

TITLE: Saliva-Dry LAMP: A rapid near-patient detection system for SARS-CoV-2.

[Noah B. Toppings](#)¹, [Abu Naser Mohon](#)¹, [Yoonjung Lee](#)², [Hitendra Kumar](#)^{2,3}, [Daniel Lee](#)², [Ratik Kapoor](#)², [Gurmukh Singh](#)², [Lisa Oberding](#)¹, [Omar Abdullah](#)¹, [Keekyoung Kim](#)^{2,4}, [Byron M. Berenger](#)^{5,6}, [Dylan R. Pillai](#)^{*1,5,6,7}

1. Department of Microbiology, Immunology, and Infectious Diseases, University of Calgary, AB, Canada
2. Department of Mechanical and Manufacturing Engineering, University of Calgary, AB, Canada
3. School of Engineering, University of British Columbia, Kelowna, BC, Canada
4. Biomedical Engineering Graduate Program, University of Calgary, AB, Canada
5. Clinical Section of Microbiology, Alberta Precision Laboratories, Calgary, AB, Canada
6. Department Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada
7. Clinical Section of Infectious Diseases, Department of Medicine, University of Calgary, Calgary, AB, Canada

*Corresponding author:

Dylan Pillai MD, PhD

Departments of Pathology, Medicine, and Microbiology, Immunology, and Infectious Diseases
University of Calgary

9-3535 Research Road NW, 1W-416

Calgary, AB, Canada, T2L2K8

drpillai@ucalgary.ca

T: +1 403-770-3338

F: +1 403-770-3347

Supplementary Materials:

Table S1. Saliva-LAMP demonstrates no cross-reactivity with a panel of 11 respiratory pathogens

Pathogen	Strain	Concentration	Result	Agreement with Expected Results
<i>Staphylococcus aureus</i>	COL (MRSA)	10-fold dilution of stock that yields a Ct value of 18.5-21 by PCR	Negative	100% (1/1)
<i>Streptococcus pneumonia</i>	Z022	10-fold dilution of stock that yields a Ct value of 26-28 by PCR	Negative	100% (1/1)
Influenza A H3	A/Brisbane/10/07	10-fold dilution of stock that yields a Ct value of 22-25 by RT-PCR	Negative	100% (1/1)
Influenza B	B/Florida/02/06	10-fold dilution of stock that yields a Ct value of 19-22 by RT-PCR	Negative	100% (1/1)
RSV A2	N/A	10-fold dilution of stock that yields a Ct value of 29-32 by RT-PCR	Negative	100% (1/1)
Coronavirus 229E	N/A	10-fold dilution of stock that yields a Ct value of 25-28 by RT-PCR	Negative	100% (1/1)
Coronavirus NL63	N/A	10-fold dilution of stock that yields a Ct value of 27-30 by RT-PCR	Negative	100% (1/1)
MERS	Florida/USA-2_Saudi Arabia_2014	10-fold dilution of stock that yields a Ct value of 25-28 by RT-PCR	Negative	100% (1/1)
Coronavirus OC43	N/A	10-fold dilution of stock that yields a Ct value of 25-28 by RT-PCR	Negative	100% (1/1)
SARS	2003-00592	10-fold dilution of stock that yields a Ct value of 25-28 by RT-PCR	Negative	100% (1/1)
Coronavirus HKU1	N/A	10-fold dilution of stock that yields a Ct value of 22-25 by RT-PCR	Negative	100% (1/1)
Positive Control (SARS-CoV-2, MS2)		10 X LOD	Positive	100% (1/1)
Negative Control		-	Negative	100% (1/1)

Table S2. Saliva-LAMP is impervious to a wide variety of household medications and substances that may be in patient samples.

†Samples were prepared to 9 X LOD before they underwent an additional freeze-thaw step, further reducing the viral titre by an unknown factor

Substance/Trade Name	Active Ingredient	Ingredient Concentration in Spiked Saliva-UTM Sample	Viral Titre in Input Saliva-UTM Sample†	% Agreement with Expected Results
Tylenol Extra Strength	Acetaminophen	Approximately 1.7 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Aspirin Extra Strength	Acetylsalicylic Acid	Approximately 12.3 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Colgate Total Mouthwash	Cetylpyridinium Chloride	0.005% (w/w)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Cepacol® Sore Throat Lozenges	Benzocaine and Menthol	Approximately 0.08 mg/mL benzocaine and 0.02 mg/mL menthol	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Chloraseptic® Sore Throat Spray	Phenol	0.07% (w/v)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Dristan Tablets	Chlorpheniramine Maleate, Acetaminophen, Phenylephrine HCl	Approximately 0.06 mg/mL (Chlorpheniramine maleate), Approximately 6.5 mg/mL (Acetaminophen), Approximately 0.1 mg/mL (Phenylephrine HCl)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Dimetapp	Bromopheniramine Maleate, Phenylephrine HCl	0.064 mg/mL (Bromopheniramine Maleate), 0.16 mg/mL (Phenylephrine HCl)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Colgate Triple Action Fluoride Toothpaste	Sodium fluoride	Approximately 0.075% (w/w)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Benadryl Extra Strength	Diphenhydramine HCl	Approximately 2.7 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)

HALLS® Kids Sore Throat Lozenges Strawberry Flavoured	Menthol	Approximately 0.08 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Extra Strength Ibuprofen Liquid Gel Capsules	Ibuprofen	Approximately 6 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Listerine Freshburst	Menthol, Thymol, and Eucalyptol	0.0042% (w/v) menthol%, 0.0063 (w/v) thymol, 0.0091% (w/v) eucalyptol	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Rub A535	Methyl Salicylate, Eucalyptus Essential Oil	0.0060% (w/w) (Methyl Salicylate), 0.0006% (w/w) (Eucalyptus Essential Oil)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Benylin Extra Strength Dry Cough	Dextromethorphan HBr	0.2 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Robitussin Cough & Cold Extra Strength	Dextromethorphan HBr, Guaifenesin, Pseudoephedrine Hydrochloride	0.2 mg/mL (Dextromethorphan Hydrobromide), 2.67 mg/mL (Guaifenesin), 0.4 mg/mL (Pseudoephedrine Hydrochloride)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Robitussin Mucus and Phlegm	Guaifenesin	2.0 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Jamieson Extra Strength Zinc Supplement (50 mg)	Zinc Gluconate	Approximately 1 mg/mL	9 X LOD	100% (1/1)
			NTC	100% (1/1)
Human Blood	N/A	10% (v/v)	9 X LOD	100% (1/1)
			NTC	100% (1/1)
No Interfering Substances	N/A	N/A	9 X LOD	100% (1/1)
			NTC	100% (1/1)

Table S4. Assessment of precision of saliva-LAMP using two samples twice per day for twenty days (20 X 2 X 2).

		Negative Control	Positive Control	Replicate 1 (10 copy/ μ L)	Replicate 2 (10 copy/ μ L)
Day 1	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 2	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 3	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 4	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 5	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 6	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 7	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 8	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 9	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 10	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 11	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 12	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 13	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 14	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 15	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 16	Round 1	Negative	Positive	Positive	Positive

	Round 2	Negative	Positive	Positive	Positive
Day 17	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 18	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 19	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive
Day 20	Round 1	Negative	Positive	Positive	Positive
	Round 2	Negative	Positive	Positive	Positive

Table S5. Capital cost of Saliva-dry LAMP commercially acquired instrument.

Instrument	Cost Per Unit (CAD)
mySPIN™ 12 Mini Centrifuge	\$1582.20 [36]
IncuBlock™ Mini Dry Bath	\$252.00 [37]
2 X Aluminum blocks (for 0.2 mL and 2 mL tubes)	\$174 [37]
Ultra Slim LED Transilluminator	\$526.75 [38]
Total Capital Cost	\$2534.95 (US\$1977.44, Dec 8, 2020 exchange rate)

Table S6. Capital cost of Saliva-dry LAMP Biobox (major items listed)

Instrument	Cost Per Unit (CAD)
Fan	\$56.19
Cable	\$46.38
Heat block & machining	\$130.29
Motor	\$38.43
Other	\$224.46
Total Capital Cost	\$495.75 (US\$386.72, Dec 8, 2020 exchange rate)

Figure S1. Cycle threshold (Ct) values based on reference RT-PCR methods of the concomitant NP swab for all SARSCoV2-positive saliva samples used in the clinical validation (n=30).

