The easy-to-use SpeciMAX Saliva Collection Kit

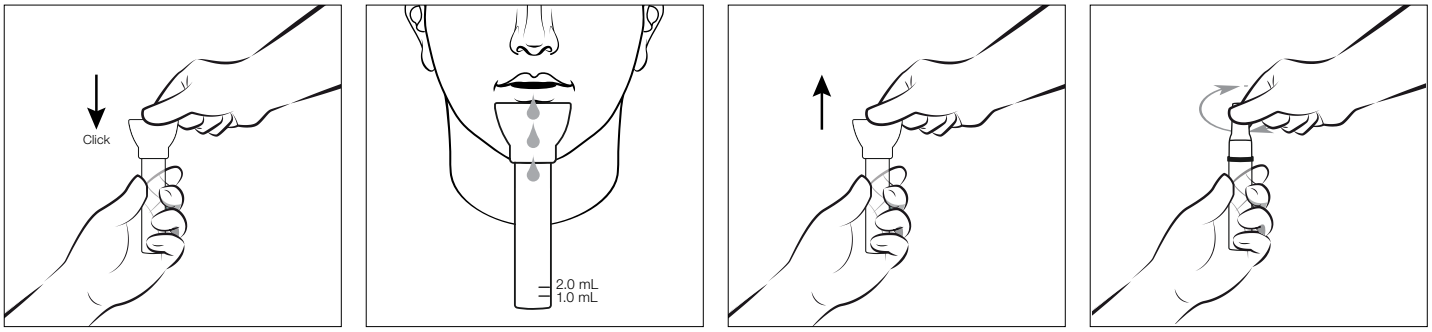
Straightforward pairing with semi-automated and fully automated systems

Introduction

Saliva is a suitable specimen type for the detection of various respiratory viruses, including SARS-CoV-2 [1]. DNA can be extracted from saliva for downstream applications, such as polymerase chain reaction (PCR) amplification, quantitative PCR (qPCR), whole-genome sequencing, genotyping-by-sequencing, and loop-mediated isothermal amplification (LAMP). Unlike nasal swabs, nasopharyngeal swabs, or blood samples, saliva specimens can be collected noninvasively to detect DNA or RNA from respiratory viruses. Invasive sampling techniques like drawing blood and collecting nasopharyngeal swabs must be performed by professionals and can cause discomfort. Collection of nasopharyngeal swabs can also cause patients to cough or sneeze, which could make a respiratory virus become airborne [2]. Raw saliva can be self-collected and easily contained with the Thermo Scientific™ SpeciMAX™ Saliva Collection Kit. The SpeciMAX kit can be used by most adults, and solutions are available for both raw and stabilized saliva samples. Using the SpeciMAX kit to collect saliva can reduce variability between users, compared to common home-brew methods for collecting saliva specimens. The SpeciMAX kit can also be paired with most commonly used saliva workflows. In this study, we collected saliva specimens using the SpeciMAX kit and compared its functionality for downstream RNA isolation and analysis to that of low-cost raw saliva collection kits currently on the market. An anonymous survey was conducted to obtain feedback about the usability of the SpeciMAX kit.

Background

Raw and stabilized saliva samples can be stored at room temperature, but raw saliva degrades human RNA over time unless a stabilization buffer is added [3]. Stabilized saliva specimens may be more suitable for RNA or DNA analysis, because stabilized saliva keeps RNA and DNA intact and inhibits bacterial growth. For research studies that do not require a longer shelf life, analyzing raw saliva may be more appropriate and cost-effective. In a virus-related crisis, sample collection kits may be assembled in-house to control costs. Some collection kits include 50 mL conical tubes and urine cups, which are commonly used in the field due to their wide mouths, ease of use, and low cost [4]. Although these kits are viable options for saliva collection, they have some drawbacks. For example, they can be awkward and difficult to hold. Instructions for use may be inconsistent, and the size and shape of the collection device may not allow samples to be processed with fully automated systems. The SpeciMAX kit was designed to circumvent these drawbacks and to enable easy use at all ages and skill levels. The collection tube is comfortable to hold, and the kit comes with simple step-by-step instructions (Figure 1). The SpeciMAX kit can also be paired with automated decappers and liquid handlers, such as Tecan™ and Hamilton™ liquid handling systems.



1. Press funnel firmly onto the tube.

2. Fill until saliva (not bubbles) is between the 1.0 and 2.0 mL lines.

3. Remove and discard the funnel.

4. Attach the cap and twist to close.

Figure 1. Instructions for the SpeciMAX Saliva Collection Kit. The instructions are conveniently located on the label of each blister pack.

The SpeciMAX saliva collection tubes fit directly into standard automation racks. In contrast, saliva in a typical 50 mL conical tube, urine cup, or tube with a straw must be manually transferred. Manual transfers significantly increase labor costs and employee strain. Each SpeciMAX collection tube can hold up to 6 mL of liquid, providing plenty of sample for various standard applications and workflows. Unlike 50 mL conical tubes or urine cups, SpeciMAX collection tubes are conveniently sized for storage in small spaces like refrigerators and common tube racks. The wide-mouthed funnel is specifically designed to allow saliva of all types and viscosities to flow easily into the collection tube.

Materials and methods

Matched saliva specimens were collected in urine cups, 50 mL conical tubes, and SpeciMAX saliva collection tubes from 14 anonymous donors at a volume of 2 mL per kit. The collection devices used in the study are shown in Figure 2. Users were provided with general instructions on how to passively drool into the urine cups and 50 mL conical tubes. To collect saliva using the SpeciMAX Saliva Collection Kit, users were asked to follow the instructions on the label. After donating their specimens, users were asked to fill out an anonymous survey to rate their experience with the SpeciMAX kit. Responses were received from 10 of the 14 donors, and the ratings were averaged for each question.

The collected specimens were spiked with low and medium concentrations of gamma-irradiated inactivated SARS-CoV-2 virus. MS2 RNA bacteriophage was spiked directly into each sample to serve as an extraction control. Extraction was performed with 200 µL of each sample using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (RUO) and the Thermo Scientific™ KingFisher™ Flex Purification System. Standard Applied Biosystems™ TaqMan® viral RNA reagents were used to perform qPCR for viral detection on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System. A typical workflow for RNA and DNA extraction from saliva in a SpeciMAX collection tube is shown in Figure 3.

Figure 2. Raw saliva collection kits used in the study.

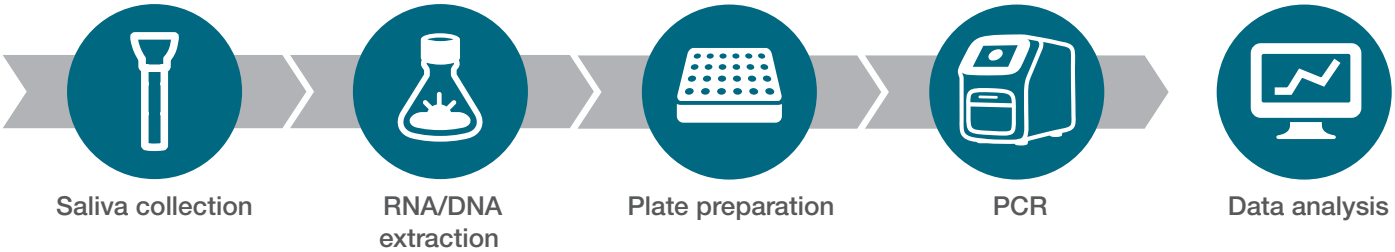


Figure 3. Workflow for the SpeciMAX kit, from sample collection to data analysis.

Results and discussion

The C_t values for saliva samples from all 14 donors containing a medium concentration of inactivated SARS-CoV-2 virus indicated there was little variation between samples collected with urine cups, 50 mL conical tubes, or the SpeciMAX kit (Figure 4). There was some donor-to-donor variability among samples spiked at the lower SARS-CoV-2 concentration. This is typical of saliva samples, due to wide variations in viscosity and genomic DNA content. The MS2 C_t values at both SARS-CoV-2 concentrations indicated there was more variation between samples collected from donors 13 and 14 when urine cups were used. There were natural variations between the donors, but variability was more pronounced with the urine cups due to their larger diameter and surface area. Since saliva is spread over a large surface area in a urine cup, mucus and particulates in more viscous samples are unable to settle at the bottom of the collection device. The mucus and particulates cannot be avoided during pipetting, which results in inconsistent extraction. Particulates could easily settle at the bottoms of the 50 mL conical tubes and SpeciMAX tubes, because the tubes were relatively long and had smaller diameters than the urine cups. The higher C_t values for MS2 and the N, S, and *orf1ab* genes, particularly with samples collected in urine cups, indicated possible PCR inhibition. This could have been caused by an excess of genomic DNA or mucin.

Ten of the fourteen individuals anonymously responded to the user experience survey. Collection of raw saliva with the SpeciMAX kit was compared to collection with standard 50 mL conical tubes and urine cups based on the responses to eleven questions. Experiences were rated on a scale of 0% to 100%, and the ratings from all ten donors were averaged (Table 1). A rating of 0% indicated the device was not user friendly, while a rating of 100% indicated ideal user friendliness. Users gave the instructions for the SpeciMAX kit a rating of 98.6% for clarity, and the general passive drooling experience got a rating of 95.7%. The “fit in hand” feature of the SpeciMAX kit received an averaging rating of 97.1%. Users were also asked to rate their experiences holding all devices, including the 50 mL conical tubes and urine cups. The average rating was 82.9%, which was 14.2% below the rating for the SpeciMAX device. This suggested that holding the SpeciMAX device was preferable to holding a urine cup or 50 mL conical tube.

Table 1. Responses of 10 donors to a SpeciMAX Saliva Collection Kit usability survey.

Description	SpeciMAX kit average rating (%)
Clarity of instructions	98.6%
Fit in hand	97.1%
Opening tube cap	98.6%
Closing tube cap	97.1%
Placing funnel on tube	92.9%
Removing funnel from tube	94.3%
Saliva flow in funnel	98.6%
Stability of funnel on tube	84.3%
Overall kit rating	91.4%

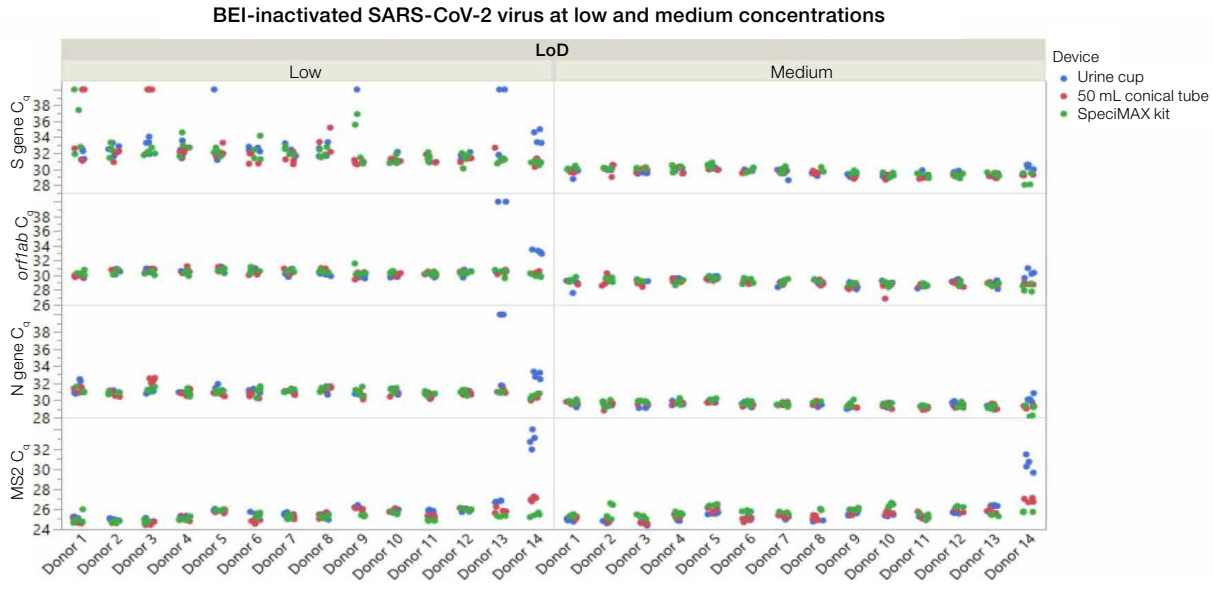


Figure 4. C_t values of saliva specimens spiked with low and medium concentrations of SARS-CoV-2. The samples were collected from 14 donors with urine cups, 50 mL conical vials, and the SpeciMAX Saliva Collection Kit.

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Opening the cap on the SpeciMAX collection tube received an average rating of 98.6%, and closing the cap received an average rating of 97.1%. Securing the funnel on the SpeciMAX collection tube received an average rating of 92.9%, while removing the funnel received an average rating of 94.3%. The flow of saliva through the kit tube was rated at 98.6%, which indicated that clogging of saliva was minimal as it traveled from the funnel into the tube. The stability of the funnel when placed on the tube received a rating of 84.3%, but the average overall rating for the kit was 91.4%.

Conclusion

Analysis of the qPCR results indicates that urine cups, 50 mL conical tubes, and the SpeciMAX Saliva Collection Kit are functionally equivalent for raw saliva collection.

Responses to a usability survey, consistent RNA detection results, and the ease of the user-friendly workflow for the SpeciMAX kit indicate that the SpeciMAX kit performs as well as or better than currently used standard kits for raw saliva collection in 50 mL conical tubes and urine cups. One distinguishing feature of the SpeciMAX kit is that it can be easily paired with semi-automated and fully automated systems and workflows, while 50 mL conical tubes and urine cups cannot. The data suggest that larger containers like urine cups may not be suitable for collecting highly viscous saliva, as they do not allow debris and mucin to settle. The SpeciMAX Saliva Collection Kit has been shown to be a suitable and affordable option for collecting raw saliva specimens.

Ordering Information

Description	Cat. No.
SpeciMAX Saliva Collection Kit (raw saliva)	A50696
SpeciMAX Saliva Collection Kit (stabilized saliva)	A50697
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
KingFisher Flex Purification System with 96 Deep-Well Head	5400630

References

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