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Handheld Biosensor for COVID-19 Screening

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ABSTRACT

We have made significant progress toward the development of an integrated nucleic acid amplification system for Autonomous Medical Devices Incorporated (AMDI's) Optikus handheld diagnostic device. In this effort, we developed a set of loop-mediated isothermal amplification (LAMP) primers for SARS-CoV-2 and then demonstrate amplification directly on a surface acoustic wave (SAW) sensor. We built associated hardware and developed a C-code to control the amplification process. The goal of this project was to develop a nucleic amplification assay that is compatible with SAW sensors to enable both nucleic and serological testing in a single handheld diagnostic device. Toward this goal, AMDI is collaborating Sandia National Laboratories to develop a rapid, portable diagnostic screening device that utilizes Sandia's unique surface acoustic wave biosensor (SAW) for COVID-19 detection. Previously, the SANDIA- AMDI SAW sensor has successfully detected multiple high-profile bacteria viruses, including Ebola, HIV, Sin Nombre, and Anthrax. Over the last two years, AMDI and SANDIA have significantly improved the sensitivity and detection capability of the SAW biosensor and have also developed a modular hand-held, portable platform called the Optikus, which uses CD microfluidics and handheld instrumentation to automate all sample preparation, reagent introduction, sample delivery, and measurement for a number of different assay targets. We propose to use this platform for the development of a rapid (<30 minutes), point-of-care diagnostic test for detection of COVID-19 from nasal swab samples.

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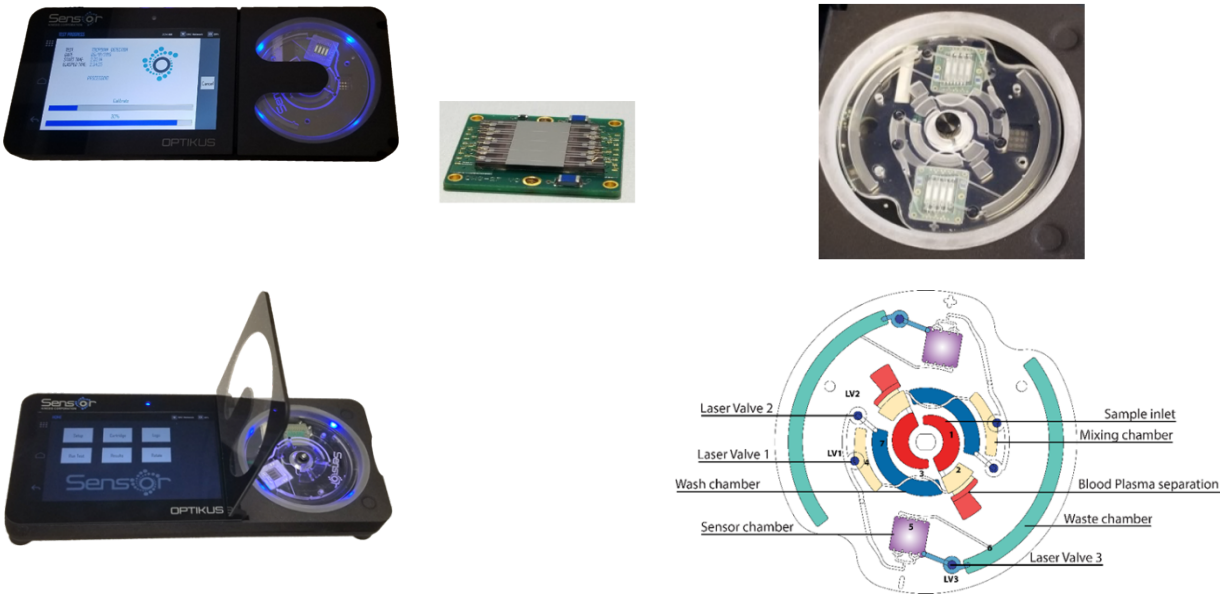
ACRONYMS AND DEFINITIONS

Abbreviation	Definition
CD	Compact disk
SAW	Surface acoustic wave
LAMP	Loop-mediated isothermal amplification
RNA	Ribonucleic acid
DNA	Deoxynucleic acid
NC	Negative control
PC	Positive control
MOSFET	Metal oxide silicon field effect transistor
PWM	Pulse width modulation

1. INTRODUCTION

Autonomous Medical Devices Incorporated (AMDI) is collaborating with Dr. Darren Branch at Sandia National Laboratories through a CRADA to develop a rapid handheld diagnostic screening device that utilizes surface acoustic wave sensors (SAW) developed at Sandia. AMDI has developed a modular, portable platform called the Optikus utilizing the SAW sensor as the core technology, which utilizes CD microfluidics to automate all sample preparation, reagent introduction, sample delivery, cleanup and analysis. The Optikus is unique in its ability to rapidly accommodate new tests via changes in the microfluidic disk design. AMDI has successfully detected viruses on the SAW using an ELISA-like immunoassay approach and is currently developing and testing an immunoassay for COVID-19 in collaboration with the University of New Mexico (UNM, BSL 3 facilities) for use on the Optikus. Ideal antibody targets for COVID-19 have already been identified by the CDC and are currently being delivered to AMDI for use in testing. At present, the SAW performs direct detection of virus and other targets via an immunological assay, thus minimizing the need for reagents. Even though the LOD is ~ 10 pg/mL which is close to the required LOD for Troponin assays, we seek to reduce the LOD to a ~ 100 copies of RNA/DNA of SARS-CoV-2 using the loop-mediated isothermal amplification (LAMP) assay.



a) **b)** **c)**

Figure 1-1. AMDI Optikus Biosensor Platform and detection method. a) The Handheld AMDI Optikus is a portable, point-of-care instrument, b) The SAW biosensor detects low concentrations (pg/mL) of analyte in multiple body fluids, and c) The microfluidic disc

automates all sample preparation steps and houses the SAW biosensor for detection.

The Optikus (Figure 1-1) contains thermoelectric heaters and coolers for performing DNA amplification via microfluidics for detection on the SAW. AMDI's microfluidic and engineering teams, in collaboration with our team at Sandia, are near completion of a device capable of performing PCR (both thermocycling and isothermal) and are performing validation of the microfluidic DNA amplification. AMDI can utilize this proof of concept of DNA amplification and detection, and rapidly develop a reverse transcriptase (RT) step upstream from the PCR microfluidic module to allow for on-disk RT-PCR to be performed by the Optikus. AMDI is currently in possession of the RT-PCR primers distributed by the CDC and is in the process of validating its RNA-DNA amplification step on disk.

The gold standard for detection of respiratory viruses, including SARS-CoV-2, are nucleic acid amplification tests, such as reverse transcriptase PCR (RT-PCR) and isothermal amplification-based diagnostics [1]. The Optikus instrument contains thermoelectric heating and cooling elements, and a temperature control mechanism, for performing RNA and DNA amplification using either thermocycling or isothermal amplification directly on the microfluidic disc. Over the past several months, AMDI's assay development and engineering teams (at AMDI Menlo Park and AMDI LA), in collaboration with the team at Sandia National Laboratories, have been working on using the SAW sensor to detect amplified DNA from viral targets. At the same

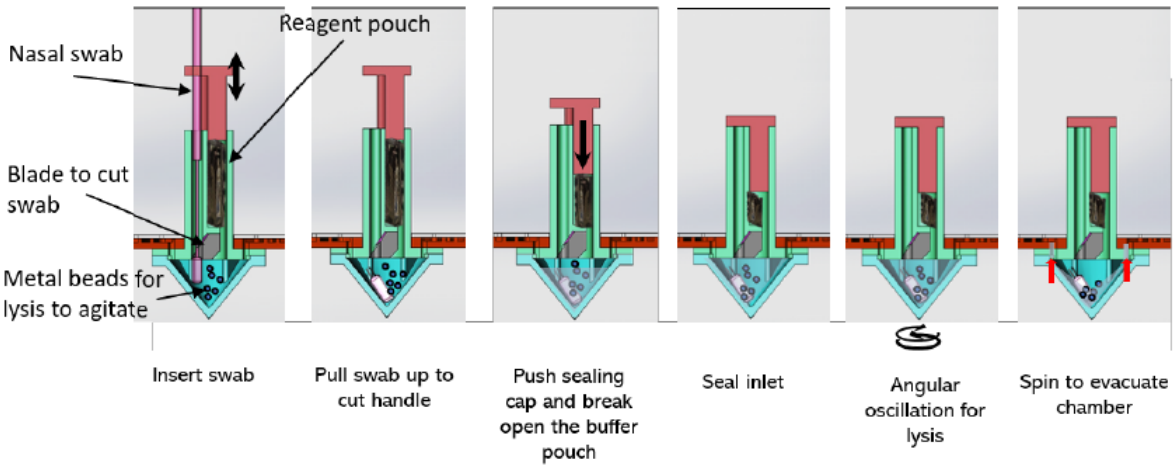


Figure 1-2. Schematic design for sample interface system that extracts and processes viral RNA from a nasal swab sample.

time, the microfluidic team at AMDI has been developing a microfluidic system for extraction and purification of viral RNA from a nasal swab sample, see scheme in Figure 1-2.

We aim to re-direct the current work for detection of COVID-19 by utilizing the appropriate primers and probes available for SARS-CoV-2 using the commercially available loop-mediated isothermal amplification (LAMP) assay. The milestones for this study were:

Milestone 1: Achieve successful amplification and detection of synthetic SARS-CoV-2 RNA/DNA using the SAW sensor (Sandia).

Milestone 2: Optimization of the Optikus instrument to perform efficient temperature control of a microfluidic chamber with 50 uL sample volume. (performed at AMDI)

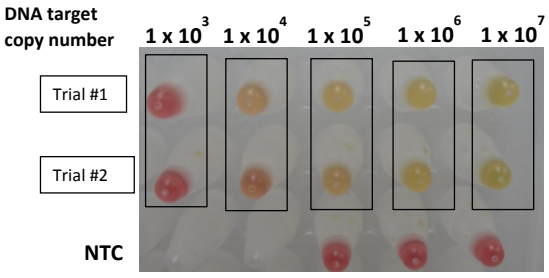
Milestone 3: Development of a microfluidic disc for SARS-CoV-2 RNA extraction/purification from a nasal swab sample. (performed at AMDI)

2. DETECTION APPROACH FOR SARS-COV-2

2.1. LAMP RT-qPCR

To validate the detection of SARS-CoV-2 DNA for integration into the Optikus platform, the loop-mediated isothermal amplification (LAMP) assay was implemented using a commercial colorimetric assay. The LAMP assay was performed using five different sets of primers (Appendix A) on an Applied Biosystems ProFlex PCR system for validation (Figure 2-1).

To evaluate detection efficiency, the SARS-CoV-2 DNA amplicon was serially diluted from 10 million copies down to 1000 copies (per 25 µL reaction) at 10-fold intervals selecting the 1D21 LAMP primer set (Appendix A). These serial dilutions were evaluated with real time measurements using LAMP with NEB's Warm Start Colorimetric LAMP 2X Master Mix (cat# M1800) at manufacturer's recommended starting reagent concentrations, qPCR fluorescence detection with 1 uM SYTO -9 double stranded DNA binding dye (Thermo Fisher S34854) ([2]), LAMP primers



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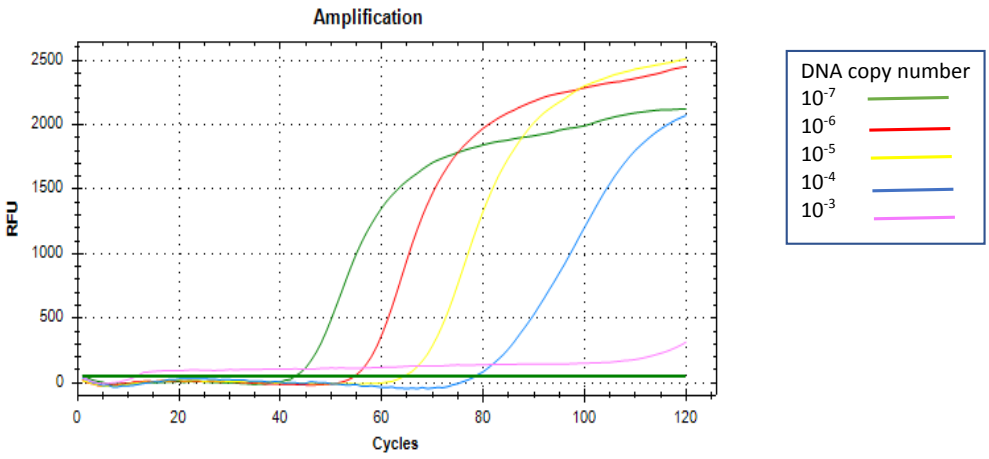


b)

Figure 2-1. RT-qPCR Results. Image of RT-qPCR color changes for DNA dilutions ranging from 10^3 to 10^6 copies; n=2 (Trials 1 and 2). Pink indicates a negative result and yellow indicates a positive result. Trials 1 and 2 and b) Primer set (ID21) trial 2 was used in the following graph amplifying SARS-CoV-2 DNA fragment concentrations ranging from 10 million to 1000 copies. Each cycle represented 15 seconds for a total runtime of 48 minutes.

designed by Primer Explorer software, LAMP primers synthesized by Integrated DNA Technologies (IDT), thermal block qPCR reaction on BioRad CFX Connect RT-PCR system for 120 cycles with 15 seconds image captures each cycle (total ~48 minutes) at 65°C. SARS CoV-2 DNA amplicon was detected as low as 1 x 10^3 copies of DNA using RT-qPCR (Figure 2-1). The yellow color change is indicative of a positive detection and a negative result is represented by pink color.

2.2. LAMP Isothermal PCR

The SARS-CoV-2 DNA amplicon concentrations tested were 10 x 10^7 and 10 x 10^{10} total copies per 50 µL reaction. The LAMP assay was run using New England Biolab's (NEB's) Warm Start Colorimetric LAMP 2X Master Mix (cat# M1800) at manufactures recommended starting reagent concentrations and incubated at 65 C for 30 minutes.

ID21 and ID07 LAMP primers detected SARS CoV-2 DNA amplicon at 1 x 10^7 and 1 x 10^{10} copies. (Figure 2-2). The yellow color change is indicative of a positive detection and a negative result is represented by pink color (negative control. The target DNA was detected within ~ 11 minutes at 1 x10^7 copies of DNA.

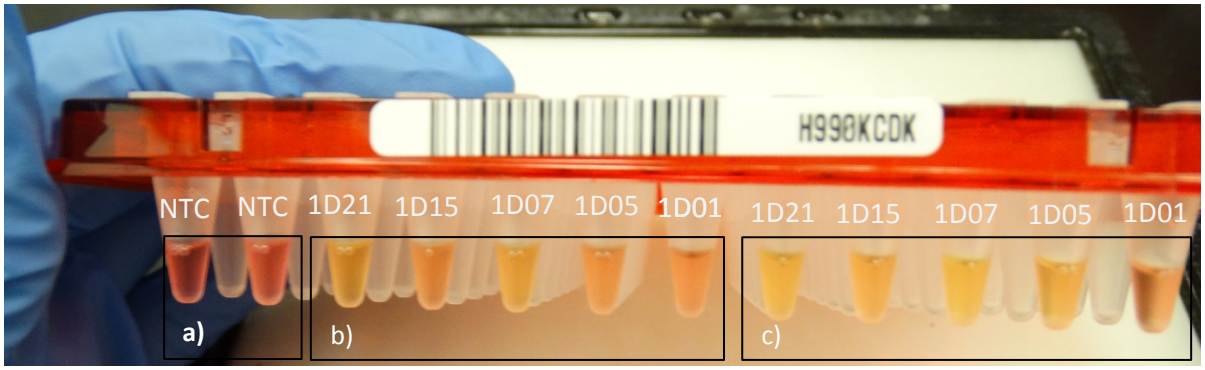


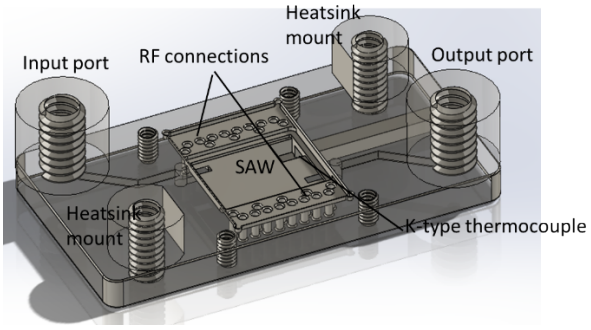
Figure 2-2. Detected SARS-CoV-2 DNA target using LAMP system for primers a) negative control, b) 10^7 copies, and b) 10^{10} copies. The yellow color change is indicative of a positive detection and a negative result is represented by pink color.

2.3. Microfluidic Heater with Embedded SAW Sensor

To implement LAMP directly on the surface of the SAW, a microfluidic cell was printed using the MIICraft 3D printer, Model 125. The MIICraft printer has a XY resolution of 30 um and z resolution of 5 um. The printed microfluidic cell was fabricated using BV-007 clear resin, allowing embedded microfluidics channels to be printed directly into the structure Figure 2-3.The SAW sensor was placed into the recess as shown in Figure 2-3. Fluidic connections were made via the large pillars using #6-32 flat bottom Upchurch connectors. The diagonal set of pillars allowed a thermofoil heating element from Minco (HAP6944) and Peltier cooler from Thor Labs to be placed directly in contact with the SAW sensor.

2.3.1. *Miniaturized Heater Circuit Operation for LAMP*

The PWM output of the microcontroller was connected to the JP2 driving a bipolar NPN and PNP transistors Figure 2-3. Managing high-frequency signals on a MOSFET transistor requires extra care in the design due to the switching from the PWM output of the microcontroller. Under transient conditions, the gate current will be non-zero since the gate capacitance will charge or discharge, which requires current. The larger the gate current, the faster the gate voltage changes and the faster the device switches. Once the switch transition is completed, then the gate current approaches zero which is mostly due to the leakage current. For low frequency switching (PWM), the rms gate current will be small, where higher switching frequencies will increase the rms current. The Zener diode (D2, 15V) was used to limit Gate voltage (Vgs) of the MOSFET while it is open because the power supply voltage can be much higher than maximum value of MOSFET Vgs voltage. Since desired operating temperature was reach within 50 seconds, additional voltage was not required. The diode FR107 removes the power from the heater when the MOSFET is closed. The temperature of the buffer on the SAW was measured using an Omega micro-K-type thermocouple (5TC-TT-K-3-36) connected to a MAX31855 digital thermometer connected to the microcontroller's SPI pins. A custom C-code controller code was written to perform a timed heating process. The C-code implemented a software-based PID controller using an Amtel microcontroller. The temperature stability of the completed system was 0.1 °C. The entire system can be miniaturized further for



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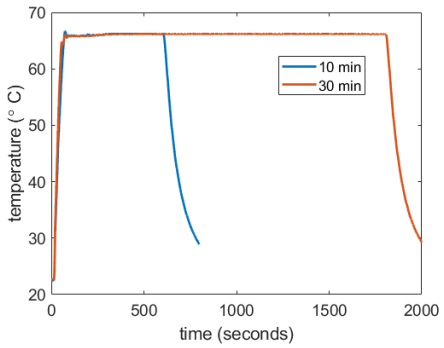

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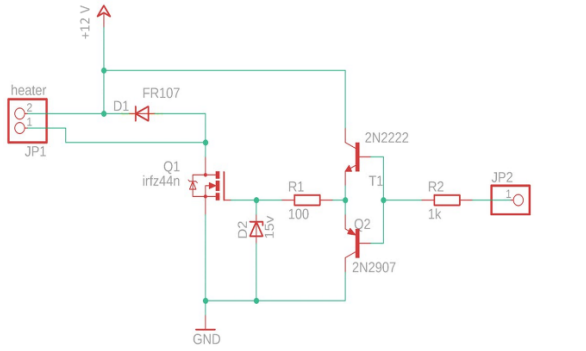

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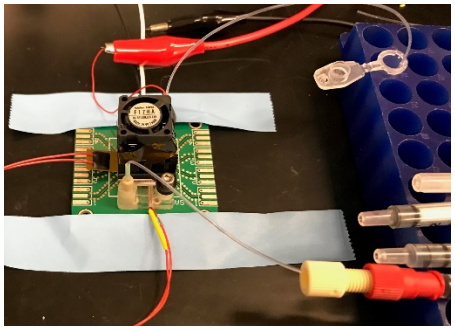


d)

Figure 2-3. 3D printed microfluidic sensor assembly, thermal behavior for the LAMP assay, and heater circuit operation for detecting SARS-CoV-2. a) The microfluidic cell has a volume of 50 uL, b) thermal response for 10 min and 30 min run. The time to reach 65 °C was ~50 seconds. The time to cooldown to 30 °C was 185 seconds, c) heater driver circuit connected to microcontroller. The rail voltage was +12V, d) completed assembly with passive fan cooling after heater is switched off.

integration with the Optikus platform, which was the primary goal of maintaining operational simplicity, while achieving the required performance.

It was determined through several heating trials that the Peltier cooler was not required since the LAMP reaction terminates below ~50 °C, which simplified the hardware and power requirements tremendously. In addition, the isothermal reaction terminates quickly due to the low thermal mass of the system. By reducing the combined thermal mass of the SAW and buffer solution, the temperature (i.e. 65 °C) to initiate LAMP occurred within 50 seconds Figure 2-3. Given further refinements, the LAMP temperature could be reached within 10 seconds.

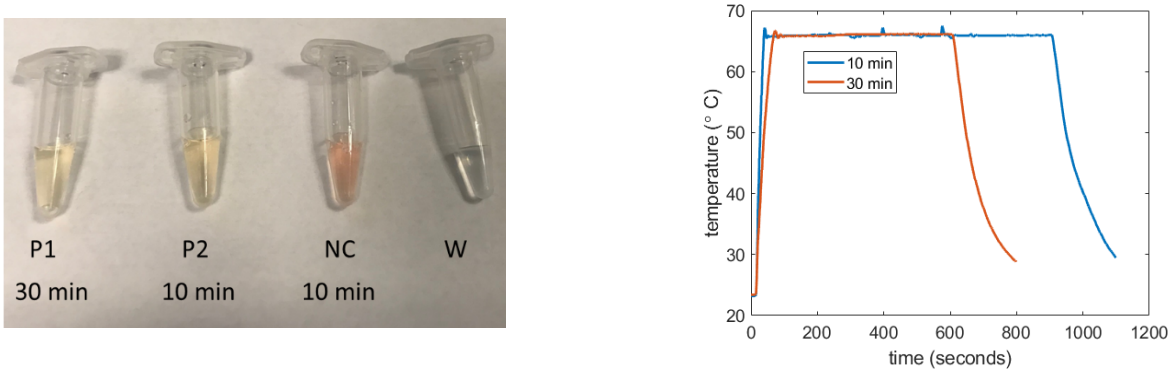
2.4. LAMP Assay on SNL Heater Platform

LAMP reaction was set-up following NEB's Warm Start Colorimetric LAMP protocol at an increased volume of 200 µL to account for dead volume in the 20mil microfluidic tubing. SARS-CoV-2 DNA amplicon was tested at 1 x 10^7 and 1 x 10^{10} copies. A syringe was used to introduce reaction mixture into platform and held from 8 to 30 minutes at 65°C (Figure 2-3).

2.4.1. Lamp Results using Custom Thermofoil Heater

The first trial used the recommended time of 30 minutes at 65°C. However, it was observed that 65°C for 30 min appeared excessive. It was determined that a sample containing 1 x 10^7 copies of SARS-CoV-2 produced a positive result as early as 10 minutes. Further experiments are required to investigate the DNA concentration and time dependencies using LAMP. When reducing the time from 30 min to 10 min, the positive control (P2) turned a light yellow.

Reaction with target SARS-CoV-2 DNA produced a change in color (light yellow) after only 10 minutes incubation at 65°C at 1.0 x 10^{10} copies. Reaction with no target DNA had no color change (light pink) with a 10-minute incubation at 65°C. In Figure 2-4a, NC (negative control) did not contain SARS-CoV-2 DNA, where P1 and P2 contained SARS-CoV-2 DNA. Although more optimization is required to determine the amplification time dependence on the sample concentration, the approach appears to yield results within 10 minutes.



a) b)

Figure 2-4. LAMP Amplification of SARS-CoV-2 DNA. a) shows that 30 min was excessive for the positive control (P1), where a shorter run for 10 min amplified the target (P2). (W) was a water sample for visual comparison, NC did not contain SARS-CoV-2 DNA, b) the heating cycle for the 10 min and 30 min runs.

3. CONCLUSIONS

We have made significant progress toward the development of an integrated nucleic acid amplification system for AMDI's Optikus handheld diagnostic device. A set of loop-mediated isothermal amplification (LAMP) primers for SARS-CoV-2 was designed and then experimental demonstrated to amplify targets directly on a surface acoustic wave (SAW) sensor. First, we demonstrated successfully amplification and detection of SARS2 where DNA amplicon was detected as low as 1 x 10^3 copies of DNA using RT-qPCR. Second, we designed and verified amplification and detection of SARS-CoV-2 DNA using the primers ID21 and ID07 at 1 x 10^7 and 1 x 10^{10} copies. The target DNA was detected within ~ 11 minutes at 1 x10^7 copies of DNA. Next, custom hardware was designed to incorporate the LAMP assay into a liquid well above the SAW to amplify and then perform detection in real-time. We prove feasibility of amplifying SARS-CoV-2 DNA using LAMP above the SAW sensor, producing a positive result as early as 10 minutes. Moreover, we determined through several heating trials that the Peltier-based cooling was not required since the LAMP reaction terminates below ~50 °C, which simplifies the hardware and power requirements tremendously. We are in the process of performing real-time detection of SARS-CoV-2 directly on the SAW during the LAMP amplification process. Given the low LOD of the SAW diagnostic platform, it is expected that the time-to-answer will be significantly faster than optical detection methods.

REFERENCES

[1] J. D. Fox, "Nucleic acid amplication tests for detection of respiratory viruses," *Journal of Clinical Virology,* vol. 40, pp. S15-S23, 2007.

[2] Y. Zhang *et al.*, "Rapid Molecular Detection of SARS-CoV-2 (COVID-19) Virus RNA Using Colorimetric LAMP," *medRxiv,* p. 2020.02.26.20028373, 2020.

APPENDIX A. LAMP PRIMERS

LAMP primers used in this study for amplifying SARS-CoV-2

Name	Sequence
F3 ID7	TGGCTACTACCGAAGAGCT
B3 ID7	TGCAGCATTGTTAGCAGGAT
FIP ID7	TCTGGCCCAGTTCCTAGGTAGTGACGAATTCGTGGTGGTGA
BIP ID7	AGACGGCATCATATGGGTTGCAGCGGGTGCCAATGTGATC
F3 ID15	AGATCACATTGGCACCCG
B3 ID15	CCATTGCCAGCCATTCTAGC
FIP ID15	TGCTCCCTTCTGCGTAGAAGCCAATGCTGCAATCGTGCTAC
BIP ID15	GGCGGCAGTCAAGCCTCTTCCCTACTGCTGCCTGGAGTT
F3 ID21	GCCAAAAGGCTTCTACGCA
B3 ID21	TTGCTCTCAAGCTGGTTCAA
FIP ID21	TCCCCTACTGCTGCCTGGAGGCAGTCAAGCCTCTTCTCG
BIP ID21	TCTCCTGCTAGAATGGCTGGCATCTGTCAAGCAGCAGCAAAG
F3 ID1	TGGACCCCAAAATCAGCG
B3 ID1	GCCTTGTCCTCGAGGGAAT
FIP ID1	CCACTGCGTTCTCCATTCTGGTAAATGCACCCCGCATTACG
BIP ID1	CGCGATCAAAACAACGTCGGCCCTTGCCATGTTGAGTGAGA
F3 ID5	CCAGAATGGAGAACGCAGTG
B3 ID5	CCGTCACCACCACGAATT
FIP ID5	AGCGGTGAACCAAGACGCAGGGCGCGATCAAAACAACG
BIP ID5	AATTCCCTCGAGGACAAGGCGAGCTCTTCGGTAGTAGCCAA

Figure A-1. LAMP Primer Sets for SARS-CoV-2

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