Sample prep

From noninvasive sample collection to SARS-CoV-2 detection

Compatibility of SpeciMAX Saliva Collection devices with the Colorimetric ReadiLAMP Kit

Introduction

The SARS-CoV-2 crisis has enormously impacted the livelihoods of people around the world. The high number of cases and severe transmissibility of the virus warrant massive testing to keep up with the demand. Currently, collection of nasopharyngeal swab specimens followed by reverse-transcription quantitative PCR (RT-qPCR) is the gold-standard method for detection of SARS-CoV-2 [1]. Nasopharyngeal swab collections require increased resources including trained personnel and additional personal protective equipment. Incorrect collection of nasopharyngeal swab samples could sacrifice sample integrity, quality, and downstream results. These limitations suggest the need for an alternative sample type and collection method to reduce resource requirements and provide an easy workflow solution from sample collection to detection. Human saliva has been sufficient for the detection of previous coronaviruses such as SARS-CoV and MERS-CoV [2]. For SARS-CoV-2, the viral load in saliva is highest during the first week after symptom onset, and viral particles can be detected in saliva up to 25 days after symptom onset, which suggests saliva is an effective, noninvasive sample type for detection of SARS-CoV-2 [3].

The Thermo Scientific™ SpeciMAX™ Stabilized Saliva Collection Kit and the SpeciMAX™ Saliva Collection Kit are now offered for collection of raw saliva or stabilized saliva, respectively. Both kits are designed for ease of use and compatibility with manual or fully-automated workflows. After collection, the devices are designed to conveniently fit in automation equipment such as liquid handlers and decappers. The SpeciMAX Stabilized Saliva Collection Kit preserves the contents of the specimen and inactivates common respiratory viruses.

The ideal assay to detect the presence or absence of SARS-CoV-2 would require no specialized equipment and would have fast and easy-to-interpret results. The Invitrogen™ Colorimetric ReadiLAMP™ Kit offers a rapid assay based on reverse-transcription loop-mediated isothermal amplification (RT-LAMP). Results are visualized as a simple color change from purple to blue in the reaction mix. In this study, we report the compatibility of SpeciMAX products (both raw and stabilized kits) with the Colorimetric ReadiLAMP Kit. Isolated RNA and raw saliva samples are evaluated, the latter of which eliminates the need for RNA purification. In addition, the performance of SpeciMAX kits is compared with other commercially available saliva collection kits. This complete workflow from sample collection to RT-LAMP assay enables faster, simpler, and more accessible detection of SARS-CoV-2.

Experimental design

Sample collection and preparation

The SpeciMAX Saliva Collection Kit and 2 other general collection devices (designated as collection tube A or B) were used to collect fresh saliva from 12 healthy donors. Contrived samples were created by spiking the saliva samples with different copy numbers of gamma-irradiated SARS-CoV-2 (BEI Resources, Cat. No. NR-52287). After an overnight at ambient temperature, the 12 samples were used for direct RT-LAMP or processed for nucleic acid isolation on the Thermo Scientific™ KingFisher™ Flex Purification System using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (RUO) according to the saliva protocol.

The SpeciMAX Stabilized Saliva Collection Kit and 3 commonly used stabilized saliva collection kits on the market (designated as A, B, or C) were also evaluated. Fresh saliva samples were collected from 12 donors, spiked with gamma-irradiated SARS-CoV-2 (BEI Resources, Cat. No. NR-52287), and left overnight in the collection tubes at ambient temperature. The 12 samples were processed for nucleic acid isolation on the KingFisher Flex Purification System using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (RUO) according to the saliva protocol.

RT-LAMP assays

Raw saliva samples and isolated nucleic acid were tested for detection of SARS-CoV-2 using the Colorimetric ReadiLAMP Kit (Figure 1). For both RT-LAMP using isolated RNA and direct RT-LAMP using raw samples, 0.2 mL 96-well–PCR plates or 8-tube strips with tight lids were used to set up the reactions. For direct RT-LAMP, raw saliva samples were diluted to a 1:1 ratio with the diluent recommended in the Colorimetric ReadiLAMP Kit user guide (TCEP, pH 8.0). Once diluted, the raw saliva samples were heated at 95°C for 2 minutes and immediately cooled on ice before setting up the direct RT-LAMP assay. Reactions for RT-LAMP with isolated RNA or direct RT-LAMP with preheated raw saliva samples were prepared with reagents from the Colorimetric ReadiLAMP Kit. Reactions were incubated on an Applied Biosystems™ Veriti™ Thermal Cycler with a heated lid at 65°C for 30 min and 25°C (room temperature) for 2 min. The initial reaction color was purple for all the samples before incubation; following the incubation, reactions positive for SARS-CoV-2 were expected to turn blue, and negative reactions were expected to stay purple as shown in Figure 1. Non-contrived samples (0 copies of SARS-CoV-2) were used as negative controls.

Results

RT-LAMP assay using purified RNA—detection of low copy numbers of SARS-CoV-2

The compatibility of the SpeciMAX Saliva Collection Kit with the Colorimetric ReadiLAMP Kit was tested using saliva samples collected from multiple donors. The saliva samples were spiked with gamma-irradiated SARS-CoV-2 and processed for nucleic acid isolation. Figure 2A shows the expected purple color for all RT-LAMP reactions before undergoing incubation. Figure 2B shows the reactions after incubation at 65°C for 30 min. Samples positive for SARS-CoV-2 underwent a color change to blue, while negative samples had no color change and remained purple. For all 12 samples, SARS-CoV-2 was detected from 20 to 250 copies (cp) with 100% sensitivity and specificity. These results indicate that the SpeciMAX Saliva Collection Kit is compatible with the Colorimetric ReadiLAMP Kit for detection of SARS-CoV-2 using RNA isolated from a collected sample.

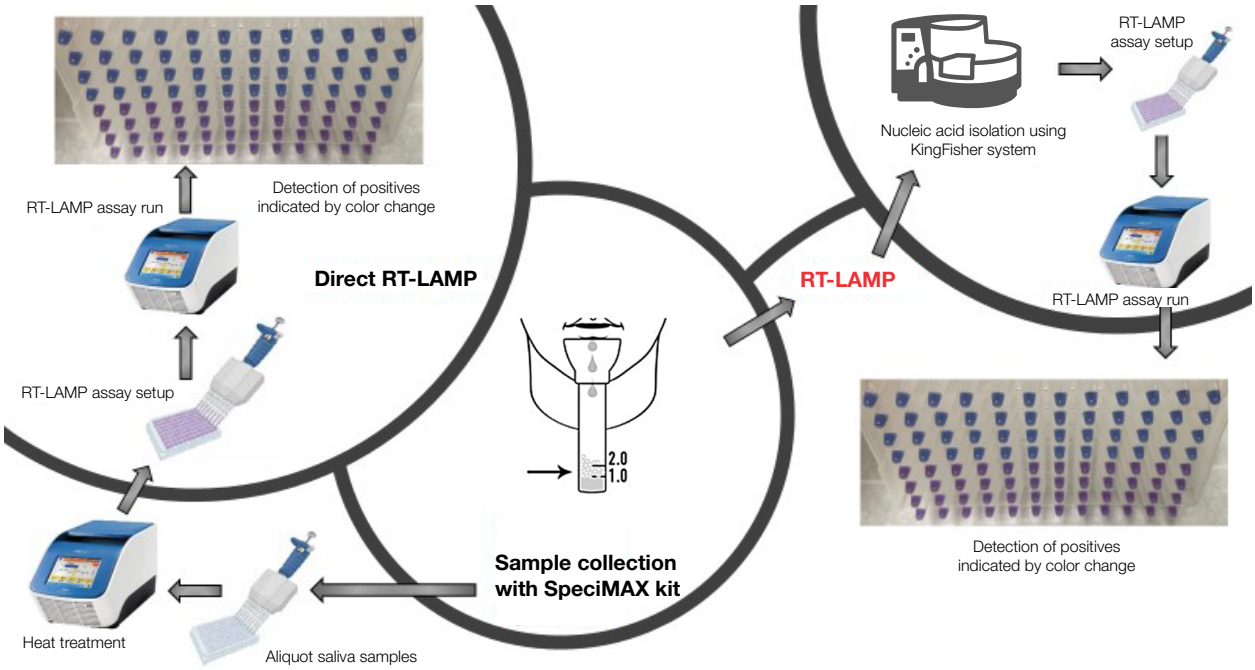

Figure 1. Workflow for sample collection with SpeciMAX kits for detection of SARS-CoV-2 with the Colorimetric ReadiLAMP Kit. Either raw saliva (direct RT-LAMP) or RNA isolated from saliva (RT-LAMP) is used in the workflow.

RT-LAMP assay using purified RNA—comparison of saliva collection devices

The compatibility of the SpeciMAX Saliva Collection Kit with the Colorimetric ReadiLAMP Kit was compared with 2 generally used saliva collection devices (designated as collection tube A or B). The RT-LAMP assay was performed using isolated nucleic acid with 0 copies of SARS-CoV-2 spiked in or 100 copies of SARS-CoV-2 spiked in. Figure 3A shows the RT-LAMP assay setup where all samples before incubation are purple. Figure 3B shows the reactions after a 65°C incubation for 30 min where samples positive for SARS-CoV-2 underwent a color change from purple to blue. Samples that remained purple were negative for the virus. Results show that 100% sensitivity and specificity was achieved with 100 copies of SARS-CoV-2 RNA for all the collection devices tested. Results indicate that the SpeciMAX Saliva Collection Kit utilized for raw saliva collection performs similarly to other generally used saliva collection devices for downstream detection of SARS-CoV-2 with the Colorimetric ReadiLAMP Kit.

Direct RT-LAMP assay—comparison of saliva collection devices

The compatibility of the SpeciMAX Saliva Collection Kit with the Colorimetric ReadiLAMP Kit was compared with 2 generally used saliva collection devices (designated as collection tube A or B) in a direct RT-LAMP assay. Saliva collected from 12 different donors was diluted with a diluent (25 µL raw saliva with 25 µL TCEP, pH 8.0) as recommended by the Colorimetric ReadiLAMP Kit user guide. The diluted raw saliva samples were heated at 95°C for 5 min and immediately cooled on ice. Direct RT-LAMP assays were set up with the preheated raw saliva samples containing 0 or 250 copies of SARS-CoV-2 spiked in. Figure 4A shows the RT-LAMP assay setup for SARS-CoV-2–contrived raw saliva samples as purple for all samples before incubation. Figure 4B shows the reactions after 65°C incubation for 30 min in which samples positive for SARS-CoV-2 had undergone a color change from purple to blue. Negative samples had no color change and remained purple. Results show that 100% sensitivity and specificity was achieved with 250 copies of SARS-CoV-2 for all the collection devices tested. Results indicate that the SpeciMAX Saliva Collection Kit utilized for raw saliva collection performs similarly to other generally used saliva collection devices for detection of SARS-CoV-2 in direct RT-LAMP with the Colorimetric ReadiLAMP Kit.

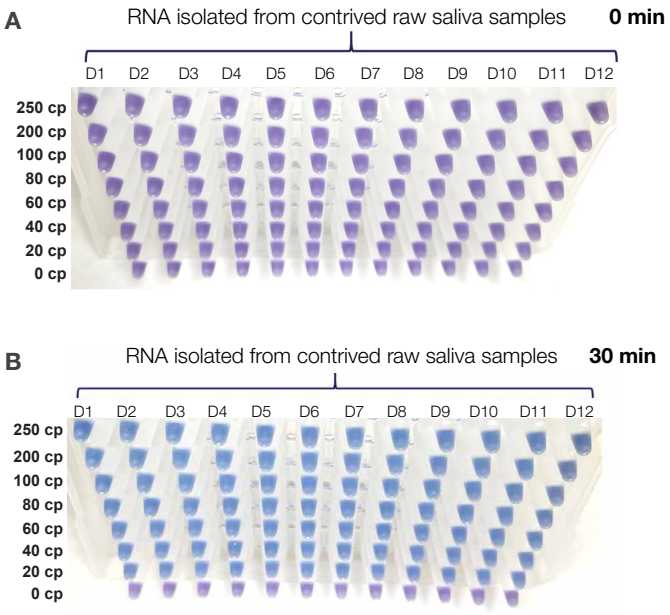

Figure 2. Compatibility of the SpeciMAX Saliva Collection Kit for downstream RT-LAMP assays with the Colorimetric ReadiLAMP Kit. Reaction mixes are shown **(A)** at 0 min and **(B)** after 30 min incubation. D1–D12: donors of saliva samples.

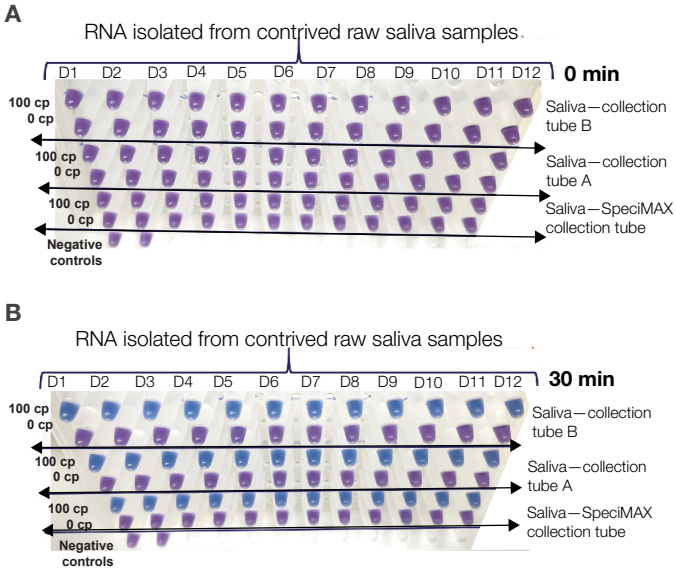

Figure 3. Comparison of RT-LAMP assay performance for raw saliva samples collected with the SpeciMAX Saliva Collection Kit and 2 other generally used collection devices. Reaction mixes are shown **(A)** at 0 min and **(B)** after 30 min incubation. D1–D12: donors of saliva samples.

RT-LAMP assay using purified RNA—comparison of stabilized saliva collection kits

The compatibility of the SpeciMAX Stabilized Saliva Collection Kit with the Colorimetric ReadiLAMP Kit was compared with 3 commonly used stabilized saliva collection kits (designated as kit A, B, or C). Stabilized raw saliva samples collected from 12 different donors were processed for nucleic acid isolation, and then tested in RT-LAMP assays using the Colorimetric ReadiLAMP Kit. RT-LAMP assays were set up with the isolated nucleic acid containing 0 or 100 copies of SARS-CoV-2 spiked in. Figure 5A shows the RT-LAMP assay setup for SARS-CoV-2 contrived samples as purple for all samples before incubation. Figure 5B shows the reactions after 65°C incubation for 30 min in which samples positive for SARS-CoV-2 had undergone a color change from purple to blue. Negative samples had no color change and remained purple. Some of the isolations had leftover beads in their elution (for example, sample D9 for kit C in the 100-copy row); bead carryover did not affect performance in the RT-LAMP assay. Results show that 100% sensitivity and specificity was achieved with 100 copies of SARS-CoV-2 for all the collection kits tested. Results also indicate that the SpeciMAX Stabilized Saliva Collection Kit utilized for raw saliva collection performs similarly to other commercially available saliva stabilization devices for downstream detection of SARS-CoV-2 with the Colorimetric ReadiLAMP kit.

Summary

This streamlined, noninvasive process of SARS-CoV-2 detection using a SpeciMAX device, with or without nucleic acid extraction, and RT-LAMP assay compares favorably to existing methods for viral detection from nasal and nasopharyngeal swabs. The procedure can be used for a fast turnaround time from sample collection to results as compared to traditional methods.

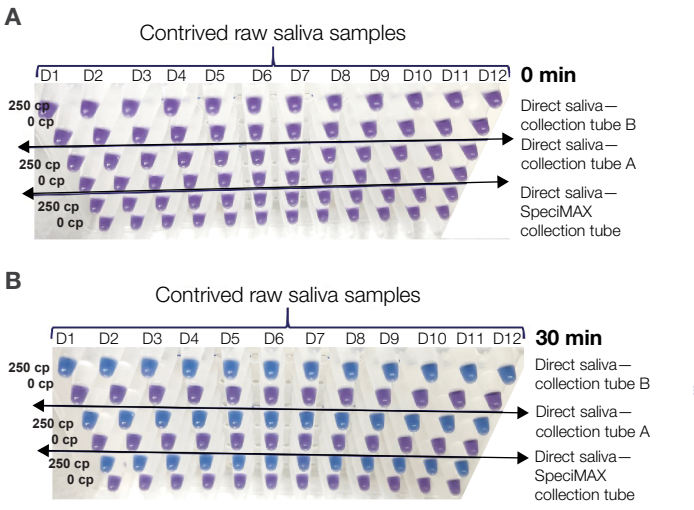

Figure 4. Direct RT-LAMP assay performance for raw saliva samples collected with the SpeciMAX Saliva Collection Kit and compared to other commonly used saliva collection devices. D1–D12: donors of saliva samples.

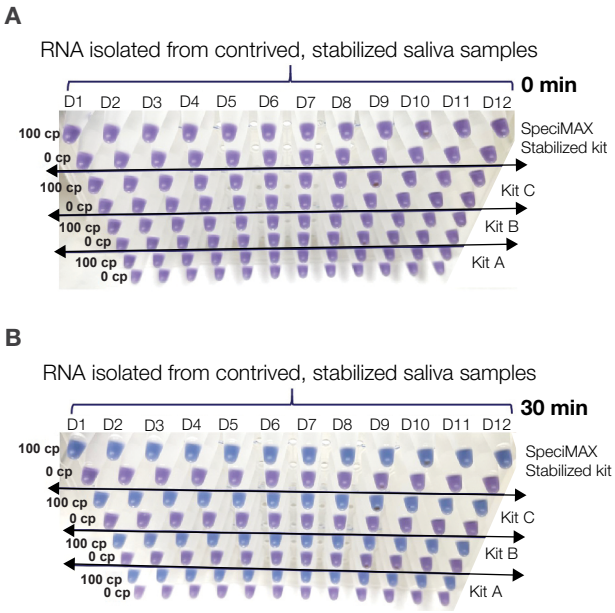

Figure 5. RT-LAMP assay performance for the SpeciMAX Stabilized Saliva Collection Kit compared to other commercially available saliva stabilization kits. D1–D12: donors of saliva samples.

Ordering information

Description	Cat. No.
SpeciMAX Saliva Collection Kit (RUO)	A50696
SpeciMAX Stabilized Saliva Collection Kit (RUO)	A50697
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (RUO)	A48383R
KingFisher Flex Purification System with 96 Deep-Well Head	5400630
Colorimetric ReadiLAMP Kit, SARS-CoV-2	A52539, A52544

References

1. Lippi G, Simundic AM, Plebani M (2020) Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). *Clin Chem Lab Med* 58(7):1070–1076.
2. Niedrig M, Patel P, El Wahed AA et al. (2018) Find the right sample: a study on the versatility of saliva and urine samples for the diagnosis of emerging viruses. *BMC Infect Dis* 18(1):707.
3. To KK-W, Tsang OT-Y, Leung W-S et al. (2020) Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 20(5):565–574.

Acknowledgment

This product contains components that are manufactured under a license from Isohelix, a division of Cell Projects Limited.

Learn more at **thermofisher.com/sarscov2**