One Hour Covid Test using LAMP
Round 5 Grant Application, March 2021

proj-nucleic-acid-amplification, # proj-jogl-fitz
Google folder with supplementary materials for the project: https://drive.google.com/open?id=1ahzaeP2Z8Kh3TW0qkF1ijUBgGAvR7yZX. See also https://www.facebook.com/onehourcovidtest.
See Documents section of this project for more supplementary materials including protocols, literature and budgets.
Please note that there have been three project updates: one in May 2020; one in October 2020; and one in March 2021. Stand-alone summaries can be found in the Documents tab on the project page.

1.0 Introduction

1.1 Problem and Background (200 words max)
There is a shortage of SARS-CoV-2 testing throughout the world. Test protocols that diagnose infections or detect environmental contamination by the virus are not yet simple, affordable or widely accessible enough. Currently, most diagnostic testing is accomplished by Reverse Transcription–quantitative Polymerase Chain Reaction (RT-qPCR). RT-qPCR is expensive, laborious and not scalable or accessible to many people living in low-resource settings. Over-reliance on this complicated technique of molecular amplification limits people in many regions of the globe from knowing whether the virus is present and hinders public health efforts. Loop-mediated Isothermal Amplification (LAMP) is an affordable, robust, simple and open detection method which requires only a single incubation temperature and can be done in a cup of hot water.

When this project began in 2020, New England Biolabs (NEB) had recently published preliminary results on a simple SARS-CoV-2 test based on RT-LAMP, a molecular detection method that allows identification of specific nucleic acids in samples with colorimetric visualization of results in 30 minutes or less. We set out to determine If this method could be optimized and compared favorably to the RT-qPCR test so that a single-tube nucleic acid amplification method could offer rapid, accurate, and cost-effective detection of SARS-CoV-2 that could be deployed anywhere.

Over the past year, we have done extensive testing and now have an optimized pipeline that includes safe sample collection using an oral swab with a buffer, a rapid method

for purifying and concentrating the viral RNA and a sensitive detection method for SARS-CoV-2 using LAMP.

1.2 Solution summary in simple terms (150 words max)

We have developed a simple test for SARS-CoV-2, the causal agent of COVID-19. The test is safe, sensitive and does not require expensive lab equipment. Results can be obtained within one hour of sample collection and can be read via the naked eye as either pink (negative) or yellow (positive).

1.3 Solution summary in technical terms (200 words max)

We optimized the NEB LAMP test protocol by varying the sample collection method, purification and concentration of RNA, primer sequences and ratios, magnesium concentration, sample buffers, and incubation temperature and time. Furthermore, we tested the ruggedness of the method through an interlab study across three labs in the U.S. (West Chicago, IL; Brooklyn, New York; and Atlanta, GA.). We now want to move towards further clinical validation of our test and to obtaining CLIA approval so we can begin scaleable testing of patients.

1.4 State of advancement of the project (100 words max)

We have successfully optimized the One Hour COVID-19 Test using LAMP. We have been able to detect both positive and negative samples for the SARS-CoV-2 virus from oral swabs of both symptomatic and asymptomatic individuals. Some of these results have been compared with results of rapid antigen tests and RT-qPCR tests, and results are consistent for RT-qPCR tests, which are the gold standard. Furthermore, we were able to detect a positive sample from an asymptomatic individual that was negative with the rapid antigen test but positive with the RT-qPCR test, which seems to suggest preliminarily that the One Hour COVID-19 Test using LAMP is more sensitive than the rapid antigen test. We have submitted a publication to the Journal of Biomolecular Techniques (JBT) describing our test and preliminary results.

We would now like to apply for Clinical Laboratory Improvement Amendments (CLIA) approval so that we can begin scaled community testing. CLIA approval requires a laboratory director with specific qualifications. We have identified such a laboratory director. In addition, CLIA approved labs must come into compliance with standard operating procedures (SOPs) for clinical testing and with specific requirements for the physical laboratory space. Related to this, we need to set up a secure database for patient information.

We would also like to further run clinical validation testing of our One Hour Covid Test directly alongside RT-qPCR testing. A Methodist hospital system in Indiana is willing to collect samples of suspected COVID-19 positive patients who come to their Emergency Department.

1.5 Project Timeline

- Weeks 1 & 2 - Apply for CLIA certification (fill out paperwork and obtain signatures of laboratory owner and laboratory director). Review the requirements for the physical laboratory space and SOPs. Order a laboratory sink and have it installed in the laboratory. Send oral swab collection kits to the Methodist hospital system in Indiana for clinical validation. Order testing reagents for downstream testing of oral swab samples.
- Weeks 3 & 4 - Set up a secure database for patient registration information; to make appointments; to link patients with their sample collection tubes; and to report results.
- Weeks 5 & 6 - Perform downstream testing of oral swab samples from the Methodist hospital system in Indiana.
- Weeks 7 & 8 - Begin scaled testing of patients.

2.0 Project Implementation

2.1 Solution (1000 words max)

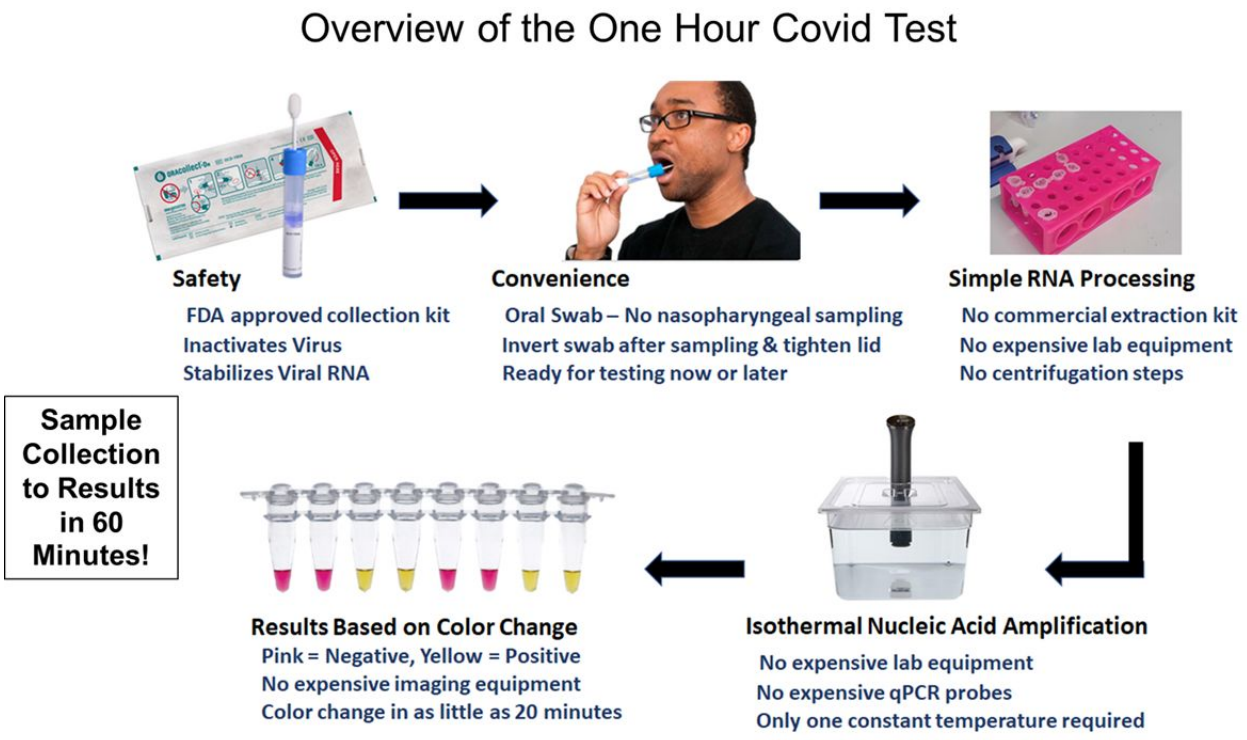
We now have an optimized testing pipeline that includes safe sample collection, a rapid method for purifying and concentrating the viral RNA and a sensitive detection method for SARS-CoV-2 using LAMP. The next steps include 1) further clinical validation of the One Hour Covid Test; 2) applying for CLIA approval so we can begin scalable testing of patients; and 3) set-up of a secure infrastructure for patient information.

2.2 Methodology (500 words max)

1. **Further clinical validation of the One Hour Covid Test**. We would like to further run clinical validation testing of our One Hour Covid Test directly alongside RT-qPCR testing. A Methodist hospital system in Indiana is willing to collect samples of suspected COVID-19 positive patients who come to their Emergency Department. The hospital system will obtain the required patient consent as required via the Institutional Review Board. Based on the hospital system's reported positivity rate of 11% for patients who visit their Emergency Department with COVID-19 symptoms, we will initially send 200 oral swab collection kits for validation.

2. **Obtainment of CLIA approval.** We would now like to apply for Clinical Laboratory Improvement Amendments (CLIA) approval so that we can begin scaled community testing. CLIA approval requires a laboratory director with specific qualifications. We have identified such a laboratory director. In addition, CLIA approved labs must come into compliance with standard operating procedures (SOPs) for clinical testing and with specific requirements for the physical laboratory space. CLIA approval varies per state in the U.S. We propose to begin with CLIA approval at Blossom Bio Labs, Inc. in West Chicago, IL, at a physical laboratory location and possibly mobile lab extensions.
3. **Setting up a secure database.** We must set up a secure database for patient registration information; to make appointments; to link patients with their sample collection tubes; and to report results.

Note: Methodology of the optimized testing procedure can be found in previous documentation, but the purpose of this grant proposal is for validation of the optimized test and obtaining CLIA approval. Below is a schematic overview of the test.



2.3 Results/Expected results (500 words max)

1. **Further clinical validation of the One Hour Covid Test**. The Methodist hospital system of Indiana has reported a current SARS-CoV-2 positive testing rate of 11%

for patients who visit their Emergency Department with COVID-19 symptoms. Preliminary testing with the One Hour COVID Test alongside the RT-qPCR test were very promising. Therefore, we expect to get good validation results for both positive and negative samples upon initially testing 200 patient samples using direct comparison of both methods. The hospital system will perform in-house testing of samples using RT-qPCR, and they will send the corresponding patient samples collected for the One Hour Covid Test to Blossom Bio Labs, Inc. in West Chicago, IL, for downstream testing there.

2. **Obtainment of CLIA approval.** Blossom Bio Labs, Inc. in a Biosafety Level 2 (BSL-2) molecular biology lab, and our identified lab director has all of the qualifications necessary for CLIA approval. We will thoroughly review the physical lab requirements and SOPs. Therefore, we expect to be granted CLIA approval.
3. **Setting up a secure database.** We have identified Orca Scan (https://orcascan.com) as a provider for our secure database. Orca Scan is endorsed by major corporations, including Amazon, UPS and NASA. In addition, Orca Scan has excellent customer service, so we are confident this company can provide the secure database infrastructure required.

3.0 Safety, quality assurance and regulation

3.1 What steps have you taken to ensure your solution's safety? How advanced are you in this process (if applicable)? Please check the Biosafety and Biosecurity guideline of OpenCovid19

Sarah Ware and Ellen Jorgensen both serve on the Biosafety and Biosecurity Advisory Board of JOGL. In addition, both Sarah and Ellen are founders of independent laboratories, and Sarah is a biology professor who teaches laboratory safety at the university level.

Please note that a key decision was to use the DNA Genotek OR-100 swab kit to collect samples because the buffer in the tube inactivates any virus present and renders the samples very safe to handle at the first step of the protocol. Positive controls for testing are either purified viral RNA or heat-inactivated viral cultures, both of which are non-infectious. Because of these safety factors, this testing can be done in a BSL-1 lab, but Blossom Bio Labs, Inc. is BSL-2. All personnel performing the experiments have received appropriate safety training through their host laboratories to handle the minimal-risk chemical reagents involved in this protocol and use good laboratory hygiene practices.

3.2 Have you planned the conduct of your manufacturing process that ensures quality, what are the steps you have taken? How advanced are you in this (if applicable)?
All three parts of our testing protocol use commercially-available components provided by reputable established companies. Sample collection is with the OR-100 oral swab kit from DNA Genotek. Viral purification and concentration is with PrepIT.q2a also from DNA Genotek. RT-LAMP nucleic acid amplification is with WarmStart® Colorimetric LAMP 2X Master Mix from New England Biolabs. Primers are from IDT, and guanidine hydrochloride can be ordered from any reputable scientific company that supplies lab-grade chemicals.

3.3 Will you need assistance with the regulation system? If not, which regulatory system do you plan on using to distribute the product? Please elaborate (please see: Regulatory-Strategies) (if applicable)
A company called IVDiagnostics has offered to handle the regulatory process for applying for FDA-EUA for the One Hour COVID Test.

3.4 Have you talked to medical staff about the feasibility of your project? What did they say?
A Methodist hospital system in Indiana has agreed to participate in validation of the One Hour Covid Test. They have indicated that they are interested in a quicker, simpler testing protocol than RT-qPCR for their Emergency Department patients who are suspected of having COVID-19. They are also interested in such a testing protocol for general admissions so that they don't need to send samples to a third party for testing that can take one or more days to receive results. Furthermore, other physicians have expressed great interest in a test with an oral swab because it is painless and less invasive than the deep nasal swab.

3.5 Have you planned the testing, verification and validation of your solution? How advanced are you? (if applicable)
Yes. See above.

4.0 Impact, issues and risks

4.1 What impact do you feel your project could have? (100 words max)
There is a shortage of SARS-CoV-2 testing throughout the world. New test methods are urgently needed that are simple, affordable and widely accessible. Over-reliance on the

complicated technique of RT-qPCR limits people in many regions of the globe from knowing whether the virus is present and hinders public health efforts. We have developed a simple RT-LAMP method that compares favorably to the current RT-qPCR test. It offers rapid, accurate, and cost-effective detection of SARS-CoV-2 for increased testing capabilities.

4.2 What do you think would make your project a success?(100 words max)
Funding of this grant proposal will make this project a success. For clinical validation, funding is needed to purchase oral swab collection kits and reagents for downstream testing. For CLIA approval, funding is needed to bring the lab into final compliance with physical structures such as a sink.

4.3 Please list the known issues, potential risks, grey-areas, etc in your project
- Some of the reagents are available only from single sources. However, we do have verbal assurances from both DNA Genotek and New England Biolabs that they do not foresee any shortages.
- Although preliminary results show good correlation of results using the One Hour Covid Test and RT-qPCR, we may find a greater discrepancy with a larger clinical sample.
- The test may not be easily shippable to low-resource areas despite the reports that these types of reagents can be freeze dried.

5.0 Originality

5.1 What other projects on JOGL are like yours? Search for them and Link them!
The following projects on JOGL use LAMP methodology: Do-It-Together SARS-CoV-2 Detective (https://app.jogl.io/project/18) andALERT: Accessible LAMP-Enabled Rapid Test (https://app.jogl.io/project/187).

5.2 Is this an innovative project? What makes this project different if it's unique on JOGL?
Yes, our product is innovative. We fully support the above projects that use LAMP technology. However, both of those projects use more DIY components. We have chosen to use commercially-available components for safety, consistency and quality control.

5.3 Is there already an open source version of this project?

See above.

6.0 Team experience

6.1 Please cite your team members and their roles in the project.
(if applicable) If the project involves several locations or labs, list them too.

Ellen Jorgensen has a Ph.D. in molecular biology and has worked in the biotechnology industry for over 30 years, including in FDA-regulated assay development. She has founded two community labs, Genspace and Biotech Without Borders. Her thesis work was on Newcastle Disease virus, a negative strand RNA paramyxovirus. She was a founding staff member of the Sabin Vaccine Foundation, and currently is the Chief Science Officer at Aanika Biosciences (Brooklyn, NY), a company offering environmental testing for SARS-CoV-2. https://www.linkedin.com/in/ellenjorgensen

Chris Monaco earned his Masters degree in bioinformatics at the Georgia Institute of Technology. He is currently a microbiologist in the CDC's Division of Scientific Resources' Biotechnology Core Facility Branch (BCFB) in Atlanta, GA. https://www.linkedin.com/in/cmonaco

Sarah Ware has a doctorate degree and has 20 years of research experience as a geneticist/molecular biologist. She is the founder of three independent labs in the Chicagoland area and is currently involved in BioBlaze Community Bio Lab and Blossom Bio Labs, Inc., both in West Chicago, IL. Sarah also teaches biology and humanities at the university level. https://www.linkedin.com/in/sarahblossomware

The above members wish to also acknowledge other members of the # proj-nucleic-acid-amplification group within JOGL

7.0 Funding and Costs

7.1 Please provide a costing of your project be as detailed as you can, all funding requests must be transparent and be for specific needs. The maximum grant is 2000 euros for new projects and 4000 euros for already established JOGL projects.

See also “Blossom Bio Labs Inc One Hour Covid Test Budget Round 5 Grant” in the Documents section of the project page.

1	**Blossom Bio Labs Inc One Hour Covid Test Budget Round 5 Grant**						
2	Item	Description	Quantity	Supplier	Price/Unit	Total Price USD	Total Price EUR
3	LAMP Primers (3 sets of 6)	3 LAMP primer sets, 100 nM each	3	IDT	$125.00	$375.00	$315.00
4	WarmStart® Colorimetric LAMP 2X Master Mix	cat. #M1800S	3	NEB	$213.00	$639.00	$536.76
5	OraCollect OR-100 Oral Swab Kit	oral swab plus buffer RNA collection kit	100	DNA Genotek	$9.95	$995.00	$835.80
6	Stainless Steal Sink for CLIA compliance	cat. #079-ES1181218R	1	Katom	$205.00	$205.00	$172.20
7	Sink Installation for CLIA compliance	labor	1	private contractor	$300.00	$300.00	$252.00
8	Preplt.Q2A	96 sample preps, cat. #PT-Q2A-96	3	DNA Genotek	$178.00	$534.00	$448.56
9	Orca Scan	1 year subscription for secure patient database, business plan	1	Orca Scan	$1,200.00	$1,200.00	$1,008.00
10	Total Without Shipping					$4,248.00	$3,568.32
11	10% Shipping					$424.80	$356.83
12						$4,672.80	$3,925.15
13							

7.2 How is your project being funded so far?
This project has received past funding via JOGL, Aanika Biosciences, BioBlaze Community Bio Lab, Blossom Bio Labs, Inc. and Isabella Zora.

7.3 How much funding do you need and how do you plan to use that funding?
We are requesting 3925 euros in funding for the Round 5 grant. This funding will be used to purchase oral swab kits and reagents for downstream testing for clinical validation and to obtain CLIA approval and a secure infrastructure to begin scalable testing of patients in the Chicago suburbs. The grant money for this round will be distributed to Blossom Bio Labs, Inc. in West Chicago, IL. See 7.1 for a detailed budget.

8.0 Achievement and Benefits of funding [only for projects already funded by JOGL]

We have successfully optimized the One Hour COVID-19 Test using LAMP. Past funding received via JOGL grants has culminated in a test with an optimized pipeline that includes safe sample collection using an oral swab with a buffer, a rapid method for purifying and concentrating the viral RNA and a sensitive detection method for SARS-CoV-2 using LAMP. We have been able to detect both positive and negative samples for the SARS-CoV-2 virus from oral swabs of both symptomatic and asymptomatic individuals. Some of these results have been compared with results of rapid antigen tests and RT-qPCR tests, and results are consistent for RT-qPCR tests, which are the gold standard. Furthermore, we were able to detect a positive sample from an asymptomatic individual that was negative with the rapid antigen test but

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