ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 MOLECULAR DETCTION ASSAY (MAYO CLINIC)

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

(The SARS-CoV-2 Molecular Detection Assay will be performed at the Mayo Clinic, Rochester, MN, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a as per the Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 Molecular Detection Assay is a real-time PCR test using TaqMan chemistry and is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens (such as nasopharyngeal swabs, throat swabs, nasal swabs, sputum, tracheal secretions, BAL fluid, and bronchial washings) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Mayo Clinic Laboratories, Rochester, MN, 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. SARS-CoV-2 RNA is generally detectable in upper and lower 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.

Testing with the SARS-CoV-2 Molecular Detection 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 SARS-CoV-2 Molecular Detection Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SARS-CoV-2 Molecular Detection Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test using the Roche LightCycler 480 amplification system. The SARS CoV-2 primer and probe sets are designed to detect RNA from the SARS-CoV-2 nucleocapsid and ORF1ab genes in a clinical sample. The nucleocapsid target was determined to be specific to SARS-CoV-2. The ORF1ab target detects SARS-related coronaviruses (i.e., including SARS-CoV [2003] and SARS-CoV-2).

RNA is isolated/purified from upper and lower respiratory specimens using the bioMérieux easyMAG and EMAG and is reverse transcribed to cDNA and subsequently amplified using Roche LightCycler 480. During the amplification process, the probes anneal to specific target sequences 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 (FAM) 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. The dye labeled probe allows for detection of SARS CoV-2 virus in the corresponding detector channel (465-510nm) of the LightCycler 480 instrument.

INSTRUMENTS USED WITH TEST

The SARS-CoV-2 Molecular Detection Assay is to be used with the bioMérieux easyMAG and EMAG extraction instruments and Roche LightCycler 480 version 1.5.1.62.

REAGENTS AND MATERIALS

Reagent	Manufacturer	Catalog #
SuperScript™ III Platinum™ One-step qRT-PCR Kit	Invitrogen	11732-088
SARS-CoV-2 NUC Primers	IDT	266468584, 266468585
NUC TaqMan Probe	IDT	265179054
SARS-CoV-2 ORF1ab Primers	IDT	264724919, 264724921
ORF1ab TaqMan Probe	IDT	264727889
Internal Control Forward Primer	IDT	Custom
Internal Control Reverse Primer	IDT	Custom
Internal Control TaqMan Probe	IDT	Custom
Internal Control	ATCC	VR764
Positive Control	IDT	Reference numbers: NUC-265183573 & ORF-264724309
Negative Control	Sigma	W4502

CONTROLS TO BE USED WITH THE SARS-CoV-2 MOLECULAR DETECTION ASSAY

1) **Negative Extraction (EXT)/Amplification (AMP) Control:** It is PCR-grade, nuclease free water which serves as the negative control (reagent contamination and extraction control). It is processed and tested on each assay run to ensure that no contamination has taken place in either the extraction or amplification phases of testing. This control contains all of the reagents for amplification but does not contain the targeted nucleic acid template.

2) **Positive Extraction (EXT)/Amplification (AMP) Control:** The extraction/amplification control contains synthetic DNA constructs of both target sequences and is used on every extraction run to ensure that adequate nucleic acid isolation has taken place. This control is processed through the extraction protocol followed by amplification and detection to ensure the reagents are functioning as expected, and that the run conditions and master mix were adequate for amplification to occur.

3) **Internal Control (IC):** IC contains a 1,000 copies/µL of an “artificial” RNA molecule with no homology to any other pathogens. It is added to the SARS-CoV-2 master mix and is reverse-transcribed, amplified and detected in each reaction. The MHV internal control ensures the integrity of the RT-PCR results by assessing for potential RT-PCR inhibition.

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) ***SARS-CoV-2 Molecular Detection Assay Controls – Positive, Negative and Internal***

   The negative template control must be negative (i.e., Undetected). The positive extraction/amplification control must be positive (i.e., Detected) with a crossing point (Cp) value within ± 3 cycles of the average Cp value established prior to the control being placed into use. The internal control must be positive in all negative samples, including the negative template control, to ensure no inhibition has occurred.

2) ***Examination and Interpretation of Patient Specimen Results:***

   The two targets (NUC and ORF1ab) are placed in separate reactions with their respective FAM-labelled probes, using the same Cy5-labelled internal control. Assessment of clinical specimen test results should be performed after the positive, negative and internal controls have been examined and determined to be valid and acceptable. If the positive and negative controls are not valid, the patient results cannot be interpreted/reported.

Positive (i.e., Detected) results are those that have a Cp value ≤ 45 and a valid amplification curve, which is defined as having a clear upward inflection rising above the baseline. Negative (i.e., Undetected) results will not have a Cp value and will not yield an amplification curve.

a. Negative extraction/amplification control:
   i. If the negative control is positive for either PCR reaction, all specimens must be re-extracted.
b. Positive extraction/amplification control:
   i. If the positive control is negative in both PCR reactions, all specimens must be re-extracted.
   ii. If the positive control is positive for one PCR reaction but negative for the other, the same extracts should first be re-amplified for the reaction that failed. If the positive control is negative a second time, all specimens must be re-extracted.
c. Internal control (IC) failure:
   i. If no IC amplification curve is present in the negative control:
      - Repeat entire run (extraction and amplification) including controls.
      - If negative control repeats with a valid IC amplification curve, results can be reported.
      - If the negative control repeats with no IC amplification curve, hold results and notify management staff for further review and direction.
   ii. If no internal control amplification curve is present in negative patient sample:
      - Repeat sample once (extraction and amplification).

Reporting criteria for SARS-CoV-2 Molecular Detection Assay

NUC	ORF	NUC IC	ORF IC	Result
+	+	+/-	+/-	Detected
+	+/-	+/-	+/-	Detected
-	+	+	+/-	Indeterminate
-	-	+	+	Undetected
-	+	-	+/-	Invalid
-	-	-	-	Invalid

NUC, nucleocapsid; ORF, open reading frame; +, Positive; -, Negative; +/-, Positive or Negative

LIMITATIONS

- The use of SARS-CoV-2 Molecular Detection Assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

- The SARS-CoV-2 Molecular Detection Assay performance was established using specimen types listed. Other specimen types have not been evaluated and should not be tested with this assay.
- Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
- The detection of viral nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedure in any one of these steps can lead to incorrect results.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- 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.
- 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. The SARS-CoV-2 Molecular Detection Assay cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.
- A trained health professional should interpret assay results in conjunction with the patient's medical condition, clinical signs and symptoms, and the results of other diagnostic tests.

PERFORMANCE EVALUATION

1) __Analytical Sensitivity/Limit of Detection (LoD)__:

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies/µL) that can be detected by the SARS-CoV-2 Molecular detection Assay at least 95% of the time. The LoD of the SARS-CoV-2 Molecular Detection Assay was determined using a quantified SARS-CoV-2 positive clinical specimen. A preliminary LoD was determined by testing 2-fold serial dilutions of quantified SARS-CoV-2 clinical sample spiked into pooled SARS-CoV-2 negative nasopharyngeal (NP) and sputum samples, tested in replicates of 20. The LoD was confirmed by testing 20

replicates of 2-fold serial dilutions. The samples of 2-fold dilutions were prepared by spiking the quantified SARS-CoV-2 positive clinical specimen into negative respiratory clinical matrices (NP swabs and sputum). The samples were extracted by bioMérieux easyMAG and tested by the SARS-CoV-2 Molecular Detection Assay per the protocol. The study results showed that the LoD of the SARS-CoV-2 Molecular detection Assay is 0.156 copies/µL in NP swabs and 12.5 copies/µL in sputum samples. Additionally, a comparative study demonstrated that the LoDs obtained with two bioMérieux extractions (easyMAG and EMAG) are comparable.

SARS-CoV-2 Molecular Detection Assay LoD Results in Upper and Lower Respiratory Samples

Test	Source	Concentration (copies/mL)	No. tested	No. (%) Positive	Average Cp	Standard Deviation Cp
NUC	Sputum	25,000	20	20 (100)	27.0	0.6
		12,500	20	20 (100)	27.2	0.4
		6,250	20	17 (85)	27.6	0.4
		3,125	20	15 (75)	28.2	0.6
	NP Swabs	2,500	20	20 (100)	30.5	1.2
		1,250	20	20 (100)	32.0	1.3
		625	20	20 (100)	30.9	0.8
		312	20	20 (100)	31.3	0.9
		156	20	20 (100)	30.7	0.9
		78	20	16 (80)	31.0	0.8

Test	Source	Concentration (copies/mL)	No. tested	No. (%) Positive	Average Cp	Standard Deviation Cp
ORF	Sputum	25,000	20	20 (100)	27.6	1.1
		12,500	20	20 (100)	28.0	0.7
		6,250	20	19 (95)	27.7	0.5
		3,125	20	17 (85)	28.1	0.5
	NP Swabs	2,500	20	20 100)	30.5	1.4
		1,250	20	20 (100)	31.9	1.5
		625	20	20 (100)	30.3	0.7
		312	20	20 (100)	30.4	0.7
		156	20	20 (100)	31.2	0.5
		78	20	16 (80)	31.2	0.4

NUC, nucleocapsid; ORF, open reading frame; Cp, crossing point

2) <u>Inclusivity</u>:

Inclusivity was tested by performing an *in silico* analysis using all publicly available sequences of SARS-CoV-2 (taxid 2697049) from the National Center for Biotechnology

Information (NCBI) located in GenBank as of April 9th, 2020. The analysis showed 100% homology with all available SARS-CoV-2 sequences when compared to ORF1ab primers and probe sequences. The analysis showed 99.6% homology when compared to nucleocapsid forward primer sequence, 99.2% homology when compared to nucleocapsid reverse primer sequence, and 92.1% homology when compared to nucleocapsid probe sequence with all available SARS-CoV-2 sequences. The mismatches in nucleocapsid primers and probe sequences showed single base pair mismatch; the majority of which occurred at the extreme terminus of the primers that should have minimal impact on the detection of the strains harboring this mismatch.

***3) Analytical Specificity*:**

Cross-reactivity of the SARS-CoV-2 Molecular Detection Assay was evaluated using both *in silico* analysis and by testing a cross-reactivity panel consisting of microorganisms that may be present in respiratory samples. High priority pathogens from the same genetic family as SARS-CoV-2, as well as organisms likely to be circulating in the population and found in the relevant clinical matrices were evaluated *in silico.*

Pathogen	Strain	GenBank Acc#
SARS-CoV	Urbani isolate icSARS-C7-MA	MK062184.1
MERS-CoV	NL13845	MG021451.1
HCoV-229E	HCoV_229E/Seattle/USA/SC9724/2018	MN369046.1
229E-related bat CoV	BtKY229E-8	KY073748.1
HCoV-OC43	HCoV-OC43/USA/ACRI_0052/2016	MF314143.1
HCoV-HKU1	SI17244	MH940245.1
HCoV-NL63	HCoV_NL63/Seattle/USA/SC0768/2019	MN306040.1
Legionella clemsonensis	CDC-D5610	CP016397.1
Human adenovirus C1	SH2016	MH183293.1
Human metapneumovirus	bj0123	MK820375.1
Human parainfluenza virus 1	E0-033	MH685717.1
Human parainfluenza virus 2	HPIV2/Seattle/USA/SC9949/2018	MN369034.1
Human parainfluenza virus 3	HPIV3/USA/629-D00687/2008	KF530242.1
Human parainfluenza virus 4b	HPIV4b/Seattle/USA/SC0496/2019	MN306032.1
Influenza A	(A/chicken/Taiwan/1843/2012(H6N1))	KJ162810.1
Influenza B	(B/Guangzhou/01/2007)	EU305612.1
Enterovirus D68	USA/CA/1962-23234	MN240508.1
Human respiratory syncytial virus	MaxFLC	MK733769.1
Rhinovirus C	3430-MY-10	KJ675507.1
Influenza C	(C/Iwate/2/2016)	LC327663.1

Pathogen	Strain	GenBank Acc#
Parechovirus A	CDMS	MK904604.1
Chlamydophila pneumoniae	LPCoLN	CP001713.1
Haemophilus influenzae	NCTC8468	LR590465.1
Legionella pneumophila	ERS1305867	CP048618.1
Mycobacterium tuberculosis	TCDC11	CP046728.2
Streptococcus pneumoniae	M26365	CP031248.1
Streptococcus pyogenes	MGAS6180	CP000056.2
Bordetella pertussis	J348	CP033416.1
Mycoplasma pneumoniae	16-734	CP039761.1
Pneumocystis jirovecii (PJP)	RU7	GCF_001477535.1
Candida albicans	SC5314-P0	GCA_002837675.1
Corynebacterium diphtheriae	BQ11	CP029644.1
Neisseria elongata	M15910	CP031255.1
Neisseria meningitidis	M18727	CP031333.1
Staphylococcus epidermidis	NCCP 16828	CP043847.1
Leptospirosis	FMAS_KW2	CP039256.1
Staphylococcus aureus	79_S10	CP010944.1
Bacillus anthracis (Anthrax)	BF1	CP047131.1
Moraxella catarrhalis	MC8	CP010902.1
Pseudomonas aeruginosa	VIT PC9	CP048791.1
Streptococcus salivarius	ICDC2	CP018187.1
Chlamydia psittaci	AMK	CP047319.1
Coxiella burnetii (Q-Fever)	RSA439	CP040059.1

When the *in silico* analysis showed ≥80% homology of any SARS-CoV-2 primer or probe with another pathogen, a single replicate of the organism was tested using the SARS-CoV-2 Molecular Detection Assay to assess cross-reactivity. These results are summarized in table below:

Cross-reactivity panel tested by the SARS-CoV-2 Molecular Detection Assay

Organism	Source	NUC PCR Assay	ORF PCR Assay	Genomes /µL
SARS-CoV N gene[1]	IDT 10006624	Negative	Negative	2.00E+05
SARS-CoV ORF1ab gene[2]	IDT Ref #264728633	Negative	Positive	2.00E+05
MERS-CoV	ATCC VR-3248SD	Negative	Negative	1.00E+06
HCoV-OC43	ATCC VR-3263SD	Negative	Negative	1.00E+06
HCoV-HKU1	ATCC VR-3262SD	Negative	Negative	1.00E+06
HCoV-NL63	ATCC VR-1558SD	Negative	Negative	1.00E+06
Hemophilus influenzae	ATCC 10211	Negative	Negative	4.36E+09
Streptococcus pneumonia	ATCC 49619	Negative	Negative	1.60E+10

Streptococcus pyogenes	ATCC 19615	Negative	Negative	2.96E+09
Candida albicans	ATCC 60193	Negative	Negative	5.70E+08
Staphylococcus aureus	ATCC 25923	Negative	Negative	4.24E+09
Bacillus anthracis (Anthrax)	LRN Survey Strain	Negative	Negative	2.15E+08
Moraxella catarrhalis	ATCC 8176	Negative	Negative	1.66E+09
Pseudomonas aeruginosa	ATCC 27853	Negative	Negative	1.55E+10

[1] This control from IDT consists of the N gene from SARS-CoV (2003). This is NOT detected by the SARS-CoV-2 MDA assay, as the N target is specific for SARS-CoV-2.
[2] This control from IDT consists of the ORF1ab gene from SARS-CoV (2003), which is detected by the SARS-CoV-2 MDA assay, as the ORF1ab target was designed to detect SARS-related CoVs, including SARS-CoV (2003) and SARS-CoV-2.

4) Clinical Evaluation:

A contrived clinical study was performed to evaluate the performance of the SAS-CoV-2 Molecular Detection Assay. 20 analyte-negative NP swabs and 20 analyte-negative sputum samples were spiked with a quantified, positive clinical sample at 1x LoD (i.e., 0.156 copies/µL for NP swabs; 12.5 copies/µL for sputum) and 2x LoD (i.e., 0.312 copies/µL for NP swabs; 25 copies/µL for sputum). In addition, 10 analyte-negative throat swabs and 10 analyte-negative BAL fluid samples were spiked with a quantified positive clinical sample across the range of the assay (3x-300x LoD). These samples were then tested by both the SARS-CoV-2 Molecular Detection Assay and the CDC EUA assay in a blinded fashion and compared to the expected results. The results of the SARS-CoV-2 Molecular Detection Assay showed 100% concordance with the CDC EUA assay and the expected results.

Clinical Performance of the SARS-CoV-2 Molecular Detection Assay with upper respiratory swab samples (NP and throat swabs)

		Expected results (as well as CDC EUA Assay)	
		Pos	**Neg**
SARS-CoV-2 MDA	**Pos**	50 [a]	0
	Neg	0	30 [b]
Positive Percent Agreement	100% (95% CI: 92.87% - 100%)		
Negative Percent Agreement	100% (95% CI: 88.65% - 100%)		

[a] These samples consisted of 40 spiked NP swabs (20 spiked at 1x LoD; 20 spiked at 2x LoD) and 10 throat swabs spiked at 3x-300x LoD.
[b] These samples consisted of 15 non-spiked NP swabs and 15 non-spiked throat swabs.

Clinical Performance of the SARS-CoV-2 Molecular Detection Assay with lower respiratory samples (sputum and BAL fluid)

		Expected results (as well as CDC EUA Assay)	
		Pos	**Neg**
SARS-CoV-2 MDA	**Pos**	50 [a]	0
	Neg	0	30 [b]
Positive Percent Agreement	100% (95% CI: 92.87% - 100%)		
Negative Percent Agreement	100% (95% CI: 88.65% - 100%)		

[a] These samples consisted of 40 spiked sputum samples (20 spiked at 1x LoD; 20 spiked at 2x LoD) and 10 BAL fluid samples spiked at 3x-300x LoD.
[b] These samples consisted of 15 non-spiked sputum and 15 non-spiked BAL samples.

In addition to testing spiked/contrived samples, 45 clinical NP samples were tested by the SARS-CoV-2 Molecular Detection Assay and an EUA-approved SARS-CoV-2 assay (CDC assay or Roche cobas SARS-CoV-2). Among these 45 samples, 15 were determined to be positive for SARS-CoV-2 at the Minnesota Department of Health (n=7), the Wisconsin Department of Health (n=1) and by the Roche cobas SARS-CoV-2 EUA method (n=7) performed at Mayo Clinic. All results were concordant and fulfills the requirement for confirmatory testing of at least 5 positive and 5 negative specimens.

Comparison of the SARS-CoV-2 Molecular Detection Assay to an EUA SARS-CoV-2 assay using clinical samples (n=45).

		EUA SARS-CoV-2 assay	
		Pos	**Neg**
SARS-CoV-2 MDA	**Pos**	15	0
	Neg	0	30
Positive Percent Agreement	100% (95% CI: 79.62% - 100%)		
Negative Percent Agreement	100% (95% CI: 88.65% - 100%)		