
5	33.35	34.17	34.61	32.78	32.91	33.77
2.5	32.59	33.53	33.22	31.82	33.09	32.39
1.25	35.42	34.51	34.29	34.25	33.95	33.51
0.63	NA	34.19	NA	NA	33.24	NA
0.31	35.16	NA	NA	33.16	NA	NA
0.16	NA	NA	35.13768	NA	NA	33.89

Bio CFX96 Opus

Copies/uL	N1 (FAM)			N2 (HEX)		
	1	2	3	1	2	3
20	34.05	34.11	33.96	33.14	33.19	33.06
10	36.58	35.33	35.52	35.35	34.31	34.76
5	35.45	36.30	35.81	34.32	34.60	35.09
2.50	37.41	37.20	36.03	36.04	36.55	35.38
1.25	38.03	39.56	36.08	35.98	37.78	35.54
0.63	37.67	39.76	37.08	36.44	42.17	36.77
0.31	40.17	NA	NA	41.12	NA	NA
0.16	40.03	NA	NA	37.98	NA	NA

LoD Confirmation QuantStudio 12K:

Initial LOD was hypothesized to be 1.25 copies./uL. However N1 and N2 were detected in fewer than 50% of 20 replicates at this concentration. N1 was detected in 17/20 samples (85%) and N2 was detected in 19/20 samples = 95% at 2.5 copies/uL, so confirmatory LOD was repeated 5 copies/uL.

Target	N1	N2
Virus Concentration	5 cp/uL	5 cp/uL
Positives/Total	20/20	20/20
% Detected	100%	100%
Mean Ct	34.23	35.42
Mean SD	0.49	0.52
CV	1.42%	1.48%

Data confirmed that LoD on the QuantStudio 12K is **5 copies/uL.**

LoD Confirmation CFX Opus:

Initial LOD was hypothesized to be 0.63 copies./uL. However N1 and N2 were detected in fewer than 50% of 20 replicates at this concentration and at 1.25 copies/uL. So confirmatory LOD was repeated 2.5 copies/uL and N1 and N2 were detected in 19/20 of the samples.

Target	N1	N2
Virus Concentration	2.5 cp/uL	2.5 cp/uL
Positives/Total	20/20	20/20
% Detected	100%	100%
Mean Ct	35.5	34.9
Mean SD	1.52	2.19

CV	4.3%	6.3%

Data confirmed that LoD on the CFX Opus is **2.5 copies/uL.**

2) ***Inclusivity (analytical reactivity):***

On December 1, 2022 a primer and probe alignment was performed with the oligonucleotide primer and probe sequences of the Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay with 1,112,818 publicly available SARS-CoV-2 sequences (including mutation variants of high prevalence, i.e., B.1.617.2 and sub-lineages at the time of issuance of this letter) from GISAID to demonstrate the predicted inclusivity of the assay. Of the mutations found, only one was prevalent in more than 5% of the publicly available SARS-CoV-2.

Gene	N1 Forward								N1 Probe		
Substitution	N*	N	N	N	N	N	N	N	N	N	N
Location	4	5	13	14	22	24	25	26	14	18	21
Mutation Rate %	0.101	0.171	2.497	0.177	0.123	0.254	38.595	0.188	0.469	3.512	0.105

Gene	N2 Forward			Rev	N2 Probe	
Substitution	Y*	N	N	N	N	N
Location	4	8	15	13	14	17
Mutation Rate %	0.208	0.191	0.36	0.127	0.292	0.213

*N = Any nucleotide substitution, Y = pyrimidine substitution

The only significantly prevalent mutation (N1 probe nucleotide 25) should not change the ability of the N1 probe to anneal to the target gene. We used an open source calculator to determine how the change affects the annealing temperature of the probe and it was minimally affected (2 degrees C).

3) Cross-reactivity (Analytical Specificity):

Analytical specificity of the primer/probe combination for Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay was evaluated by conducting sequence alignment of the primer/probe sequences of the test with publicly available genome sequences for potential cross-reacting microorganisms. The following organisms were tested with Scope Molecular

Laboratory SARS nCoV-2019 Multiplexed Assay primer probe set and yielded no cross-reactivity:

Microorganism	N1 Target	N2 Target
Human adenovirus 2	No Similarity	No Similarity
Coronavirus 229E	No Similarity	No Similarity
Coronavirus HKU1	No Similarity	No Similarity
Coronavirus NL63	No Similarity	No Similarity
Coronavirus OC43	No Similarity	No Similarity
Human Metapneumovirus	No Similarity	No Similarity
Human Rhinovirus/Enterovirus	No Similarity	No Similarity
Influenza A virus	No Similarity	No Similarity
Influenza A Virus A/H1	No Similarity	No Similarity
influenza A virus A/H3	No Similarity	No Similarity
Influenza A virus A/H1-2009	No Similarity	No Similarity
Influenza B virus	No Similarity	No Similarity
Parainfluenza 1	No Similarity	No Similarity
Parainfluenza 2	No Similarity	No Similarity
Parainfluenza 3	No Similarity	No Similarity
Parainfluenza 4	No Similarity	No Similarity
Respiratory syncytial virus	No Similarity	No Similarity
Bordetella parapertussis	No Similarity	No Similarity
Bordatella pertussis	No Similarity	No Similarity
Chlamydia pneumoniae	No Similarity	No Similarity
Mycoplasma pnemoniae	No Similarity	No Similarity

4) Clinical Evaluation:

Clinical evaluation of the Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay was conducted with individual anterior nasal swab clinical specimens collected from patients suspected of SARS-CoV-2 infection by a healthcare provider in COVID-19 disease endemic region(s). This study was performed using retrospective samples. Samples were confirmed positive or negative using the FDA Emergency Use Authorized tests from Thermo Fisher TaqPath Combo Kit, or the Biofire Respiratory Panel 2.1 or the Cepheid GeneXpert Test. Of the Samples tested, 30 were confirmed positive for Sars-Cov-2 and 30 were confirmed negative.

Nucleic acid was extracted from all specimens using the Applied Biosystems MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit on the Kingfisher Flex and reverse transcription Real-time-PCR was performed using the Empirical reagents on the QuantStudio 12K Thermocycler. Clinical results yielded 29 positive for SARS-CoV-2 and 31 negative for SARS-CoV-2.

Scope Molecular Laboratory SARS nCoV-2019 Multiplexed Assay
Test Summary: ***1/5/2023***

Scope Multiplex Test	Comparative Kit		Agreement		Performance
	Positive	Negative			
Positive	29	1	PPA = 29/30	96.7%	Sensitivity
Negative	1	30	NPA = 30/31	96.8%	Specificity

The Empirical reagents on the BioRad CFX96 Opus Thermocycler. Clinical results yielded 30 positive for SARS-CoV-2 and 30 negative for SARS-CoV-2.

Scope Multiplex Test	Comparative Kit		Agreement		Performance
	Positive	Negative			
Positive	30	0	PPA = 30/30	100%	Sensitivity
Negative	0	30	NPA = 30/30	100%	Specificity

LIMITATIONS

The initial performance of this test was established based on the evaluation of a limited number of clinical specimens collected between April 2021 and August 2021 and were collected from various collection sites throughout the state of Oregon within the United States.

The clinical performance of this test has not been established in all circulating variants but is anticipated to be reflective of the variants in circulation at the time and location(s) of the clinical evaluation. As such, 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.

Clinical performance has been established in specimens collected from subjects suspected of COVID-19 by a healthcare provider. Performance of specimens collected from individuals without symptoms or other reasons to suspect COVID-19 has not been established. A study to determine the performance in individuals without symptoms or other reasons to suspect COVID-19 will be completed.

WARNINGS:

This product has not been FDA cleared or approved but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by the laboratory that developed the test and 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.

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.