Bachelor of Science in Medicine Degree Program
End of Term Final Report

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Project Title: Street LAMP: loop-mediated isothermal amplification testing as an easily modifiable point of care rapid testing platform in at-risk populations

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Summary (250 words max single spaced):
The COVID-19 pandemic has shifted rapid testing for infectious disease into a cultural spotlight, and interest and demand for rapid testing has subsequently increased as a result. The ability to confirm diagnoses at point of care and immediately deploy contact tracing, prevention, and treatment has proven advantages, especially in at-risk and vulnerable communities. This report investigates loop-mediated isothermal amplification (LAMP) assays as a platform for rapid testing with the intention of using it at homeless shelters and drop-ins for unsheltered people. This platform would allow community-based testing programs to test individuals cheaply, rapidly, and accurately. By using a single reaction mixture and swapping in or out primers for different pathogens, these testing programs allow community-informed testing. We validated reverse transcriptase LAMP (RT-LAMP) with previously RT-PCR tested SARS-CoV-2 swab samples (N=200) and, by using primers for multiple gene targets, determined a sensitivity of 75.00% (95% CI 65.34-83.12%), and a specificity of 98.99% (95% CI 94.50-99.97%). Additionally, we determined a sensitivity of 95.24% (95% CI 68-99%) and a specificity of 100% (95% CI 80-100%) for the use of LAMP testing in swabs of syphilis lesions (N=40). Finally, Bartonella quintana pooled lice samples (N=27) showed a sensitivity of 76% (95% CI 50-93%) and a specificity of 100% (95% CI 69-100%). These early findings and the ability to quickly respond to population specific needs with a modular, modifiable testing platforms merit further validation of these tests in real-world conditions, where they could be assessed for ease of use, cost-benefit, patient acceptability, and health outcomes.

Student Signature

Primary Supervisor Signature

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Introduction & Background

People who experience unstable housing are at higher risk for an array of communicable diseases, including respiratory, gastrointestinal, sexually transmitted and blood-borne pathogens (1). They are also at a higher risk for poor health outcomes from COVID-19 infection, with significantly higher rates of hospitalization compared to the population at large (2). Early diagnosis of SARS-CoV-2 allows for isolation, cohorting, and treatments that can reduce risk of hospitalization.

People with insecure housing are also at a greater risk of contracting syphilis, caused by the spirochete *Treponema pallidum pallidum*. This is a growing infectious crisis with cases surging in the last decade in Canada and rising in the United States during the first year of the SARS-CoV-2 pandemic (3,4).

Infectious diseases carried by the human body louse (*Pediculosis corporis*), in particular *Bartonella quintana*, the causative agent of trench fever and culture negative bacterial endocarditis, are also important diagnostic targets in vulnerable insecurely housed populations. (5)

The increased burden of these infectious diseases in insecurely housed populations demands innovative and efficient interventions to improve health outcomes. Rapid testing allows us to quickly diagnose infectious diseases and immediately link them to care.

Loop-mediated isothermal amplification (LAMP) testing is a form of nucleic acid amplification test (NAAT) that relies on using primers to create self-generating stem-loop structures. These structures act as templates for further DNA amplification, allowing LAMP assays to create massive amounts of double-stranded DNA at a single amplification temperature (6). Because there is no need for thermocycling, results can be obtained using less complex machinery than with other NAAT (7). LAMP has been shown to be less sensitive but highly specific when compared to standard reverse transcription polymerase chain reaction (RT-PCR), all while remaining simpler and more affordable (7, 8). The ability to develop new LAMP primers facilitates quick adjustment for emerging infectious diseases testing in high-risk or otherwise vulnerable populations, and to modify the test to meet specific epidemiology of infectious diseases within a given population.

LAMP testing can be supplemented with a co-occurring reverse transcriptase step (RT-LAMP) to act as a diagnostic test for RNA viruses such as SARS-CoV-2. Because of this, much research has been done on RT-LAMP testing with SARS-CoV-2 as a target (9, 10). Due to its high diagnostic accuracy, rapidity, low cost, easily adjustable nucleic acid targets, relatively low laboratory complexity, and ability to be read visually by colorimetric change, LAMP is also an excellent candidate for rapid testing in the community and at point-of-care for infectious diseases that are both common and devastating in vulnerable populations. (7, 8) This includes SARS-CoV-2 in populations who are insecurely sheltered and living in congregate settings, as it creates an environment in which it is difficult to create space to isolate oneself. (11)

Our research study seeks to show that LAMP has promising applications for the above infectious diseases in at-risk populations by measuring its diagnostic accuracy and showing that multiplexed LAMP testing in these populations is a viable model for an accurate, community-informed and controlled, and adaptable testing regime. This application of ‘Street LAMP’ testing

will allow for rapid test and response programs to be carried out in the most vulnerable populations.

Materials & Methods

Samples

Nasopharyngeal swabs were collected from walk-in or drive-through testing sites in Winnipeg, Manitoba, collected from symptomatic or asymptomatic individuals and stored in viral transport medium (N=200). They were previously tested by RT-PCR using E gene primers and purified RNA template on either the Cobas or Panther platforms at Cadham Provincial Laboratory. Samples with a fluorescence threshold between 10 and 37 were considered positive. SARS-CoV-2 positive saliva samples stored in collection buffer (DNA Genotek) and Ct values were generously provided by Dr. Zahra Kashi (Kashi Clinical Laboratories, Portland, OR), The samples were defrosted, centrifuged, and an aliquot incubated at 95°C for 5-10 minutes. Another aliquot of the same sample was mixed with an equal volume of QuickExtract reagent (Lucigen Inc) and incubated at 95°C for 5-10 minutes. Purified DNA from direct tissue sampling of syphilitic lesion swabs and louse pools were kindly provided by the National Microbiology Laboratory.

Primers

SARS-CoV-2 primers targeted two nucleocapsid regions (“N” and “N2”), the E gene (“E”) and the human RNaseP gene (“R”) (New England Biolabs) (Table 1). We used primers published by various groups for *Treponema pallidum pallidum* (“S2”), and *Bartonella quintana* (“B”) (Table 1). All primers were resuspended in molecular-grade water to a concentration of 100 µM (freezer stocks), and then 10 µM working stocks prepared by mixing 16 µl each FIP/BIP primers, 2 µl each F3/B3 primers and 8 µl each LB/LF primers, and 48 µl molecular-grade water (final volume 100 µl).

Controls

Positive controls for SARS-CoV-2 (Integrated DNA Technologies) and *T. pallidum* (American Type Culture Collection) were purchased, while positive controls for *B. quintana* were kindly provided by the National Microbiology Laboratory. Negative controls for SARS-CoV-2 were SARS-CoV-1 and MERS-CoV DNA (Integrated DNA Technologies). Negative controls for *B. quintana* included DNA for *Anaplasma* and *Borrelia* ssp. kindly provided by the National Microbiology Laboratory. All controls were used directly as template in LAMP assays or after serial dilution in molecular-grade water. “No template” controls using molecular-grade water were included with every experiment.

LAMP

LAMP and RT-LAMP reactions consisted of 10 µL of 2X Colorimetric WarmStart LAMP Master Mix (New England Biolabs), 1 µL of Green Fluorescent Dye (Lucigen), 2 µL of 10 µM primers, 6 µL of molecular grade water and 1µl template (final volume 20 µl). Reactions were incubated at a temperature of 60-65°C for 30-60 minutes on a CFX96 thermocycler (Applied Biosystems, Inc.), with relative fluorescence measured at 30 second intervals, followed by melt curve analysis for some samples. A sample that increased above an RFU threshold of 100 in less than 30 minutes (time to threshold 100, Tt_{100}) was considered to be positive. Sensitivity and specificity values compared to PCR/RT-PCR Ct values were calculated using an online calculator at MedCalc.

Results

Optimizing RT-LAMP for SARS-CoV-2 detection

Sensitivity and specificity of RT-LAMP compared to RT-PCR testing was calculated using 200 SARS-CoV-2 nasopharyngeal swab samples in VTM (Condition A: unextracted sample, N primers) (Figure 2). Samples that were RT-LAMP negative but RT-PCR positive (N= 33) were pre-treated with QuickExtract and amplified with N2, E and combined N2/E primers (Condition B), which improved sensitivity by detecting several additional samples with Ct values above 20. With the use of multiple primer sets, sensitivity was 75% (95% CI 65-83%) and specificity was 99% (95% CI 95-100%). Integrity of discrepant samples was also verified by amplifying human RNaseP (not shown).

Comparing LAMP and PCR in purified DNA extracts

For syphilis samples (N=40), LAMP using S2 primers detected 19/20 PCR-positive (sensitivity 95%) and 0/20 PCR-negative samples (specificity 100%) (Figure 3.). A second freeze-thaw of the DNA resulted in only 17/20 PCR-positive being detected (sensitivity 77%) (not shown). For louse pools (N=27), 13/17 PCR-positive samples and 0/10 PCR-negative samples were also LAMP-positive (sensitivity 76%, specificity 100%) (Figure 4).

Quantitative LAMP/RT-LAMP

RT-LAMP Tt_{100} was compared to RT-PCR Ct values in 14/33 samples that were re-tested with multiple primer sets. Only samples that were positive by both methods were included. A coefficient of determination was calculated, showing moderate correlation between Tt_{100} and Ct values (Figure 5). This association was only seen with simultaneous use of the primer sets and not when they were used independently.

Translation to field use

18 saliva samples all of which were SARS-CoV-2 positive were assessed with LAMP to determine its validity on this sample type. Without pre-treatment, only 3/18 underwent a clear colour change, however this increased to 18/18 when heat-treated with QuickExtract (not shown).

Discussion:

Our findings confirmed the highly specific nature of LAMP assays. For each target organism, regardless of which gene was used as a target and whether a single or multiple primer sets were used, the reaction was highly specific. Additionally, we confirmed the sensitivity of LAMP, with use of multiple primer sets within the same assay further increasing sensitivity.

Another pattern emerging regardless of target type was the tendency for LAMP sensitivity to drop with lower amounts of target present in the sample, as seen by comparing samples with high PCR Cts. This may make LAMP assays suboptimal diagnostic tests for pathogens or scenarios where low copy numbers is anticipated in a given sample. For some infections, this may make it more clinically relevant, eliminating detection of low-level contamination or colonization, or a decaying “tail” of prior infection, resulting in better prediction of active infections that may be clinically or public health relevant. For Covid-19, there is data to show that PCR positives with high Ct values may not be strongly correlated with infectious potential on viral culture assays (12). Further research targets are to reassess our 200 NP samples with multiple simultaneous primer sets to confirm the results seen with the use of E and N2 primers in our discrepant sample set. This could be further expanded to include other primer sets for gene targets not mentioned in this report.

Preliminary testing has begun on the use of LAMP testing in SARS-CoV-2 saliva samples. The initial results show high specificity when combined with QuickExtract pre-treatment of the samples, however only 18 samples, all of which were positive, have been assessed. Further experiments are necessary to determine sensitivity and specificity.

The universally highly specific results are promising for rapid and effective use of LAMP testing for rule-in testing at point-of-care. At a community institution, coupled with minimal equipment requirement and ease of reading the results, this could help determine need for treatment, as evidence to help convince a patient that treatment is needed, and whether infectious spread precautions should be undertaken especially in congregate living situations.

Additionally, the use of a single reaction mixture with primer sets that can be switched in or out allows tailoring of the assay rapidly in response to changing epidemiolocal dynamics. With many LAMP primer sets currently available in the online literature, it is easy to create tests for a wide array of pathogens. The potential of multiplexing, i.e testing for a modifiable set of pathogens allows the adaptation of a LAMP based panel based on the context where the test is used. This may be leveraged for the development of syndemic LAMP assays for specific high incidence settings (unstably housed populations, corrections facilities, or other specific community epidemiology).

To finish validation of our results, we must also determine their accuracy in real-world settings. This would involve collection, processing, and analysis of results on-site in community drop-ins and homeless shelters. Additionally, further work needs to be done to validate LAMP testing in different fluids. Shallow nasal swabs should be assessed for COVID-19 infection, as should plasma or capillary whole blood samples for testing for syphilis if no chancre exists. Testing of pooled and extracted body lice, capillary whole blood, or skin lesion swabs may all be necessary for *Bartonella quintana*, which has a poorly understood infection process.

Early work into determining LAMP use for detection of *T. pallidum pallidum* in syphilis positive patient serum and detection of *Acinetobacter baumani* in louse pools has already begun, with results to be published in a later paper.

Ease of use, satisfaction of healthcare workers and clients, and cost should all be evaluated at point of care as well. In the future, our lab plans to begin studies on patient cohorts in order to determine the above qualities. Additionally, we would like to develop and validate the use of LAMP testing for emerging global infectious diseases such as monkeypox, which is currently spreading primarily in vulnerable communities (13).

Figures:

Primer set	Primer name	Sequence (5' -> 3')	Citation
N	FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG	14
	BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT	
	LF	GGACTGAGATCTTTCATTTTACCGT	
	LB	ACTGAGGGAGCCTTGAATACA	
	F3	TGGCTACTACCGAAGAGCT	
	B3	TGCAGCATTGTTAGCAGGAT	
N2	FIP	TTC CGA AGA ACG CTG AAG CGG AAC TGA TTA CAA ACA TTG GCC	15
	BIP	CGC ATT GGC ATG GAA GTC ACA ATT TGA TGG CAC CTG TGT A	
	LF	GGG GGC AAA TTG TGC AAT TTG	
	LB	CTT CGG GAA CGT GGT TGA CC	
	F3	ACC AGG AAC TAA TCA GAC AAG	
	B3	GAC TTG ATC TTT GAA ATT TGG ATC T	
E	FIP	ACC ACG AAA GCA AGA AAA AGA AGT TCG TTT CGG AAG AGA CAG	15
	BIP	TTG CTA GTT ACA CTA GCC ATC CTT AGG TTT TAC AAG ACT CAC GT	
	LF	CGC TAT TAA CTA TTA ACG	
	LB	GCG CTT CGA TTG TGT GCG T	
	F3	TGA GTA CGA ACT TAT GTA CTC AT	
	B3	TTC AGA TTT TTA ACA CGA GAG T	
R	FIP	GTGTGACCCTGAAGACTCGGTTTTAGCCACTGACTCGGATC	16
	BIP	CCTCCGTGATATGGCTCTTCGTTTTTTTCTTACATGGCTCT GGTC	
	LF	ATGTGGATGGCTGAGTTGTT	
	LB	CATGCTGAGTACTGGACCTC	
	F3	TTGATGAGCTGGAGCCA	
	B3	CACCCTCAATGCAGAGTC	
S2	FIP	CAG CGC TTC TTT TAA GGA ATA GGT AGC ACA TCT TCT CCA CTG T	17
	BIP	CGC ACG AAG ATA GTG TGT GGA CAT GGT ACA TCG TCA CG	
	LF	CGA TAA ATA CCA TCA AGT GTG CCA AA	
	LB	GAA GAA AGA TGC ATT TTT TTC TCG TTC	
	F3	ATT GGT CCT AAG ACG GCT	
	B3	GCG GAA TAC AAC AGG AAT C	
B	FIP	ACAAACGCTGATATGCTTCTTTTTTATCAAGTCCTTGATTTA GAAGGT	18
	BIP	CATTCTCTTCATAGTGCAGTGGCTACGGACTGACATATGTGC	
	LF	CTTGTGACTTTGTGAAAAATCTG	
	LB	TCGGAATGGCCGAAAAATTTGG	
	F3	TGCTTTAGTCATTTGGTTTGTTGG	
	B3	TTAATTGAACTTTTGGTTGAGAGGG	

Figure 1. Primer sets used for RT-LAMP/LAMP assays.

		Condition A		Condition B		
		RT-LAMP Pos	RT-LAMP Neg	RT-LAMP Pos	RT-LAMP Neg	Total
RT-PCR Pos	All	68 (67%)	33 (33%)	75 (75%)	26 (25%)	101
	Ct <20	41 (100%)	-	NT	NT	41
	Ct 20-24.9	25 (83%)	5 (17%)	28 (93%)	2 (7%)	30
	Ct 25-29.9	2 (17%)	10 (83%)	4 (33%)	8 (67%)	12
	Ct => 30	-	18 (100%)	2 (6%)	16 (95%)	18
RT-PCR Neg		1 (1%)	98 (99%)	NT	NT	99
		Estimate	95% CI	Estimate	95% CI	
	Sensitivity	67%	57-76%	75%	65-83%	
	Specificity	99%	94-99%	99%	95-100%	

Figure 2. Sensitivity and specificity of RT-LAMP vs. RT-PCR for SARS-CoV-2 detection in archived nasopharyngeal swab in VTM. NT: Not tested - only discrepant samples were re-analyzed using Condition B.

T. pallidum pallidum testing	LAMP positive	LAMP negative
PCR positive	20	1
PCR negative	0	19
	Sensitivity	Specificity
Point estimate	95.24%	100.00%
95% CI	68 to 99%	80 to 100%

Figure 3. Sensitivity and specificity of LAMP vs. PCR for *Treponema pallidum pallidum* detection in syphilitic lesion swabs using S2 primers. A LAMP results of ≤ 30 minutes was considered positive. PCR results were externally determined at Cadham Provincial Laboratory and reported as either positive or negative. N = 40.

B. quintana testing	LAMP positive	LAMP negative
PCR positive	13	4
PCR negative	0	10
	Sensitivity	Specificity
Point estimate	76.00%	100.00%
95% CI	50 to 93%	69 to 100%

Figure 4. Sensitivity and specificity of LAMP vs PCR for Bartonella quintana detection in louse pools using B primers. A PCR Ct ≤ 40 or a Tt_{100} ≤ 30 were considered to be positive. N = 27

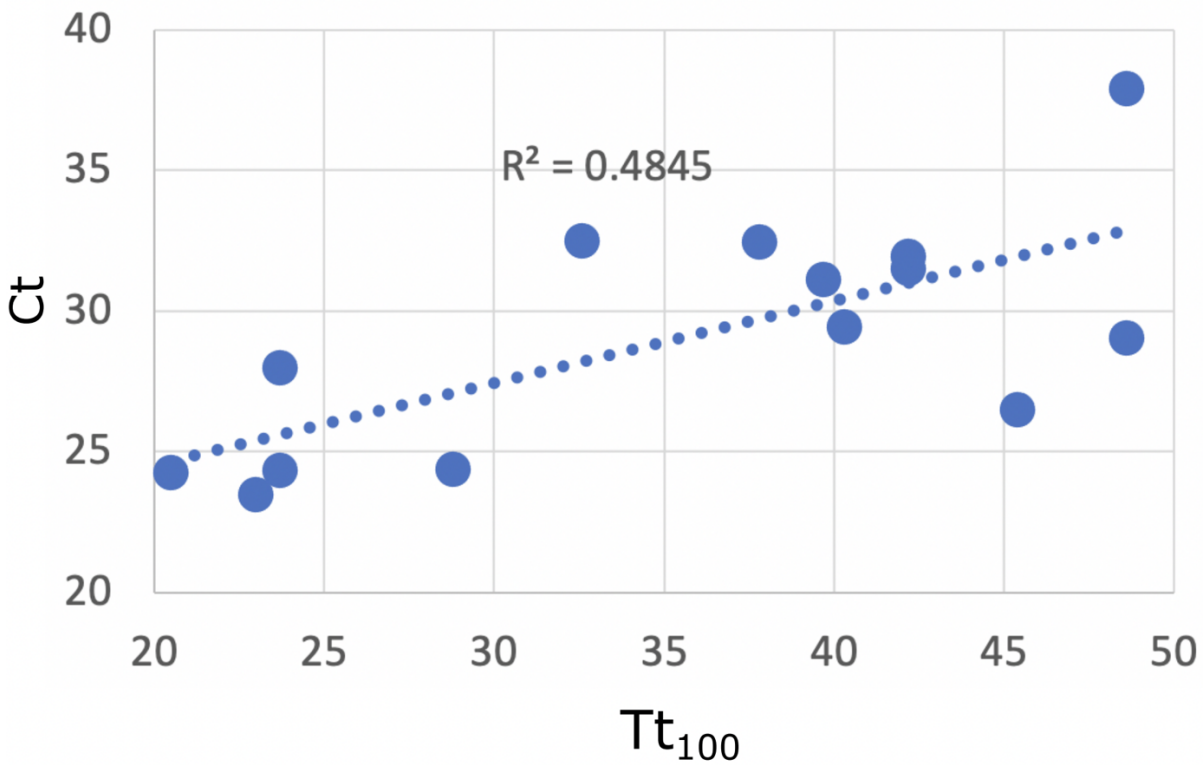

Figure 5. Association between RT-PCR Ct values and RT-LAMP Tt_{100} for 14/33 discrepant SARS-CoV-2 nasopharyngeal samples. A moderate association was seen only when using E and N2 primer sets in combination for RT-LAMP. Only samples that were positive by both methods were included.

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