EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR
(MAWD Laboratories)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

The MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay will be performed at MAWD Laboratories, located at 11070 Strang Line Rd., Lenexa, KS 66215, 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, as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR 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 swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to MAWD Laboratories, located at 11070 Strang Line Rd., Lenexa, KS 66215, 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 identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal 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 definitive cause of disease. Laboratories within the United States and its territories are required to report all test 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/or epidemiological information.

The MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures.

The MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from the SARS-CoV-2 in upper respiratory specimens (i.e., nasopharyngeal swab specimens) collected from individuals who meet COVID-19 clinical and/or epidemiological criteria.

The assay is designed for specific detection of regions of the virus nucleocapsid (N) gene, specifically N1 and N2 of SARS-CoV-2 (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) serves as an internal positive control (IPC). In addition to IPC, an external positive control (EPC) and no template control (NTC) are included in each run.

Description of Test Steps:

Nucleic acids are isolated and purified from nasopharyngeal swab specimens using the PerkinElmer Viral DNA/RNA 300 Kit H96 (CMG-1033-S) on a PerkinElmer chemagic 360 nucleic acid extractor. The extraction protocol uses 200 µL of sample combined a solution of 200 µL of lysis buffer and 10 µL of PolyA/ProK reagent that undergoes a magnetic bead purification on the instrument. The isolated RNA is eluted with 80 µL of elution buffer.

PCR plate setup is achieved on an Opentrons OT-2 liquid handling system. Isolated RNA in a 96-well microwell plate is placed on the deck of the instrument accompanied by a PCR reaction plate, pipette tips, and master mix tube. Master mix is dispensed into each well of the PCR reaction plate with a robotic single channel pipette. Subsequently, eluate of clinical samples is transferred to the plate with a multichannel pipette on board the instrument. The PCR plate is removed, sealed, briefly centrifuged, and loaded onto an Applied Biosystems QuantStudio 7 Flex Real-Time PCR system with software v1.3.

RNA isolated and purified from upper respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems QuantStudio 7 Flex Real-Time PCR system with TaqPath 1-Step RT-qPCR Master Mix. In the amplification step, the probe anneals to the 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 cleaves off 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 by Applied Biosystems QuantStudio 7 Flex Real-Time PCR system with software version 1.3.

INSTRUMENTS USED WITH THE TEST

Table 1. Instruments and Software for Use with the MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Assay

Instrument	Manufacturer	Software Version
PerkinElmer chemagic 360 nucleic acid extractor	PerkinElmer	6.3.0.3
PerkinElmer JANUS G3 Primary Sample Reformatter	PerkinElmer	5.5.56
Opentrons OT-2 Liquid Handling Robot	Opentrons Labworks	3.20.1
Applied Biosystems QuantStudio 7 Flex Real-Time PCR System	Thermo Fisher	1.3

REAGENTS AND MATERIALS

Table 2. Reagents and Materials Used for the MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Assay

Reagent/Material	Manufacturer/ Supplier	Catalogue/Part Number
Positive SARS-Cov-2 control, 2019-nCoV_N_Positive Control	Integrated DNA Technologies	10006625
Positive internal control, Hs_RPP30 Positive Control	Integrated DNA Technologies	10006626
2019-nCov_N1 forward primer, 1000 nmol	Biosearch Technologies	nCoV-N1-F-1000
2019-nCoV-N1 reverse primer, 100 nmol	Biosearch Technologies	nCoV-N1-R-1000
Dual-labeled probe, 5' FAM/3' BHQ-1 N1-P, 3000 nmol	Biosearch Technologies	DLO-FB1-1sp
nCOV_N2 Forward Primer Aliquot, 100 nmol	Integrated DNA Technologies	10006833
nCOV_N2 Reverse Primer Aliquot, 100 nmol	Integrated DNA Technologies	10006834
nCOV_N2 (SUN) Probe, 50 nmol	Integrated DNA Technologies	10007049
Human RNase P Extraction Control Forward Primer,1000 nmol	Biosearch Technologies	RNP-F-1000
Human RNase P Extraction Control Reverse Primer,1000 nmol	Biosearch Technologies	RNP-R-1000
Dual-labeled probe, 5' CAL Fluor Red 160/3' BHQ-2 RP-P3, 1031 nmol	Biosearch Technologies	DLO-CAB2-1sp
TE Buffer, 1X Solution pH 8.0, low EDTA, Molecular Biology Grade, 500 mL	Thermo Scientific	J75793.AP
Invitrogen Nuclease-Free Water, 1000 mL (or equivalent)	Thermo Scientific	4387936
TaqPath 1-Step Multiplex Master Mix (No ROX), 10 mL	Applied Biosystems	A28523
chemagic Viral DNA/RNA 300 Kit H96	Applied Biosystems	CMG-1033-S

CONTROLS

The MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay includes a positive control, human specimen control and a negative control, described in **Table 3** below. Each control is included with each 96-well plate of patient samples.

Table 3. Assay Controls Used with the MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR

Control	Description	Manufacturer	Purpose
Positive control	2019-nCoV_N_Positive Control	Integrated DNA Technologies Catalog #10006625	Diluted to a working concentration of 1500 copies/mL; used to assess amplification performance
Human specimen control	Hs_RPP30 Positive Control	Integrated DNA Technologies Catalog #10006626	Used to assess cellular adequacy of samples, rule out PCR inhibition, and assess performance of the extraction process
Negative template control	Elution Buffer 5	--	Used to rule out contamination from extraction through amplification

INTERPRETATION OF RESULTS

Assay Controls

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. External controls should be interpreted according to **Table 4** below.

Table 4. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Results Interpretation

Control Name	Ct		
	N1	N2	RP
NTC	Undetected	Undetected	Undetected
nCoV Pos	<40	<40	<40

Clinical Specimens

Results are interpreted according to the requirements in **Table 5** below.

Table 5. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Results Interpretation

N1 (SARS-CoV-2) Ct Value	N2 (SARS-CoV-2) Ct Value	RP (Internal Control) Ct Value	Result Interpretation	Actions
Either target <=40.00		+/-	Detected	N/A
Undetected	Undetected	<=40.00	Not Detected	N/A
Undetected	Undetected	Undetected	Invalid	Re-extract primary sample and repeat PCR
Undetected or >40 for both targets on repeated invalid sample		<=40.00 on repeated invalid sample	Not Detected	N/A
Undetected or >40 for both targets on repeated invalid sample		Undetected on repeated invalid sample	Invalid	N/A
<=40.00 for either target on repeated invalid sample		+/-	Detected	N/A

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity

The LoD of the MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay was determined using SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-QA1/2020, Gamma-Irradiated, NR-52287, contributed by the Centers for Disease Control and Prevention). A preliminary LoD was determined by testing dilutions of the SARS-CoV-2 spiked into negative pooled upper respiratory swab matrix using three replicates at each target level. Spiked samples were tested with the MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay following extraction. The preliminary LoD was determined as the lowest concentration at which all three replicates were positive (**Table 6** below).

Table 6. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Preliminary LoD Results

Concentration (copies/mL)	N1 Results	N2 Results
1000	3/3 detected	3/3 detected
800	3/3 detected	3/3 detected
600	3/3 detected	3/3 detected
400	3/3 detected	3/3 detected
200	0/3 detected	0/3 detected

The LoD of the test was then confirmed by testing twenty replicates at the tentative limit of detection of 400 copies/mL where at least 19 out of 20 replicates are detected. The results of the LoD confirmation studies are summarized in **Table 7** below.

Table 7. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Confirmation LoD Study Results

Concentration (copies/mL)	N1 Results	N2 Results
400	20/20 detected	20/20 detected

2) <u>Inclusivity (Analytical Reactivity):</u>

The MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR assay utilizes oligonucleotide primer and probe sequences for the N1 and N2 target genes identical to those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. The CDC granted a right of reference to the performance data contained in the CDC's EUA request to any entity seeking an FDA EUA for a COVID19 diagnostic device including the *in silico* analysis of SARS-CoV-2 inclusivity.

Since the alignments of the CDC's primers/probes were completed in February 2020, additional *in silico* inclusivity analyses were completed in March and April 2023. These *in silico* analyses assessed the impact of the recently emerged SARS-CoV-2 variants and demonstrated that none of the observed mutations would be expected to interfere with the detection of the N1 and N2 genes and affect the homology between sequences. As indicated in **Table 8**, no mismatches were detected for N1 forward and reverse primers binding regions. For the probe, a single mismatch was detected at the 3rd nucleotide from 5' end (C>T) on some Omicron variants. Another mismatch (C>T) was also detected at the 4th nucleotide from 5' end of the N1 probe binding region on three Omicron variants (BE.1.1.1, BQ.1, BQ.1.1). These mismatches at the N1 probe binding regions are not expected to compromise N1 reactivity. No mismatches were detected in N2 primers and probe binding regions of these 21 VOI and VOC genomic sequences.

Table 8. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Inclusivity *In Silico* Analysis

PANGO Lineage	N1			N2		
	Forward Mismatch	Reverse Mismatch	Probe	Forward Mismatch	Reverse Mismatch	Probe Mismatch
B.1.1.7	None	None		None	None	None
B.1.617.2	None	None		None	None	None

PANGO Lineage	N1			N2		
	Forward Mismatch	Reverse Mismatch	Probe	Forward Mismatch	Reverse Mismatch	Probe Mismatch
AY.4.2	None	None		None	None	None
XBB	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
XBB.1	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
XBB.1.5	None	None		None	None	None
B.1.427	None	None		None	None	None
B.1.526	None	None		None	None	None
B.1.621	None	None		None	None	None
B.1.1.529	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BA.1.1	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BA.2.20	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BA.2.75.2	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BA.2.75.3	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BA.4	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BA.5	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BE.1.1.1	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BJ.1	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BM.4.1	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BQ.1	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None
BQ.1.1	None	None	C>T on 3rd nucleotide from 5'-end	None	None	None

An additional *in silico* analysis was completed in August, 2023 for the top 10 Omicron variants in circulation. Omicron variants of lineages EG.5, XBB.1.16, XBB.1.16.1, XBB.1.16.6, FL.1.5, XBB.9.1, and XBB.9.2 contain a single mismatch (T>C) at the 10th nucleotide from the 5' end of the N1 forward primer. These mismatches are not expected to impact assay sensitivity.

3) Cross-Reactivity (Analytical Specificity), Microbial Interference:

The MAWD Laboratories SARS-CoV-2 Dual Target by RT- PCR is a modified assay that uses the primers and probes sequences of the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel with right of reference to performance data contained in the CDC's EUA request (EUA200001). The cross-reactivity of the CDC 2019-nCoV EUA was evaluated through *in silico* analysis and indicated the N1 and N2 primers and probes combination displayed no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

4) Interfering Substances

A banked positive nasopharyngeal patient sample diluted to 2-3X the LoD and two pooled negative nasopharyngeal patient samples were tested in triplicate with potential interfering substances to examine the potential effect on the MAWD Laboratories SARS-CoV-2 Dual Target by RT- PCR test. Due to volume limitations after testing six of the seven substances, one additional negative nasopharyngeal patient sample was tested in triplicate with Cepacol lozenges only. No assay interference was observed with any of the negative or positive SARS-CoV-2 nasopharyngeal swabs in the presence of the substances at the concentrations indicated in **Tables 9** and **10** below.

Table 9. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Interfering Substances Study Results Using SARS-CoV-2 Positive Samples

Substance	Concentration	N1 Results	N2 Results	RP Results	Interpretation
Afrin nasal spray	15% w/v	3/3	3/3	3/3	Detected
Cepacol lozenges	3 mg/mL	3/3	3/3	3/3	Detected
Mouth wash	5% w/v	3/3	3/3	3/3	Detected
Cough syrup	5% w/v	3/3	3/3	3/3	Detected
Mucin	2.5 mg/mL	3/3	3/3	3/3	Detected
Nicotine/tobacco	0.03 mg/mL	3/3	3/3	3/3	Detected
Toothpaste (saliva)	0.5% w/v	3/3	3/3	3/3	Detected

Table 10. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Interfering Substances Study Results Using SARS-CoV-2 Negative Samples

Substance	Concentration	N1 Results	N2 Results	RP Results	Interpretation
Afrin nasal spray	15% w/v	0/3	0/3	3/3	Not detected
Cepacol lozenges	3 mg/mL	0/3	0/3	3/3	Not detected
Mouth wash	5% w/v	0/3	0/3	3/3	Not detected
Cough syrup	5% w/v	0/3	0/3	3/3	Not detected
Mucin	2.5 mg/mL	0/3	0/3	3/3	Not detected
Nicotine/tobacco	0.03 mg/mL	0/3	0/3	3/3	Not detected
Toothpaste (saliva)	0.5% w/v	0/3	0/3	3/3	Not detected

5) Specimen Stability:

Nasopharyngeal swab samples collected in viral transport media demonstrated stability at ambient temperatures (18-30°C) for up to twenty-fours after collection and at refrigerated temperatures (2-8°C) for up to three days (i.e., 72 hours) after collection consistent with the CDC COVID-19 specimen stability recommendations.

6) Clinical Evaluation:

Performance of the MAWD Laboratories SARS-CoV-2 Dual Target by RT- PCR was evaluated using 302 banked positive and negative nasopharyngeal specimens previously tested using a highly sensitive FDA-authorized molecular SARS-CoV-2 RT-PCR Assay as the comparator assay. Two samples resulted in "invalid" by the candidate test (tested negative by the comparator assay) and

were excluded from the performance evaluation. Of the remaining 123 positive samples and 177 negative samples, seven were discordant. Results of the evaluation are summarized in **Table 11** below.

Table 11. MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR Clinical Evaluation Study Summary

		Comparator Assay		
		Positive	**Negative**	**Total**
MAWD Laboratories SARS-CoV-2 Dual Target by RT-PCR	**Positive**	118	5	123
	Negative	2	175	177
	Total	120	180	
Positive Agreement		98.3% (94.1 – 99.5%)		
Negative Agreement		97.2% (93.7 – 98.8%)		

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.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- *In silico* analysis revealed that the N1 probe contains a single mismatch at the 3rd nucleotide from 5' end (C>T) on most Omicron variants. Another mismatch (C>T) was also detected at the 4th nucleotide from 5' end of the N1 probe binding region on three Omicron variants (BE.1.1.1, BQ.1, BQ.1.1). These are not expected to impact the sensitivity of the assay.
- Omicron variants of lineages EG.5, XBB.1.16, XBB.1.16.1, XBB.1.16.6, FL.1.5, XBB.9.1, and XBB.9.2 contain a single mismatch (T>C) at the 10th nucleotide from the 5' end of the N1 forward primer. This mutation is not expected to impact the sensitivity of the assay.

WARNINGS

- For in vitro diagnostic use
- Rx Only
- For use under Emergency Use Authorization (EUA) only
- This product has not been FDA cleared or approved, but has been authorized for emergency use 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 Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.