Rapid detection of SARS-CoV-2 from crude saliva and nasal swab samples using the Colorimetric ReadiLAMP Kit

Introduction

Rapid, robust, and specific detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be accomplished using the Invitrogen™ Colorimetric ReadiLAMP™ Kit. The direct protocol for this kit provides steps to inactivate and lyse SARS-CoV-2 in a sample aliquot and effectively detect the virus in a subsequent loop-mediated isothermal amplification (LAMP) reaction, without requiring prior RNA purification of the virus sample.

Depending on the laboratory setting and SARS-CoV-2 surveillance testing goals, the workflow and sample diluents may be further modified to maximize efficiency. To help guide Colorimetric ReadiLAMP Kit–based surveillance testing efforts, we outline four distinct options for SARS-CoV-2 inactivation and lysis and compare each option based on workflow and cost constraints. All suggested solutions are validated for Colorimetric ReadiLAMP assay performance across sample types using a series of medium-throughput validation assays. The four options incorporate either tris (2-carboxyethyl) phosphine (TCEP), dithiothreitol (DTT), phosphate-buffered saline (PBS) pH 7.4, or proteinase K (PK) for viral inactivation and lysis, allowing flexibility in individual surveillance testing efforts for SARS-CoV-2 detection.

Materials and methods

Experimental samples

In this work, samples types included raw saliva and nasal swabs. CLASSIQSwabs™ Dry Swabs (Copan Diagnostics) were inserted into donor nostrils and twisted in a circular motion for 15 seconds. These swabs were transferred to 1.0 mL Universal Viral Transport Media (VTM; Becton, Dickinson and Company), vigorously rotated on an orbital shaker for 1 hour at room temperature, and then the VTM solutions were stored at 4°C for up to 5 days. Raw saliva samples were collected from individual donors using the passive drool technique and stored at 4°C for up to 7 days. Contrived experimental samples were created by combining 200 µL raw saliva or nasal swab in VTM with γ-irradiated SARS-CoV-2 isolate USA-WA1/2020 (BEI Resources, Cat. No. NR-52287).

Diluent preparation

Chemical diluents and a proteolytic agent were purchased from Thermo Fisher Scientific (Table 1). These included TCEP, DTT, PBS at pH 7.4, and PK. PK and PBS solutions were used directly as supplied from the manufacturer and stored at room temperature for several months (preparation and storage outlined in Table 1). TCEP and DTT solutions were both prepared to a final concentration of 0.1 M, and 6 N NaOH was used to adjust the final pH to 8.0 ± 0.05. Working solutions of 0.1 M DTT, pH 8.0 were made immediately prior to use, while 0.1 M TCEP, pH 8.0 solutions were stored at room temperature for up to five days.

SARS-CoV-2 inactivation, lysis, and detection with the Colorimetric ReadiLAMP assay

Experimental samples were combined with an equal volume of chemical diluent (e.g., 25 µL crude sample and 25 µL of 0.1 M TCEP, pH 8.0) or with PK solution equating to 20% of the original sample volume (e.g., 25 µL crude sample and 5.0 µL PK), mixed, and centrifuged. Samples with chemical diluents were then heated to 95°C for 5 min; samples with PK were sequentially incubated at 55°C for 15 min followed by a 95°C incubation for 3 min (Table 2). All samples were then cooled to room temperature before SARS-CoV-2 detection with the Colorimetric ReadiLAMP Kit. For all sample types and diluents, the direct protocol for the Colorimetric ReadiLAMP Kit was performed according to the user guide, using 2.0 µL of experimental samples in which SARS-CoV-2 inactivation and lysis had been previously performed.

Table 1. Sample diluent guide. Direct comparison of sample diluents with respect to cost, supplied formats, solution preparation and stability, and sample compatibility.

	Chemical lysis			Enzymatic lysis
LAMP sample diluent	**0.1 M TCEP, pH 8.0**	**0.1 M DTT, pH 8.0**	**1X PBS, pH 7.4**	**Proteinase K (PK)**
Cat. No.	20490	R0862	10010049	A42363
Relative cost	$$$	$$	$	$$$$
Estimated diluent cost*	$489.00/10 g	$387.00/25 g	$37.68/1.0 L	$684.00/10 mL
Solution preparation required	Yes, with pH adjustment	Yes, with pH adjustment	No	No
Room temperature stability	1 week	Immediate use only	24 months	1 year
Estimated workflow cost for 96 x 25 µL samples*	$3.36	$0.57	$0.09	$32.82
Estimated workflow cost for 1 x 2.0 mL sample*	$2.80	$0.48	$0.08	$27.36
Compatible with raw saliva	•	•	•	•
Compatible viral transport media (select, serum-free VTM only)	•	•	•	•

* Estimates are based on the listed prices by Thermo Fisher at the launch date of the Colorimetric ReadiLAMP Kit. Prices are listed in United States dollars (USDs).

Table 2. SARS-CoV-2 inactivation and lysis protocols for recommended diluents. SARS-CoV-2 virions may be inactivated and lysed in the presence of chemical diluent with a single-temperature heat treatment, while proteolytic lysis requires an enzymatic incubation with PK followed by a high-temperature heat treatment. Samples were cooled to room temperature prior to subsequent LAMP assay.

Protocol	Step	Temperature	Time
SARS-CoV-2 inactivation using chemical diluent (TCEP, DTT, PBS)	1	95°C (lid ≥100°C)	5 min
	2	15–25°C (lid ≥100°C)	2 min
SARS-CoV-2 inactivation using proteolytic diluent (PK)	1	55°C (lid ≥100°C)	15 min
	2	95°C (lid ≥100°C)	3 min
	3	15–25°C (lid ≥100°C)	2 min

Results

The direct protocol for the Colorimetric ReadiLAMP Kit enables SARS-CoV-2 detection in crude sample types without prior RNA isolation. This workflow incorporates viral inactivation and lysis in 0.1 M TCEP, pH 8.0; however, alternative inactivation approaches may be required based on user workflows, testing site restrictions, and cost considerations. Here, we characterize and compare four diluents for saliva and swab samples, including the recommended TCEP solution provided in the Colorimetric ReadiLAMP User Guide (Table 1). First, as an alternative to TCEP, DTT is an inexpensive, common laboratory reagent that may be used as a diluent for the Colorimetric ReadiLAMP Kit. The 0.1 M DTT, pH 8.0 solution must be made immediately prior to use, and any remaining solution must be discarded due to the instability of DTT at pH 8.0. Next, 1X PBS pH 7.4 is an inexpensive, common, and stable lab reagent that can also be used as a crude sample diluent for the Colorimetric ReadiLAMP Kit. This buffer may be used directly as supplied and stored at room temperature for up to 2 years. However, as the Colorimetric ReadiLAMP Kit relies on a metal ion–sensitive dye for color change, the PBS diluent must not contain Mg^{2+}, Ca^{2+}, or phenol red. Both 0.1 M DTT, pH 8.0 and 1X PBS, pH 7.4 diluents are compatible with large-scale samples where additional processing steps are not feasible, keeping estimated costs as low as $0.05–0.50 per 2.0 mL sample. Finally, for users that prefer proteolytic inactivation and lysis of SARS-CoV-2, commercial PK solution may be directly added to crude samples at 20% of the original sample volume. This enzymatic lysis requires several incubation temperatures, extending the sample processing time by approximately three-fold (Table 2). Depending on individual SARS-CoV-2 testing constraints, users may elect to use any of the suggested diluents; however, for the greatest sensitivity and LAMP assay performance, we continue to recommend 0.1 M TCEP, pH 8.0 as the optimal choice.

The robustness of the Colorimetric ReadiLAMP Kit's workflow with each of the sample diluents was evaluated using a series of medium-throughput validation assays. In the first part of the workflow, SARS-CoV-2 was inactivated and lysed in crude sample types (Figure 1). Specifically, the experiment was divided into eight 96-well plates where half were crude saliva samples and the remaining half were crude nasal swab samples in VTM. For each sample type, half of the crude samples were designated as no-template controls (NTC, n = 48) and the other half were spiked with 100–1,000 copies of SARS-CoV-2/µL (n = 48). Each of the four recommended sample diluents were individually added to each respective sample type, and the corresponding SARS-CoV-2 inactivation and lysis heat treatments were performed (Table 2). Ultimately, the layout of the validation assays allowed for all combinations of crude sample type (i.e., saliva or nasal swab), SARS-CoV-2 copy number (i.e., NTC vs. 100–1,000 copies of SARS-CoV-2/µL, concentration dependent on the sensitivity using a respective diluent), and diluents (i.e., 0.1 M TCEP, pH 8.0; 0.1 M DTT, pH 8.0; 1X PBS, pH 7.4; and PK) to be evaluated from 48 unique donors.

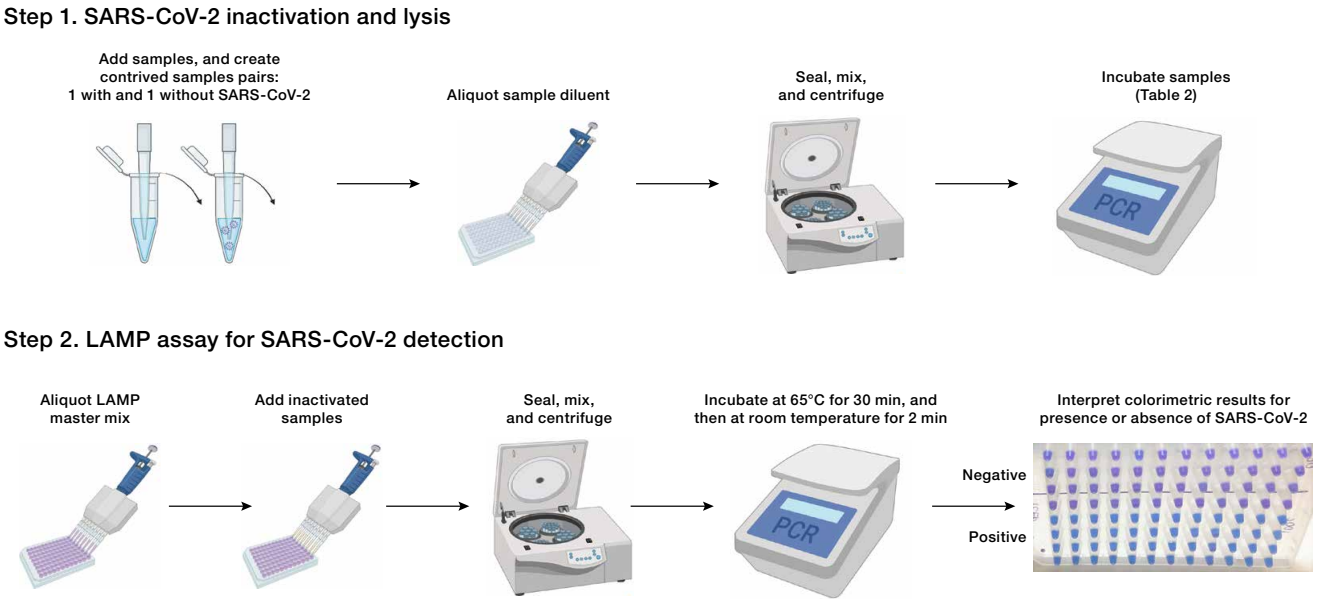

Figure 1. The Colorimetric ReadiLAMP Kit's direct protocol for crude sample types. Workflow for the medium-throughput validation testing, including representative results for positive (blue) and negative (purple) reactions.

The second component of the Colorimetric ReadiLAMP validation assays evaluated LAMP performance to directly compare the diluent types and robustness of the overall workflow (Figure 1). The LAMP assays were performed as recommended in the user guide with 2.0 µL of inactivated and lysed SARS-CoV-2 crude sample as input. LAMP reactions were incubated at 65°C for 30 min, then at 15–25°C for 2 min, and the results were visually interpreted. The presence of SARS-CoV-2 caused the reaction mixture to turn blue (positive result), and the absence of the virus caused the reaction mixture to remain purple (negative result). The accuracy of the Colorimetric ReadiLAMP assay was calculated as the percentage of true positives in reactions containing SARS-CoV-2, while specificity was calculated as the percentage of true negatives in NTC reactions. Using TCEP as a crude sample diluent, 100 copies of SARS-CoV-2 per 25 µL LAMP reaction were detected with ≥96% accuracy and ≥96% specificity in both saliva and nasal swab sample types (Table 3). Selecting DTT as an alternative sample diluent and increasing the SARS-CoV-2 concentration two-fold due to the reduced sensitivity of this diluent, resulted in ≥96% accuracy and ≥92% specificity across the two sample types. Increasing the concentration further by an additional 5- and 10-fold for PK and PBS diluents, respectively, resulted in strong performance statistics. The PBS diluent–based detection resulted in ≥88% accuracy and ≥96% specificity, while PK proteolytic–based detection resulted in ≥98% accuracy and ≥98% specificity. Ultimately, these strong LAMP assay statistics validated the four recommended crude sample diluents for SARS-CoV-2 surveillance testing using the Colorimetric ReadiLAMP Kit.

Conclusions

The Colorimetric ReadiLAMP Kit incorporates a direct protocol for analyzing crude sample types. This protocol eliminates the need for RNA isolation reagents and instrumentation, thereby reducing overall costs and turnaround time for SARS-CoV-2 detection. Herein, we verified alternative methods for the inactivation and lysis of SARS-CoV-2 to accommodate user considerations in testing site, workflow, cost, and SARS-CoV-2 detection sensitivity. The suggested sample diluents and workflows were validated using the Colorimetric ReadiLAMP Kit where all options for inactivation and lysis exhibited superior LAMP assay performance. Ultimately, this work offers flexible customization of the Colorimetric ReadiLAMP Kit in viral inactivation and lysis, thus tailoring SARS-CoV-2 detection to individual testing settings.

Table 3. Colorimetric ReadiLAMP Kit performance comparison across recommended diluents. SARS-CoV-2 can be detected in crude saliva and nasal swab samples after brief viral inactivation and lysis followed by the Colorimetric ReadiLAMP assay.

		Chemical lysis			Enzymatic lysis
Sample type		**0.1 M TCEP, pH 8.0**	**0.1 M DTT, pH 8.0**	**1X PBS, pH 7.4**	**PK**
Saliva	Copies per reaction	100	200	1,000	500
	Total samples NTC With SARS-CoV-2	n = 96 48 48	n = 96 48 48	n = 96 48 48	n = 96 48 48
	LAMP assay accuracy (%)	100	98	88	100
	LAMP assay specificity (%)	96	100	96	98
Nasal swabs transferred to VTM	Copies per reaction	100	200	1,000	500
	Total samples NTC With SARS-CoV-2	n = 96 48 48	n = 96 48 48	n = 96 48 48	n = 96 48 48
	LAMP assay accuracy (%)	96	96	98	98
	LAMP assay specificity (%)	98	92	100	98

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Ordering information

Product	Cat. No.
Pierce TCEP-HCl	20490
Bond-Breaker TCEP Solution, Neutral pH	77720
Thermo Scientific DTT (dithiothreitol)	R0862
Gibco PBS, pH 7.4	10010049
MagMAX Viral/Pathogen Proteinase K	A42363
Sodium Hydroxide Solution (10N/Certified)	Fisher Cat. No. SS255-1
Orion ROSS Ultra Refillable pH/ATC Triode Combination Electrodes for Orion Series Meters	8157BNUMD
Veriti 96-Well Thermal Cycler	4375786
MicroAmp Optical 96-Well Reaction Plate	4316813
MicroAmp Clear Adhesive Film	4306311
Fisherbrand Mini Dry Bath with Heated Lid	Fisher Cat. No. 14-955-241
MicroAmp Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL	A30588

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