Saliva sampling using the SpeciMAX Saliva Collection Kit

Introduction

Saliva is a complex mixture that continually bathes the oral cavity surface epithelium in a protective fluid. Saliva is made up of water, mucus, proteins, electrolytes, and amylase secreted by the mandibular, parotid, and sublingual salivary glands. The bicarbonate, phosphate, and urea components serve to maintain the oral cavity by modulating the pH of the mouth. Mucins and minerals protect teeth by breaking down food particles and remineralizing enamel, which mitigates tooth decay. Healthy saliva also contains nonpathogenic oral commensal bacteria, also known as the saliva microbiome. These bacteria produce antimicrobial peptides that stimulate protective immune responses and prevent colonization and invasion by harmful pathogens. On average, humans produce 1–2 liters of saliva daily, and small amounts of saliva are secreted continually.

Saliva contains abundant epithelial cells that are shed into the oral fluid, and they are a convenient source of suitable material for genetic analysis [1]. The U.S. Food and Drug Administration approved the use of saliva as a direct-to-consumer (DTC) tool for genetic research in 2017, and saliva was approved as a human diagnostic specimen to determine an individual's predisposition to 10 diseases or conditions [2]. Over the last 5 years, DTC genetics companies like 23andMe™ and AncestryDNA™ have brought discussions about saliva testing to the dinner table through marketing and promotions, making saliva the preferred genetic biospecimen over other sample types. More recently, saliva collection has emerged as a viable alternative to more invasive nasopharyngeal (NP) swabbing for the detection of respiratory viruses, such as the novel coronavirus SARS-CoV-2.

Sample collection for the detection of infectious respiratory disease agents

NP swabs are considered the optimal specimen type for detecting infectious respiratory disease agents. To collect an NP swab, a participant is first asked to clear their nasal passages. A trained medical professional in full personal protective equipment (PPE) must insert a long, thin, flexible swab into the participant's nasal cavity up to 5 inches deep to reach the nasopharynx. Most participants characterize the experience as mildly to severely uncomfortable. NP swab collection typically triggers sneezing, coughing, and tearing, which increases the risk of viral transmission. Once a participant has experienced NP sample collection, they may be reluctant to participate in repeat testing. Thus, routine collection of NP swabs for the study of SARS-CoV-2 respiratory virus is not ideal.

Saliva sampling is a more attractive way to collect biospecimens for SARS-CoV-2 research applications because it is well-tolerated. Saliva can be self-collected, which reduces the need for collection professionals and requires up to 90% less PPE than more invasive specimen collection procedures [3]. Most research-based molecular analyses can be performed with as little as 200 µL of whole saliva. Thus, collecting 1–2 mL of saliva in 3–5 minutes provides ample specimen for routine molecular analyses. Studies have demonstrated that the SARS-CoV-2 virus is present in saliva in quantities that can be detected by reverse transcription–quantitative polymerase chain reaction (RT-qPCR) analysis [4]. To demonstrate that common respiratory viruses could be detected in saliva, 2 mL of raw saliva was collected from 46 donors and diluted 1:1 in phosphate-buffered saline. The diluted samples were spiked with ZeptoMetrix™ RP Multimarker targets, which included coronavirus 229, adenovirus, coronavirus NL6, influenza A, and enterovirus. The samples were then left overnight at ambient temperature. Using the Applied Biosystems™ MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Collection Kit [5], nucleic acid from 200 µL of the spiked saliva sample from each donor was extracted on the Thermo Scientific™ KingFisher™ Flex Purification System. RT-qPCR was utilized to detect the viral RNA, DNA, and bacterial targets listed in Table 1 using Applied Biosystems™ TaqMan® assays along with TaqMan® Fast Virus 1-Step Master Mix on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System with the suggested cycling conditions.

All targets were efficiently detected across 46 different saliva samples utilizing the MagMAX Viral/Pathogen II extraction workflow. Figure 1 shows the consistency of detection across all donors. The mean C_t is reported by donor for each qPCR target.

RNA in raw saliva is stable at room temperature for up to 7 days, whereas some stabilized saliva samples can be stored for up to 14 days. To demonstrate the stability of raw saliva, 4 saliva samples were collected and spiked with inactivated SARS-CoV-2 virus from BEI Resources. All samples were spiked with the same concentration of inactivated virus and left at ambient temperature for 7 days. Nucleic acid was extracted from the samples on day 1 and day 7 using the MagMAX Viral/Pathogen II (MVP) Nucleic Acid Isolation Kit on the KingFisher Flex system. The extracted samples were run on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System to determine the C_t values. Figure 2 shows minimal variation between RNA detection on day 1 and day 7. RNA was thus stable in saliva at ambient temperature for up to 7 days. Donor-to-donor variation was observed for each target. Although the S gene and *orf1ab* targets varied more by donor than the N gene, overall consistency was observed when all three targets were averaged over a 7-day period.

Table 1. Viral RNA, DNA, and bacterial targets.

RP1 and RP2 spike-in; donor-independent	Enterovirus		(+)ssRNA
	SARS-CoV-2 target	OC43	
		NL63	
		229E	
	Influenza A pan assay		(–)ssRNA
	Adenovirus type 3		dsDNA
Donor-dependent	16S		Bacterial RNA

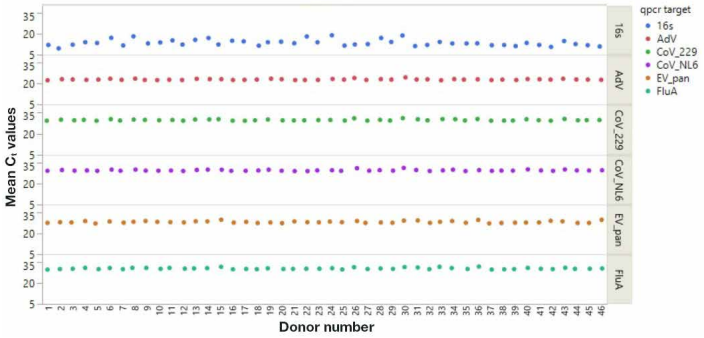


Figure 1. Mean C_t values for each target per donor. The C_t values obtained for each TaqMan Assay (16S, adenovirus, coronavirus 229, coronavirus NL6, enterovirus, and influenza A) were averaged and reported for each donor.

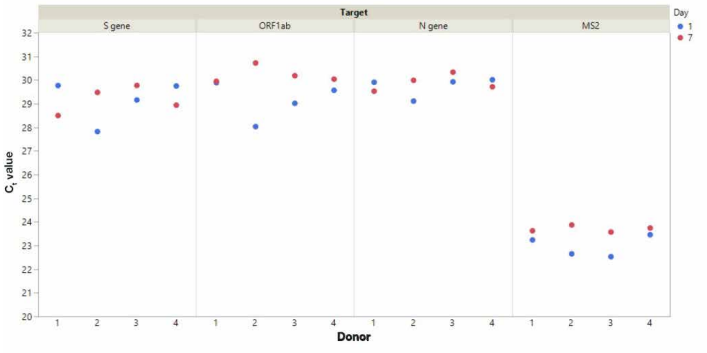


Figure 2. C_t values of samples from 4 donors stored 1 day and 7 days after collection. Minimal variation was observed after day 1 and day 7.

Saliva collection kits

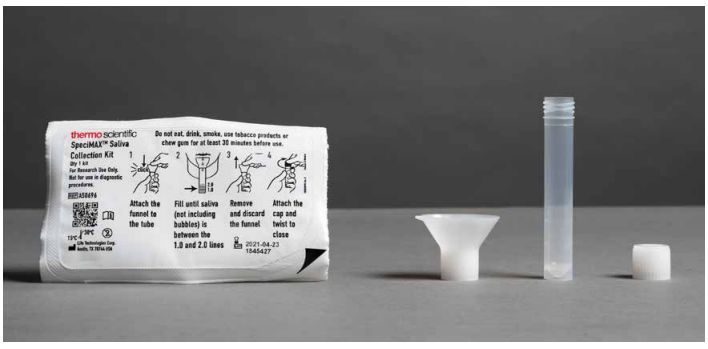
Despite the advantages of saliva as a biospecimen for detecting SARS-CoV-2 and other respiratory viruses, the scientific community continues to be hampered by shortages of oral fluid collection kits that offer convenient saliva collection features and concomitant standardized integration into automated laboratory workflows for high-throughput testing.

To cut costs in times of great financial strain, such as during the global SARS-CoV-2 crisis, large-scale routine SARS-CoV-2 studies have employed empty 15 mL or 50 mL "home-brew" centrifuge tubes, urine collection cups, suitably sized cryopreservation tubes, and standard laboratory test tubes to collect and store saliva in the absence of an affordable saliva collection kit. Saliva collected using empty home-brew tubes is referred to as raw or whole saliva. Raw saliva is convenient because it can be collected in laboratory containers that are readily available and cost only a small fraction of what commercial saliva collection kits cost. However, the convenience and price are outweighed by the heavy burden that is imposed on laboratories that process these samples. When a laboratory receives raw saliva in home-brew containers like urine cups or 50 mL conical tubes, a staff of laboratory professionals must manually transfer saliva from these containers to standard sample plates or tubes for further processing. This process can be exceptionally tedious and time-consuming. For example, if a laboratory wants to process 1,000 saliva samples, transferring the saliva out of one non-standard tube into a tube or plate that is compatible with robotics can take more than 8 hours.

The SpeciMAX Saliva Collection Kit

The Thermo Scientific™ SpeciMAX™ Saliva Collection Kit is a solution for convenient and consistent raw saliva collection. Each SpeciMAX Saliva Collection Kit is composed of a 6 mL tube, a screw cap, and a snap-on funnel packaged together in an individual blister pouch that is convenient for distribution and saliva collection (Figure 3). The SpeciMAX Saliva Collection Kit makes collecting saliva from study participants easy with convenient user instructions printed directly on the package. Participants assemble the kit by attaching the 35-mm wide funnel to the top of the collection tube and drool into the funnel. Fill volumes are indicated on the SpeciMAX collection tube to allow for accurate collection volumes. The flat-bottom collection tube allows the user to set it on a tabletop without using a tube rack or special holder. After saliva collection is complete, the user removes and discards the funnel and attaches the screw cap to secure the saliva in the tube [6]. SpeciMAX tubes are compatible with most liquid handlers from manufacturers such as Tecan™ and Hamilton™, as well as fully automated sample transfer processing systems like the automated MGISTP-7000 high-throughput sample transfer processing system from MGI Tech™ [7].

A



B

Figure 3. SpeciMAX Saliva Collection Kit. (A) Image of an individually packaged and barcoded SpeciMAX Saliva Collection Kit. **(B)** The kit includes an an easy-to-use snap-on funnel and a standard, automation-friendly 6 mL collection tube with visible 1 and 2 mL fill lines.

The small footprint of the SpeciMAX collection tube makes it conveniently designed for high-efficiency storage at 4°C or an incubator, which is needed to inactivate virus in raw saliva samples. A maximum of 108 home-brew 50 mL conical tubes can fit in a 104 L (3.64 ft.^{3}) Thermo Scientific™ Heratherm™ Incubator. In comparison, up to 648 SpeciMAX collection tubes can fit in an equivalent space due to their more compact size. When considering storage of saliva collection tubes, the smaller standard size of the SpeciMAX collection tubes allows users to store 6 times more tubes in the same space occupied by home-brew collection tubes. Table 2 details the SpeciMAX Saliva Collection Kit dimensions.

Saliva is relatively abundant and readily available, and saliva collection is noninvasive and easy to perform for adolescents, adults, and elderly populations. An individual can hold a tube and deposit 1–2 mL of saliva in less than 5 minutes with minimal assistance from a professional. Thermo Fisher offers a full saliva workflow to detect respiratory viruses for research and monitoring. The workflow includes sample collection with the SpeciMAX Saliva Collection Kit; nucleic acid extraction with the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on a KingFisher system; and detection and analysis of targets via RT-PCR using an Applied Biosystems™ QuantStudio™ Real-Time PCR system (Figure 4).

SpeciMAX kits offer solutions for the collection of raw or stabilized saliva for various downstream applications, such as qualitative or quantitative detection of RNA, DNA, and bacteria that cause respiratory conditions.

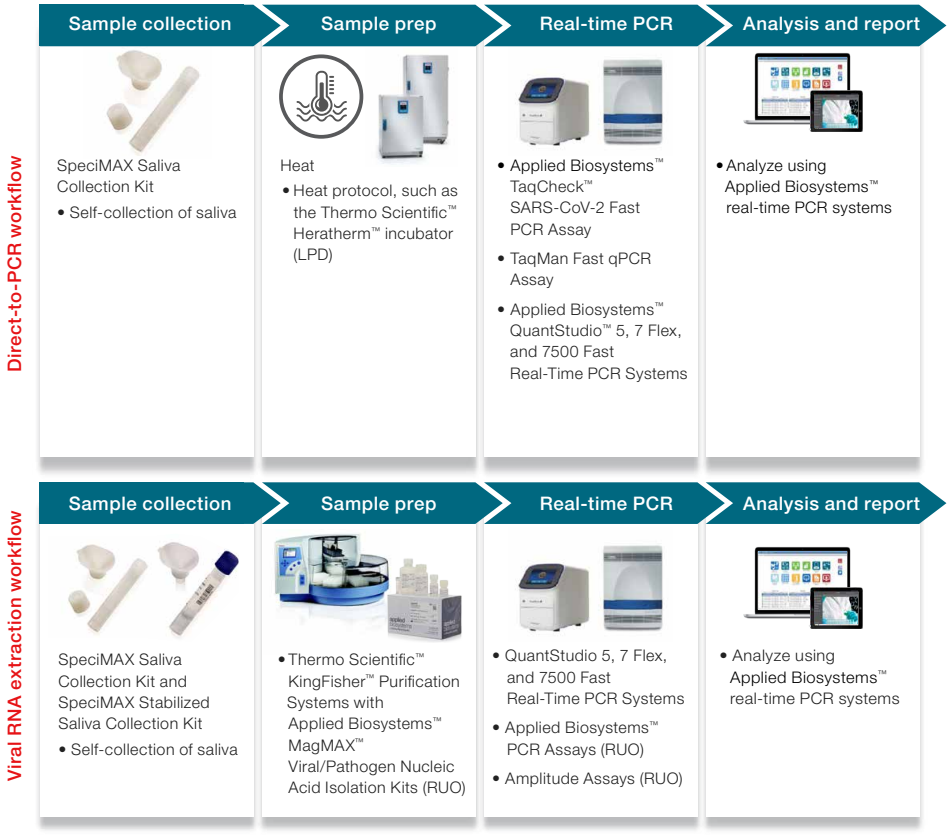


Figure 4. Workflow from sample collection to detection. A direct-to-PCR workflow allows simple and direct processing of raw saliva samples for PCR research applications. Kits for raw and stabilized saliva enable RNA extraction workflows with manual or automated bead-based extraction for downstream research applications.

Table 2. SpeciMAX Saliva Collection Kit component dimensions.

Kit components	Dimensions
Tube height	80 mm
Tube diameter	12 mm
Tube capacity	6 mL
Tube cap diameter	12 mm
Funnel width	35 mm
Funnel height	15 mm

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References

1. Endler G, Greinix H, Winkler K, Mitterbauer G et al. (1999) Genetic fingerprinting in mouthwashes of patients after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 24(1):95–98, doi:10.1038/sj.bmt.1701815.
2. U.S. Food and Drug Administration (2018) FDA allows marketing of first direct-to-consumer tests that provide genetic risk information for certain conditions. **https://www.fda.gov/news-events/press-announcements/fda-allows-marketing-first-direct-consumer-tests-provide-genetic-risk-information-certain-conditions**.
3. Karow J (2020) Alternative sample types could boost COVID-19 testing. *Modern Healthcare*, **https://www.modernhealthcare.com/patients/alternative-sample-types-could-boost-covid-19-testing**.
4. Supriya L (2021) SARS-CoV-2 viral load in saliva may be linked to COVID-19 disease severity. *News Medical Life Sci*, **https://www.news-medical.net/news/20210107/SARS-CoV-2-viral-load-in-saliva-may-be-linked-to-COVID-19-disease-severity.aspx.**
5. Applied Biosystems MagMAX Viral/Pathogen II (MVP II) Nucielc Acid Isolation Kit (RUO). Thermo Fisher Scientific, **thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/automated-purification-extraction/automated-magmax-kits-nucleic-acid-extraction/magmax-viral-pathogen-extraction-kits.html**.
6. Thermo Scientific SpeciMAX Saliva Collection Kit. **thermofisher.com/order/catalog/product/A50696#/A50696**.
7. MGISTP-7000. MGI Tech, **https://en.mgi-tech.com/products/instruments_info/16/**.

Ordering information

Product	Cat. No.
SpeciMAX Saliva Collection Kit	A50696 (400 kits) A50696P10 (10 kits)
SpeciMAX Stabilized Saliva Collection Kit	A50697 (100 kits) A50697P10 (10 kits)
KingFisher Flex Purification System	5400610
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	A48383R
TaqMan Fast Virus 1-Step Master Mix	4444436
TaqMan Gene Expression Assays	4331182
QuantStudio 12K Flex Real-Time PCR System	4471087
QuantStudio 5 Real-Time PCR System	A28575 (384-well) A28568 (96-well)

Find out more at **thermofisher.com/specimaxsaliva**

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