CardiAi

CoviLamp™
(Fluorometric assay)

For in vitro diagnostic use

Validation of this test has not been reviewed by FDA. Review under the EUA program is pending. Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2

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Please read this User Guide before using the CoviLamp™ Test Kit.

CoviLamp™

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1. Intended Use

The SARS-CoV-2 assay is a reverse transcription loop-mediated isothermal amplification (RT-LAMP) technology assay intended for the qualitative detection of SARS-CoV-2 viral RNA in nasal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to clinical laboratories licensed/accredited to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

The assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of RT-LAMP and in vitro diagnostic procedures.

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.

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1.1. Special Conditions for Use Statements:

For prescription use only

For in vitro diagnostic use only

1.2. Special Instrument Requirements:

The CoviLamp SARS-CoV-2 Fluorometric Test Kit is to be used with the following:

- Zymo Research Quick-RNA Viral Kit for extraction, and,
- the Applied Biosystems 7900HT Fast Real-Time PCR System with SDS Software 2.4.1

2. Background Information:

The global pandemic of COVID-19 has challenged health-care systems worldwide with several patients presenting with severe and life-threatening symptoms. Screening for COVID-19 infected individuals relies on sensitive and accurate detection of the SARS-CoV-2 virus. The CoviLamp™ diagnostic assay by CardiAI, provides a detection

method of SARS-CoV-2 for rapid, small and largescale patient testing.

With CoviLamp™, the viral RNA can be detected in as low as 30 minutes requiring less specialized equipment. Using reverse transcription loop-mediated isothermal amplification (RT-LAMP), CoviLamp™ amplifies SARS-CoV-2 RNA faster and with an easier-to-use platform than traditional RT-PCR. Detection of viral RNA can be performed by observing change in fluorescence. Fluorescence detection can be performed in a fluorimeter or RT-PCR instrument.

2.1. LAMP PCR Compared to RT-PCR:

LAMP differs from conventional PCR in two main ways. LAMP occurs under isothermal conditions, allowing the test to be performed with more versatile laboratory equipment (heat blocks, water baths, programmable ovens). While the clinical-scale method produced by CardiAI Inc. is designed for use with a plate reader, the kit can also be reduced to a small-scale reaction observed by eye.

3. Test Principle

CoviLamp™, is a loop-mediated amplification PCR assay that detects SARS-CoV-2 RNA. Using a proprietary mix of reagents and process, CoviLamp™ involves a timed incubation at 60°C followed by read out of fluorescence. The kits are available for small scale with test tubes (microfuge) or large-scale (high-throughput plate readers for 96 well plates).

4. Kit Components

4.1. Included in the CoviLamp™ kit

Table 1:

Kit components	Storage	Storage Conditions	Source
WarmStart LAMP 2X Master Mix for DNA & RNA – Fluorometric + Fluorescence dye (PCR MM-F)	-20°C	If stored at -20ºC this product has a shelf life of ~24 months and few months at room temperature	New England BioLabs (NEB)
E-gene - primer cocktail	-20°C to +4°C *	At –20°C oligos remain stable for 24 months and 3 months at room temperature	Integrated DNA Technologies (IDT)
RNAse P - primer cocktail	-20°C to +4°C *	At –20°C oligos remain stable for 24 months and 3 months at room temperature	Integrated DNA Technologies (IDT)
RAB solution	22°C	Stable at room temperature	Sigma
Water (RNAse/DNAse free)	-20°C to +4°C *	Stable at room temperature	Integrated DNA Technologies (IDT)
E-gene – Control (5 µL = 1000 copies)	--20°C to +4°C *	If stored at -20ºC this product has a shelf life of ~24 months and few months at room temperature	New England BioLabs (NEB)
RNase P gene –Control (5 µL = 1000 copies)	-20°C to +4°C *	If stored at -20ºC this product has a shelf life of ~24 months and few months at room temperature	New England BioLabs (NEB)

* Controls stored at 4°C should be used within one month and primer with one week. Avoid multiple cycles of freezing and thawing as it can damage the protein structure.

Self-prepared reagent: Disinfectant, such as 70% ethanol

4.2. Additional Components Required for Testing not included with the kit

These items are not included in the CoviLamp™ kit.

Table 2:

S.no.	Instrument / Consumables Name
1	Laminar flow hood or biosafety cabinet (workspace capable of maintaining an aseptic environment)
2	Zymo Research Quick-RNA Viral Kit for extraction
3	Applied Biosystems 7900HT Fast Real-Time PCR System with SDS Software 2.4.1
4	Pipettor, 1mL, 200µL, 100µL, 10µL and 2µL, Multichannel pitters with listed µL range is preferred for high throughput.
5	Sterile and filtered pipettes tips,
6	Standard polypropylene 96 well PCR plate
7	Optical Adhesive plate Cover
8	Labels
9	Vortex Mixer
10	Microcentrifuge
11	DNAZap or equivalent detergent solution
12	10% Bleach solution
13	Disposable gloves and surgical gowns

4.3. Controls that will be provided with CardiAI's CoviLamp™ SARAS-Cov-2 test kit

4.3.1. Positive control: A positive template control is needed to verify that the reaction mixture is amplifying target sequence as expected and reaching a threshold of fluorescence at a time consistent with previously controlled reaction. The positive control will be ssDNA target (E-gene and RNAse P, on the reaction plate) at a concentration of 1000 copies/reaction for each.
In Fluorometric **CoviLamp™** plate assay the positive control wells are expected to demonstrate reactions at Ct values of 15 to 17 for E-gene. The change is described in more detail in the procedure section.

4.3.2. Negative control: the negative control is needed to ascertain that the reaction mixtures is not auto-amplifying PCR structures (NTC color should not show any change in fluorescence. In Fluorometric **CoviLamp™** plate assay negative results should not demonstrate amplification or should demonstrate it only if it is observed after the

recommended window for *bona fide* reactions (for this assay, we suggest that NTC after 35 minutes is acceptable. If they are very late, they may be considered a non template reaction, similar to an NTC and can be considered negative.

4.3.3. Extraction control: human RNAse P (200 copies of ssDNA oligonucleotide) will be run **for each patient sample** to verify that enough RNA material was acquired to provide an accurate test for associated viral material. This measure is essential to describe a test result as SARS-CoV-2 negative, since it is possible that a poorly extracted sample will be below the LoD of the primer sets.

5. Procedure or Test Steps

5.1. Sample collection: Certified labs would collect samples based on their established protocols.

5.2. Sample processing: Sample will then be processed for the extraction of viral RNA. For

extraction of RNA from the clinical samples, labs can use Zymo Research Quick-RNA Viral. It is important that with this kit that RNA is eluted into water.

5.3. RT-LAMP reaction:

5.3.3. Setting up 96 well fluorometric Assay plate Kinetic assay:

5.3.3.1. Work area preparation:

a)Clean the workspace with ethanol followed by an RNase cleaning solution.
b)Use RNase/DNase free plasticware for tubes and filtered tips and ensure that they are free from contamination.
c)Please use DNase/RNase free 96 well spectrophotometry plate, ½ volume wells, flat bottom
d)Please use standard polypropylene 96 well PCR plate for this assay

5.3.3.2 Thaw reagents:

a)Remove the PCR MM-F, RAB solution, fluorescent dye and the primer cocktail from the kit, and fully thaw to the room temperature.
b)Make sure to dissolve any precipitates in the Master Mix by vertexing briefly.
c)Mix primer cocktail, and control template gently by pipetting up and down.
d)Briefly centrifuge above vials to collect contents at bottom.
e)All Nucleic acid templates should be kept on ice before use

5.3.3.3 Reagent Preparation:

a)Calculate the number of wells (table 3; column 3) to be used for each primer cocktail
b)Based on number of well user intends to use for each assay reaction mix, user should end up with following assay reaction mixes:

1. Primer assay reaction mix (primer cocktail; E-gene) with no template

2. Primer assay reaction mix (primer cocktail; RP-gene) with no template

c)Calculate the volume required for each reaction component according to Table 3; column 2 to make an assay reaction mix (ARM) Table 3, Column 4 and 5.
d)Add the calculated volumes to clean pre-labeled tubes and label according to which Primer cocktail is being used (E-gene/ RP gene)
e)Pulse vortex the ARM solutions (E-gene/RP gene) briefly and keep it on ice.

Please make separate ARM for each primer cocktail.

Table 3: Typical recipe for the Reaction Pre-mix:

Single gene-primer cocktail volume calculation setup for 32 wells.

This recipe should be repeated to make E gene and RP gene ARM.

1	2	3	4	5
Ingredients	Fluorometric assay (uL)	# of samples	Assay master Mix (AMM) assay (uL)	Assay master Mix +10% additional reagent (uL)
PCR MM	12.5	32	400	440
Primer cocktail	3	32	96	105.6
Fluorescent dye	0.5	32	16	17.6
RAB solution	1	32	32	35.2
Water	3	32	96	96.96
Total	25	32	640	695.36
RNA /Control template/water(NTC)	5	32	160	176

For NTC or control well- equivalent RNA template volume (5uL) of water or control template should be added to NTC and control wells

# of samples should be calculated for each gene -primer cocktail intends to be used for screening purposes

The reaction mixtures should be combined immediately before performing the assay to avoid nonspecific amplification.

5.3.3.4 Plate loading (Refer Fig 1)

a)Calculate the number of wells (table 3; column 3) to be used for each primer cocktail

b)Based on number of well user intends to use for each assay reaction mix, user should end up with following assay reaction mixes:

1. Primer assay reaction mix (primer cocktail; E-gene) with no template
2. Primer assay reaction mix (primer cocktail; RP-gene) with no template

c)Calculate the volume required for each reaction component according to Table 3; column 2 to make an assay reaction mix (ARM) Table 3, Column 4 and 5.
d)Add the calculated volumes to clean pre-labeled tubes and label according to which Primer cocktail is being used (E-gene/RP gene)
e) Pulse vortex the ARM solutions (E-gene/RP gene) briefly and keep it on ice. Refer to section 5.3.3.5 for measurement and analysis.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene
B	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene
C	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene
D	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene
E	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene
F	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene
G	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene	Egene	RP gene
H	Egene	RP gene	Egene	RP gene	E +ve Ctrl	E +ve Ctrl	Rp +ve Ctrl	Rp +ve Ctrl	E-NTC	E-NTC	Rp-NTC	Rp-NTC

Figure 1: Schematic representation of 96 well plate layout for a typical 3 gene RT-Lamp assay.

5.3.3.5 Fluorometric measurement and analysis:

a) Create a kinetic fluorescence protocol for RT-LAMP using a qRT-PCR machine with a block compatible with the 96-well 0.2 mL plate.

1. The type of experiment is a **Comparative CT experiment** (ΔΔCT).

- Detection: Select a fluorometric excitation/emission for SYBR green. Ensure that you select "none" for passive reference as it will interfere with the analysis protocol (plate set-up window for the Applied Biosystems 7900HT Fast Real-Time PCR System with SDS Software 2.4.1).

2. PCR method: Select a reaction volume of 25 µL. Create a single step method where the temperature ramps at 2 °C/s to stay stable at 60 °C throughout the assay. Perform 35 cycles of 1-minute duration. A melt-curve is unnecessary.

3. Plate setup: select 2 targets, representing the CardiAI primer sets of E-gene and RNAse P. Select the patient samples by assigning numbers or other preferred method to each unique sample set of 6 (according to the layout of your plate). Label each well as unknown (drop-down arrow from the task menu). The NTCs for each primer set should be labelled as a separate group and be designated as N drop-down menu). Positive control wells should have their own labels and may be listed as 1000 copies.
4. Threshold designation: At this time, we have used the default setting for the threshold (0.2), with a baseline starting at 3 cycles and ending at 10 cycles.

- Following the run, perform plate analysis for each of the samples and controls. If using the Applied Biosystems 7900HT Fast Real-Time PCR System with SDS Software 2.4.1, view the amplification window and

plate map. Highlight the control sections of the plate:

1. For wells designated as NTC, the reactions should stay at baseline throughout the course of the experiment. If any NTC reactions are observed, it should be noticeable very late in the assay, after 30 minutes.
2. Positive control wells are expected to demonstrate reactions at Ct values of 15 to 17 for E-gene.
3. After verification of the correct behavior of controls, the samples may be analysed for RT-LAMP reactions. Highlight the wells corresponding to each sample test for this step and assess whether the reaction is positive based on the criteria below:
    i. Positive results are amplifications that can be observed before 25 Ct for E-gene (this interpretation is based on our in-house experiments and may change, depending on

the LoD determined by your analysis).

ii. Negative results should not demonstrate amplification or should demonstrate it only if it is observed after the recommended window for *bona fide* reactions. If they are very late, they may be considered a non template reaction, similar to an NTC and can be considered negative.

iii. Duplicate wells for each sample/primer set should show agreement to make a conclusion of a positive or negative result. Refer to Table 4 for the interpretation of results.

5.3.3.6 Troubleshooting:

a) SYTO9 (FLORESCENT DYE) is in DMSO and it freezes in the fridge or on ice. It must be completely thawed at room temperature and vortexed to be evenly suspended. Great care

should be taken to make sure that the viscous solution is pipetting accurately throughout the assay.
b) We would also recommend that the reaction mixes are made by mixing reagents in the following order:

1. Add the dye to the master mix for a volume that will be distributed for each reaction mixture that will be used in the experiment (E-gene and RNAse P). For instance, you are needing enough reaction mix so that there is 1.3 mL of each, make 4 mL of MM and dye. Vortex briefly and pulse spin the solution.

2. Distribute the MM/dye to the three tubes you will use for each reaction mix .

3. Add the water and GuHCl (RAB) solution to each reaction mix.

4. Ensure the primer cocktails are completely thawed and well mixed. Add the primer cocktail

to each mix. Pulse vortex the solutions and/or pipette the volume ~10 times. Put on ice.

5. Load 20 microlitres to each well for the PCR, placing each primer set solution in the pattern according to the plate map design.
6. Add 5 microlitres of template solution/control/NTC.

7. Spin the plates down.

8. Load onto instrument.

9. Perform PCR

6. Interpreting Test Results based on patient symptoms

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

Table 4:

S.No.	CoviLamp™ test Results	Manifestation of Symptoms	Result interpretation
1.	Positive	Positive	COVID 19 +ve
2.	Positive	Negative	Repeat the test with CoviLamp™. In case 2nd time test is positive, repeat the test immediately with established gold standard testing procedure
3.	Negative	Positive	Repeat the test immediately with established gold standard testing procedure
4.	Negative	Negative	COVID 19- ve ***Note:*** *Negative results must be considered in the context of an individual's recent exposures, history, presence of clinical signs and symptoms consistent with COVID-19*

7. Performance Evaluation

7.1. Limit of Detection (LoD) - Analytical Sensitivity:

2-fold serial dilutions of SeraCare's AccuPlex Verification Positive Sample (spiked in COVID-19 Negative DNA/RNA Shield solution as the clinical matrix) was used to confirm LoD. Viral extraction was done using Zymo Research Quick-RNA Viral Kit and quantification was done using the Applied Biosystems 7900HT Fast Real-Time PCR System with SDS Software 2.4.

Clinical matrix used as the final LoD was determined to be the lowest concentration resulting in positive detection in 95% of the replicates (19/20). As shown in the summary table below, the final LoD determined for this test is 25,000 copies/mL. Table 5 and Table 6 enlists the preliminary and definitive LOD.

Table 5: Preliminary LoD: 2-fold Dilutions Data

Copy Number	Replicate#	E-gene	
50,000 copies/ml	1	13.024	(+)
	2	14.089	(+)
	3	14.678	(+)
25,000 copies/ml	1	15.939	(+)
	2	14.825	(+)
	3	14.955	(+)
12,500 copies/ml	1	13.797	(+)
	2	16.468	(+)
	3	15.514	(+)
6,250 copies/ml	1	20.383	(+)
	2	22.603	(+)
	3	18.533	(+)
3,125 copies/ml	1	22.357	(+)
	2	20.402	(+)
	3	25.611	(+)
1,562.5 copies/ml	1	24.959	(+)
	2	Undetermined	(-)
	3	Undetermined	(-)
781.25 copies/ml	1	Undetermined	(-)
	2	Undetermined	(-)
	3	Undetermined	(-)

Table 6: Definitive LOD

Sample	Ct (min)	Qualitative Result
25K copies/mL (1)	16.523561	Positive
25K copies/mL (2)	20.61594	Positive
25K copies/mL (3)	21.889938	Positive
25K copies/mL (4)	17.521986	Positive
25K copies/mL (5)	19.607185	Positive
25K copies/mL (6)	17.557981	Positive
25K copies/mL (7)	16.89957	Positive
25K copies/mL (8)	15.025991	Positive
25K copies/mL (9)	18.281677	Positive
25K copies/mL (10)	16.039358	Positive
25K copies/mL (11)	15.645684	Positive
25K copies/mL (12)	23.943827	Positive
25K copies/mL (13)	19.941277	Positive
25K copies/mL (14)	18.11043	Positive
25K copies/mL (15)	15.04962	Positive
25K copies/mL (16)	17.916359	Positive
25K copies/mL (17)	17.39197	Positive
25K copies/mL (18)	21.575377	Positive
25K copies/mL (19)	17.802507	Positive
25K copies/mL (20)	18.136494	Positive

7.2. Inclusivity (analytical sensitivity):

To determine inclusivity, CoviLamp manufacturer, CardiAI, performed in-silico studies of the sequences used to design the primers for the RT-LAMP assay. CardiAI used the blastn search protocol with the target sequences for primer design as queries against the Betacoronavirus Genbank database (Assay was performed on NCBI server from July 15th 2020 through October 15th 2020). CardiAI observed 100 % alignment of query sequences with the 500 entries identified in the search.

The design of LAMP primer sets centered around one genomic region of SARS-CoV-2 corresponding to E-gene. This region was used for in-silico analysis for inclusivity.

Table 7: Sequences used in the development of the CoviLamp Assay

Primer target	Nucleotide Sequence used to make LAMP primers	% inclusivity with SARS-CoV-2 sequences
E-gene (332 bp)	ACGGTTCATCCGGAGTTGTTAATCCAGTAATGGAACCAATTTATGATGAACCGACGACGACT ACTAGCGTGCCTTTGTAAGCACAAGCTGATGAGTACGAACTTATGTACTCATTCGTTTCGGAA GAGACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCTAG TTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATATTGTTAACGTGA GTCTTGTAAAACCTTCTTTTTACGTTTACTCTCGTGTTAAAAATCTGAATTCTTCTAGAGTTCC TGATCTTCTGGTCTAA	100 %
RNAse P (320 bp)	TGGCTGCCAATACCTCCACCGTGGAGCTTGTTGATGAGCTGGAGCCAGAGACCGACACAC GGGAGCCACTGACTCGGATCCGCAACAACTCAGCCATCCACATCCGAGTCTTCAGGGTCA CACCCAAGTAATTGAAAAGACACTCCTCCACTTATCCCCTCCGTGATATGGCTCTTCGCA TGCTGAGTACTGGACCTCGGACCAGAGCCATGTAAGAAAAGGCCTGTTCCCTGGAAGCCC AAAGGACTCTGCATTGAGGGTGGGGGTAATTGTCTCTTGGTGGCCCAGTTAGTGGGCCTT CCTGAGTGTGTGTATGCGGT	0 %

From the sequences of **Table 7,** LAMP primers were designed and tested for selectivity and sensitivity to SARS-CoV-2 viral genome. The E-gene set below was chosen for the RT-LAMP assay moving forward.

Table 8: Primer sequences used for the CoviLamp Assay

Gene Targeted	LAMP Primer ID	Nucleotide Sequence
	F3	acggttcatccggagttg
	B3	aggcacgctagtagtcgt
E - gene	FLP	aggtacgttaatagttaatagcgtacttc
(SARS-CoV-2)	BLP	cgcagtaaggatggctagt
	FIP	actcatcagcttgtgcttacaaaatggaaccaatttatgatgaaccg
	BIP	tgtactcattcgtttcggaagaggcaagaataccacgaaagcaag
	F3	ttgatgagctggagcca
	B3	caccctcaatgcagagtc
RNAse P	FLP	atgtggatggctgagttgtt
(human ribonuclease P)	BLP	catgctgagtactggacctc
	FIP	gtgtgaccctgaagactcggttttagccactgactcggatc
	BIP	cctccgtgatatggctcttcgtttttttcttacatggctctggtc

7.3. Cross-reactivity (analytical specificity):

To assess the effects of clinically relevant co-infections, the Sponsors tested the selected microorganisms commonly found in the claimed specimen matrix in the presence of SARS-CoV-2 at low concentration. The interference was evaluated by testing with a minimum of 3 sample replicates in

nucleic acid-free water. The results are not from an exhaustive study of all possible bacteria strains but are from common strains evaluated for COVID studies.

CardiAI tested 14 respiratory viral pathogens, and none were identified to interfere with the SARS-CoV-2 CoviLamp Fluorometric Assay. Furthermore, 10 respiratory bacterial pathogens were tested and none were identified to interfere with the SARS-CoV-2 CoviLamp Fluorometric Assay.

Table 9: Respiratory Viral Panel Cross-reactivity (Wet lab)

Respiratory Viral Panel	Concentration	Replicates
		E-gene
Twist Synthetic Measles virus RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human coronavirus OC43 RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human parainfluenza virus 1 RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human rhinovirus 89 RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Influenza H1N1 (2009) RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic MERS coronavirus 2c EMC/2012 RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human coronavirus NL63 RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Influenza B RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human parainfluenza virus 4 RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human coronavirus 229E RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Mumps virus RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human bocavirus 1 DNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Human enterovirus 68 RNA control	$1x10^6$ copies/µL	0/3
Twist Synthetic Influenza H3N2 RNA control	$1x10^6$ copies/µL	0/3

CardiAI also performed *in silico* cross reactivity experiments to demonstrate that the test does not react with related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in a clinical specimen. To address possible issues of cross-reactivity of the LAMP primer sets, blastn searches were performed with the genomic region associated with LAMP primer design. Below is the summary of global, semi-global and local sequence alignment.

The conclusion is that there is no significant cross-reactivity (greater than 80 %) between these sequences and any viruses that are prevalent in the human population, since SARS-CoV-1 is no longer an active and contagious virus.

Table 10: Respiratory Viral Panel Cross-reactivity (*In silico*)

Virus name		Alignment to E-gene 332 bp		
	Genbank Accession #	Global (%)	Semi-global (%)	Local (%)
COVID-19 (SARS-CoV-2)	MN908947.3	1.07	1.07	100
Human Coronavirus 229E	NC_002645.1	1.19	0.81	50.43
Human Coronavirus OC43	NC_006213.1	1.07	0.734	51.47
Human Coronavirus HKU1	NC_006577.2	1.1	0.75	51.26
Human Coronavirus NL63	NC_005831.2	1.18	0.82	51.83
SARS-CoV-1 (Tor2)	NC_004718.3	1.05	1.04	92.22
MERS-CoV-2c (EMC/2012)	NC_019843.3	1.09	0.74	51.26
Adenovirus, strain ad71	X67709.1	19.44	13.88	47.27
Human Metapneumovirus	NC_039199.1	2.43	1.65	53.85
Parainfluenza virus 1, strain Washington/1964	AF457102.1	2.1	1.43	51.3
Parainfluenza virus 2, strain GREER	AF533012.1	1.33	2.09	48.82
Parainfluenza virus 3, strain HPIV3/MEX/1526/2005	KF530234.1	2.11	1.37	51.09
Parainfluenza virus 4, strain M-25	NC_021928.1	1.91	1.33	46.36
Influenza A (H1N1)	FJ966079.1	12.85	9.7	46.83
Influenza A (H3N2)	KT002533.1	13	8	47.45
Influenza B (Victoria)	MN230203.1	12.93	9.05	48.05
Influenza B (Yamagata)	MK715533.1	12.47	8.86	49.75
Enterovirus D68 (EV-D68)	KP745766.1	4.36	2.87	49.11
Respiratory syncytial virus	U39661.1	2.1	1.49	52.02
Human rhinovirus 14	NC_001490.1	4.4	3.06	46.37

Table 11: Respiratory Bacterial Panel Cross-reactivity (Wet lab)

Respiratory Bacterial Panel	Concentration	Replicates
		E-gene
Streptococcus salivarius	4.19 x 10^8 CFU/mL	0/3
Streptococcus pyogenes	2.66 x 10^9 CFU/mL	0/3
Mycobacterium tuberculosis	6.32 x 10^7 CFU/mL	0/3
Streptococcus pneumoniae	4.16 x 10^8 CFU/mL	0/3
Mycoplasma pneumoniae	3.16 x 10^8 CFU/mL	0/3
Pneumocystis jiroveci - S. cerevisiae	6.34 x 10^8 CFU/mL	0/3
Legionella pneumophila	1.42 x 10^{10} CFU/mL	0/3
Haemophilus influenzae	5.43 x 10^8 CFU/mL	0/3
Candida albicans	4.5 x 10^8 CFU/mL	0/3
Streptococcus salivarius	4.19 x 10^8 CFU/mL	0/3

Table 12: Respiratory Bacterial Panel Cross-reactivity (In silico)

Bacterial Strains	Genbank Accession number	E-gene
Bordetella_pertussis	NC_002929.2	46.6
Candida_albicans	NC_032089.1	44.9
Chlamydia_pneumoniae	NC_000922.1	47.5
Haemophilus_influenzae	NC_000907.1	50.9
Legionella_pneumophila	NC_002942.5	47.9
Mycobacterium_tuberculosis	NC_000962.3	43.2
Mycoplasma_pneumoniae	NC_000912.1	47.2
Pneumocystis_jirovecii	NW_017264775.1	47.2
Pseudomonas_aeruginosa	NC_002516.2	48.8
Staphylococcus_epidermidis	NC_004461.1	46.3
Streptococcus_pneumoniae	NC_003098.1	47.3
Streptococcus_pyogenes	NC_002737.2	45.2
Streptococcus_salivarius	NZ_CP009913.1	50.3

7.4. Clinical Evaluation

Performance of the CardiAI's CoviLamp diagnostic assay was evaluated using:

a. 30 COVID-19 positive nasal swab specimens, confirmed positive on an EUA-approved RT-PCR SARS-CoV-2 test.

b. 30 COVID-19 negative nasal swab specimens, confirmed negative on an EUA-approved RT-PCR SARS-CoV-2 test.

Comparator method is the EUA-approved ThermoFisher TaqPath. Viral RNA was extracted using Zymo Research Quick-RNA Viral Kit and test was run using the Applied Biosystems 7900HT Fast Real-Time PCR System with SDS Software 2.4.1

Table 13: Data Summary of Clinical Evaluation using Nasal Swab Specimens

Samples Tested Individually	Comparator Method Result	
Candidate test result	Positive	Negative
Positive	27 (90%)	2 (6.67%)
Negative	3 (10%)	28 (93.3%)

8. Limitations

Laboratories should include a statement in test reports to patient healthcare providers such as “The test has been validated but FDA’s independent review of this validation is pending”.

9. Warnings and Precautions

1. Validation of this test has not been reviewed by FDA.

2. All samples shall be considered potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements.

3. Testing laboratories should have designated areas for reagent preparation, sample preparation and amplification detection area to avoid any type of cross contamination which can influence the test results.

4. Lab personnel performing the test should be equipped with PPE (Personal protective equipment) to avoid direct contact with reagents or infectious samples.

5. PCR machines should be calibrated regularly

6. All the kit waste and pipettes should be discarded according to local, regional or federal regulations

7. Testing areas should be thoroughly disinfected/decontaminated after running the experiment. Disinfection can be carried out in accordance to the institute/company/lab policy.

8. Quality of the test results is dependent on sample collection, transportation, storage and extraction process. If any step is improperly performed it might lead to false negative or false positive results.

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