EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
In-Dx SARS-CoV-2 RT-LAMP Assay
(Michigan State University laboratories, Department of Medicine Olin Student Health Center)

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

The In-Dx SARS-CoV-2 RT-LAMP Assay will be performed at Michigan State University laboratories, Department of Medicine Olin Student Health Center located at 463 East Circle Drive, East Lansing, MI 48824, 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 In-Dx SARS-CoV-2 RT-LAMP Assay is an in vitro reverse transcription loop-mediated isothermal amplification (RT-LAMP) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab specimens (NPS) collected in viral transport medium (VTM) from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Michigan State University laboratories, Department of Medicine Olin Student Health Center, located at 463 East Circle Drive, East Lansing, MI 48824, 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 In-Dx SARS-CoV-2 RT-LAMP Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of RT-LAMP and in vitro diagnostic procedures. The In-Dx SARS-CoV-2 RT-LAMP Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The In-Dx SARS-CoV-2 RT-LAMP Assay is a RT-LAMP Test Kit designed to detect RNA from SARS-CoV-2 in nasopharyngeal swab specimens collected in viral transport media (VTM) by a healthcare provider.

The In-Dx SARS-CoV-2 RT-LAMP Assay utilizes six (6) primers to detect the nucleocapsid (N) gene for specific detection of SARS-CoV-2 viral RNA. It also utilizes a human specific primer set to detect the human Beta Actin (BA) gene. RNA from swab specimens is reverse transcribed to cDNA and subsequently amplified using loop-mediated amplification using the Thermo Scientific Digital Dry Bath/Block Heater.

The WarmStart Colorimetric LAMP 2X Master Mix is contained in a low-buffer reaction solution containing a visible pH indicator for detection of loop-mediated isothermal amplification (LAMP) and RT-LAMP reactions. During the RT-LAMP reaction there is production of protons from the extensive DNA polymerase activity in a LAMP reaction which drops the pH and produces a change in solution color from magenta to yellow. The reaction color change initiated by amplification is spectrophotometrically quantified using the BioTek 800TS Absorbance Reader for definitive detection of SARS-CoV-2-specific RNA material by calculating the delta between the 415 nm and 550 nm measurements. If the Delta value of a well is <0.1, this well will be considered negative. If the Delta value of a well is ≥0.1, this well will be considered positive (**Table 5**).

Description of Test Steps:

1. *Specimen Transport and Storage*

Nasopharyngeal specimens are collected on nasopharyngeal swabs, placed in VTM. Nasopharyngeal swab specimens are collected, transported, stored, and processed according to CLSI MM13-A. Specimens are transported and stored according to CDC guidelines.

2. *Specimen Testing*

Prior to performing RT-LAMP, reagents from the MagMAX CORE Nucleic Acid Purification Kit are prepared by aliquoting reagents into their intended plates. Crude samples are pipetted manually into the prepared sample plate along with Proteinase K and bead binding mix. Extraction is automated by the KingFisher Flex Instrument.

The WarmStart Colorimetric LAMP 2X Master Mix, guanidine thiocyanate and primer mix is manually pipetted into the 96-well plate. Extracted patient samples are added to the 96-well plate and overlayed with mineral oil. The RT-LAMP amplification is performed using the Thermo Scientific Digital Dry Bath/Block Heater and data interpretation and analysis is performed on the the BioTek 800TS Absorbance Reader.

3. Result Reporting

All test results are reported to the requesting healthcare provider via the authorized distributor's Electronic Health Record (EHR) system and public health authorities in accordance with local, state, and federal requirements.

INSTRUMENTS USED WITH THE TEST

Table 1: Instruments and Software

Equipment	Manufacturer	Catalog Number or Software Version
KingFisher Flex Purification System with 96 Deep-well head	Thermo Fisher Scientific	5400630
Thermo Scientific Digital Dry Bath/Block Heater	Thermo Fisher Scientific	8871001
96-well/flat bottom plate block	Thermo Fisher Scientific	88871107
BioTek Absorbance Plate Reader	Agilent	BioTek 800TS
BioTek 800TS Gen5 Software	Agilent	Software: GEN5RC

REAGENTS AND MATERIALS

Reagents should not be used past their expiration date.

Table 2: Assay Reagents

Reagent	Manufacturer	Catalog Number
MagMAXViral/Pathogen II Nucleic Acid Isolation kit	Thermo Fisher Scientific	A48383
WarmStart Colorimetric LAMP 2X Master Mix	New England Biolabs	M1800L
Heat-inactivated SARS-CoV-2	ATCC	VR-1986HK
RNA/DNA Shield	Zymo Research:	R1100-250
Molecular grade Absolute Ethanol	Thermo Fisher Scientific	BP2818-4
Guanidine thiocyanate powder reconstituted in nuclease free water	Invitrogen	15502-016
Molecular grade Nuclease-Free Non-DEPC treated water	Qiagen	129112
Viral Transport Media	MedSchender	MEDSCHENKER-STM30

CONTROLS

The In-Dx SARS-CoV-2 RT-LAMP Assay utilizes a Human Beta Actin (BA), Negative Control, and Positive Control. (**Table 3**). The Internal Control is applied to each patient sample prior to extraction. One Negative Extraction Control is included in every extraction batch and the Negative and Positive Control are used on every PCR plate. The delta between the 415 nm and 550 nm measurements using the BioTek 800TS Absorbance Reader is interpreted as seen in **Table 5**.

Table 3: Control Materials

Control Type	Description	Expected Results	Purpose
Internal Control (IC)	Endogenous Human Specimen Beta Actin Control	Delta ≥ 0.1[1]	Monitors specimen quality, demonstrates that nucleic acid was generated by the extraction process and serves as an inhibition control
Negative Control	Molecular Grade Nuclease Free Water	SAR-S CoV-2 N-gene negative (3/3) and IC negative signal	Monitors non-specific amplification, cross-contamination, or contamination of RT-LAMP reagents
Positive Control	DNA/RNA Shield medium spiked with Heat-inactivated SARS-CoV-2 (ATCC VR-1986HK) (50 copies/µL)	SARS-CoV-2 N-gene positive (2/3 or 3/3) and IC negative or positive signal*	Assesses assay validity and serves as an extraction control

[1] Refer to Table 5
*Positive control Heat-inactivated SARS-CoV-2 (ATCC VR-1986HK) is made from Vero E6 cells. In a few cases that the Positive well with Human Beta Actin primer set will have a positive signal due to trance among of the Vero E6 RNA which have cross reaction with Human Beta Actin primers set. This will NOT affect the judgement of the results.

Table 4: Interpretation of Controls

Control	SARS CoV-2 N-gene	Human Beta Actin
Positive Control (PC)	Positive signal (2/3 or 3/3 wells)	Negative/Positive signal*
Negative Control (NC)	Negative signal (3/3 wells)	Negative signal

*Positive control Heat-inactivated SARS-CoV-2 (ATCC VR-1986HK) is made from Vero E6 cells. In a few cases that the Positive well with Human Beta Actin primer set will have a positive signal due to trance among of the Vero E6 RNA which have cross reaction with Human Beta Actin primers set. This will NOT affect the judgement of the results.

INTERPRETATION OF RESULTS

Assessment of clinical specimen test results are performed after positive and negative controls have been examined and determined to be valid and acceptable (**Table 6**). If the controls are not valid, patient results cannot be interpreted. Any patient specimen that produces an invalid result is retested and collection of a new specimen if the repeat result is invalid.

The delta between the 415 nm and 550 nm measurements using the BioTek 800TS Absorbance Reader is interpreted as seen in **Table 5**.

Table 5: Interpretation of BioTek 800TS Absorbance Reader

Delta 415 nm – 550 nm	Interpretation
≥0.1	Positive Signal
< 0.1	Negative Signal

Table 6: Interpretation of Patient Results

SARS-CoV-2 (2/3 or 3/3 wells)	Beta Actin	Interpretation	Action
Negative	Positive	SARS-CoV-2 Not Detected	Report results
Negative	Negative	INVALID	Repeat testing
Positive	Positive or negative	SARS-CoV-2 Detected	Report results

PERFORMANCE EVALUATION

Limit of Detection (LoD) - Analytical Sensitivity:

The LoD of the In-Dx SARS-CoV-2 RT-LAMP Assay was determined using quantified, heat inactivated SARS-CoV-2 (ATCC VR-1986HK) at a starting concentration of 4.2 x 10^5 copies/µL. To estimate the LoD, three replicate samples were contrived at 6 different concentrations using negative, clinical matrix consisting of known, negative nasopharyngeal sample matrix in VTM. Samples were extracted using the MagMAX CORE Nucleic Acid Purification Kit then amplified on the KingFisher Flex instrument. The lowest concentration at which all three replicates produced positive results was defined as the preliminary LoD (**Table 7**). The preliminary LoD was then confirmed by testing an additional 20 replicates at the estimated LoD concentration (**Table 8**). The confirmed LoD of the In-Dx SARS-CoV-2 RT-LAMP Assay was 17 copies/µL of starting sample.

Table 7: Preliminary LoD Determination

Concentration (copies/µL)	N gene	IC
132	3/3	3/3
66	3/3	3/3
33	3/3	3/3
17	**3/3**	**3/3**
8	0/0	0/0
4	0/0	0/0

Table 8: Confirmation of LoD

Concentration (copies/ µL)	N gene	IC
17	20/20	20/20

Inclusivity (analytical reactivity):

In silico inclusivity analysis was performed on May 2nd, 2023, by aligning the LAMP primer sequences with the SARS-CoV-2 sequences deposited in GenBank at NCBI on

October 7, 2022. The data set included NCBI BLAST analysis of eight regions of the six primers.

All primers used in the In-Dx SARS-CoV-2 RT-LAMP assay showed 100% identity with sequences tested by NCBI BLAST analysis. One base pair mutation was noted in sequence analysis of Delta strains B.2.617.2. Subsequent SARS-CoV-2 strains have shown reversion to the original G residue in this position. No failure or change in SARS-CoV-2 RNA detection was noted in clinical sample analysis across strains tested.

Positive samples are sent for analysis by whole SARS-CoV-2 genome sequencing as part of a collaboration with the Michigan Department of Health and Human Services (MDHHS). Tests are continually validated against known, sequenced and typed strains provided by the MDHHS.

Cross Reactivity (analytical specificity)

In silico cross-reactivity analysis was performed with alignment of the LAMP primer amplicon sequences with the sequences of common respiratory organisms (**Table 9**). SARS-CoV, which is closely related to SARS-CoV-2, showed a significant match (87%) against the amplicon region covered by the In-Dx SARS-CoV-2 RT-LAMP Assay probes which could potentially lead to amplification of SARS-CoV. The likelihood of false positive amplification is low at this time as no known circulating strains of SARS-CoV are currently known to be present in the human population.

Wet testing was performed to evaluate the potential cross-reactivity and exclusivity of the In-Dx SARS-CoV-2 Assay with other common pathogens. DNA of different organisms was tested directly by the In-Dx SARS-CoV2 RT-LAMP test in triplicate (**Table 10**).

Table 9: Cross-Reactivity *In silico* Results

Organism	NCBI Accession	Identity to N-gene Amplicon
COVID-19	NC_045512	100%
Human coronavirus 229E, complete genome	NC_002645.1	No significant similarity
Human coronavirus OC43	NC_006213.1	No significant similarity
Human coronavirus HKU1	NC_006577.2	No significant similarity
Human coronavirus NL63	NC_005831	No significant similarity
SARS-coronavirus	NC_004718.3	87%
MERS-coronavirus	NC_038294.1	No significant similarity
Human adenovirus 1	AC_000017.1	No significant similarity

Organism	NCBI Accession	Identity to N-gene Amplicon
Human Metapneumovirus	NC_039199.1	No significant similarity
Parainfluenza virus 1	NC_003461.1	No significant similarity
Parainfluenza virus 2	AF533010.1	No significant similarity
Parainfluenza virus 3	NC_001796.2	No significant similarity
Parainfluenza virus 4	NC_021928.1	No significant similarity
Influenza A	NC_002021.1	No significant similarity
Influenza B	NC_002204.1	No significant similarity
Enterovirus 68	NC_038308.1	No significant similarity
Respiratory syncytial virus	NC_001803.1	No significant similarity
Human rhinovirus 1 strain	NC_038311.1	No significant similarity
Chlamydia pneumoniae	NC_005043.1	No significant similarity
Haemophilus influenzae	NZ_CP007470.1	No significant similarity
Legionella pneuomophila	NC_002942.5	No significant similarity
Mycobacterium tuberculosis	NC_000962.3	No significant similarity
Streptococcus pneumoniae	NZ_CP020549.1	No significant similarity
Streptococcus pyogenes	NZ_LS483338.1	No significant similarity
Bordetella pertussis	BX479248.1	No significant similarity
Mycoplasma pneumoniae	NZ_LR214945.1	No significant similarity
Pneumocystis jirovecii	GCF_001477535.1	No significant similarity
Candida albicans	NC_032089.1	No significant similarity
Pseudomonas aeruginosa	NC_002516.2	No significant similarity
Staphylococcus epidermidis	NZ_CP035288.1	No significant similarity
Streptococcus salivarius	NC_017594.1	No significant similarity

Organism	NCBI Accession	Identity to N-gene Amplicon
Herpes simplex virus 1 (HSV-1)	NC_001806.2	No significant similarity
Epstein-Barr virus (EBV)	NC_009334.1	No significant similarity
Cytomegalovirus (CMV)	NC_006273.2	No significant similarity
Moraxella catarrhalis	NZ_CP018059.1	No significant similarity
Porphyromonas gingivalis	NC_010729.1	No significant similarity
Bacteroides oralis	NZ_AEPE00000000.2	No significant similarity
Nocardia sp.	NC_006361.1	No significant similarity
Streptococcus mutans	NZ_CP044221.1	No significant similarity
Streptococcus mitis	NC_013853.1	No significant similarity
Eikenella sp.	NZ_CP038018.1	No significant similarity
Neisseria sp.	NZ_AP023069.1	No significant similarity
Lactobacillus sp.	NZ_CP010432.1	No significant similarity

Table 10. Cross-Reactivity Wet Testing Results

Organism	Strain	SARS-CoV-2 Replicates Detected
Staphylococcus aureus	Sparrow Hospital Culture	0/3
Streptococcus pyogenes	Sparrow Hospital Culture	0/3
Klebsiella pneumoniae	Sparrow Hospital Culture	0/3
Pseudomonas aeruginosa	ATCC10145	0/3
Stenotrophomonas maltophilia	Sparrow Hospital Culture	0/3
Acinetobacter baumanii	Sparrow Hospital Culture	0/3
Haemophilus influenzae	ATCC 49766	0/3
Moraxella catarrhalis	ATCC 25240	0/3
Legionella pneumophila	ATCC 33152	0/3
Serratia marcscens	Sparrow Hospital Culture	0/3
Streptococcus pneumoniae	ATCC 700669	0/3
Enterobacter aerogenes	ATCC 13048	0/3
Citrobacter koseri	Sparrow Hospital culture	0/3

Organism	Strain	SARS-CoV-2 Replicates Detected
Candida albicans	Sparrow Hospital Culture	0/3

Microbial Interference:

N/A - The sponsor relies on the right of reference from the Seegene Technologies for the inclusivity data of their assay

Endogenous/Exogenous Interference Evaluation:

The impact of potential interfering substances on the In-Dx SARS-CoV-2 RT-LAMP Assay was evaluated via spiking potential interfering substances into three (3) positive and three (3) negative samples aliquoted in duplicate. Positive specimens were created via spiking heat inactivated virus into known, negative nasopharyngeal sample matrix in VTM at 2.5x LoD. Each interfering substance was tested in triplicate. No false negative or false positive results occurred during the study (**Table 11**).

Table 11: Interference Testing

Substance	Brand	Concentration	SARS-CoV-2 Concentration	Positive Sample Results (#Pos/Total)	Negative Sample Results (#Pos/Total)
Afrin Original Nasal Spray	Afrin	15% v/v	2.5x LoD	2/2	0/6
Sore throat and cough lozenges	Chloraseptic max, Benzocaine 15mg/Menthol 10mg/lozenges	3 mg/mL	2.5x LoD	3/3	0/3
Sore Throat spray	Chloraseptic	5% (v/v)	2.5x LoD	3/3	0/3
Mouth Wash	Listerine Cool Mint	5% (v/v)	2.5x LoD	3/3	0/3
Cough syrup	Robitussin	5% (v/v)	2.5x LoD	3/3	0/3
Bovine Submaxillary Gland, Type I-S	Sigma Aldrich (M3895)	2.5 mg/mL	2.5x LoD	3/3	0/3
Nicotine or Tobacco	Sigma, (N3876-5ML)	0.03 mg/mL	2.5x LoD	3/3	0/3
Toothpaste	Crest fluoride anticavity toothpaste	0.5% (v/v)	2.5x LoD	3/3	0/3

Sample Stability:

N/A - Specimens are collected, stored, and handled according to CDC guidelines and manufacture's protocol.

Clinical Evaluation for Patients Suspected of COVID-19:

Clinical performance of the In-Dx SARS-CoV-2 RT-LAMP Assay was evaluated by testing a total of 68 clinical nasopharyngeal swabs specimens collected in VTM from patients suspected of COVID-19 by a healthcare provider and by a highly sensitive FDA-authorized molecular SARS-CoV-2 RT-PCR Assay. Among these specimens, 38 were positive and 30 were negative as determined by the comparator method. The positive percent agreement was 97.4% (37/38) and the negative percent agreement was 100% (30/30). Based on the Ct values obtained with the comparator method, 7 comparator positive samples have Ct values within 3 cycles of the average Ct at the LoD of the comparator assay and were considered "weak positive". The results of this study support the use of the In-Dx SARS-CoV-2 RT-LAMP Assay for SARS-CoV-2 testing for individuals suspected of COVID infection and are presented in **Table 12**.

Table 12: Clinical evaluation results for patients suspected of COVID-19

		EUA Authorized Comparator Test	
		Positive	**Negative**
In-Dx SARS-CoV-2 RT-LAMP Assay	**Positive**	37	0
	Negative	1	30
Positive Agreement		97.4% (CI: 86.5%, 99.5%)	
Negative Agreement		100% (CI: 88.7%, 100%)	

WARNINGS

- For use under Emergency Use Authorization (EUA) only.
- For *in vitro* diagnostic use.
- For prescription use 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 Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

LIMITATIONS

- The In-Dx SARS-CoV-2 RT-LAMP Assay is intended and validated for use only with nasopharyngeal swab specimens. Testing of other sample types may result in inaccurate results.

- Primers for the In-Dx SARS-CoV-2 RT-LAMP Assay target highly conserved regions within the genome of SARS-CoV-2. Mutations rarely occur in these highly conserved regions, but if a mutation did occur in these regions, SARS-CoV-2 RNA could become undetectable.
- Negative results in the In-Dx SARS-CoV-2 RT-LAMP Assay 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.
- If the virus mutates in the regions targeted by the RT-PCR assay, SARS-CoV-2 may not be detected or may be detected less predictably.
- A false positive result may occur if there is cross-contamination by target organisms, their nucleic acids or amplified product.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- This test detects pan-sarbecovirus from the sarbecovirus subgenus family and cannot distinguish between SARS-CoV-1 and SARS-CoV-2. SARS-CoV-1 has not been in circulation since 2004.
- 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.