Improved total RNA and DNA detection and preservation with the SpeciMAX Stabilized Saliva Collection Kit

Introduction

The Thermo Scientific™ SpeciMAX™ Saliva Collection Kit and the Thermo Scientific™ SpeciMAX™ Stabilized Saliva Collection Kit are now offered for raw and stabilized saliva collection, respectively. Depending on the research need, either the raw collection kit or the stabilized kit may be more appropriate. In some instances, RNA and DNA extraction occurs within days of specimen collection, so a stabilizer may not be necessary. In other cases, sensitive RNA and DNA may be stored at ambient temperature for longer periods of time before extraction. In this case, a stabilizer is necessary to reduce the risk of microbial growth degrading RNA and DNA in the specimen. Due to the crisis caused by SARS-CoV-2 infections, saliva sampling has become a major focus. Saliva collection can be a high-quality and noninvasive alternative to more invasive specimen collection methods, such as nasopharyngeal and nasal swabbing [1]. This study compares the SpeciMAX Stabilized Saliva Collection Kit to other kits currently on the market for several research applications.

Background

The SpeciMAX Stabilized Saliva Collection Kit was designed for easy collection and compatibility with workflows ranging from manual to fully automated for the detection of RNA, mRNA, and DNA. The dimensions of the collection tube (12.9 x 80 mm) allow it to conveniently fit in automation equipment like liquid handlers and decappers. The chemistry of the SpeciMAX Stabilized Saliva Collection Kit preserves the contents of the specimen and inactivates most respiratory viruses. Post-collection, the specimen can be stored at ambient temperature due to stabilization. Compared to two other on-the-market saliva collection kits, the SpeciMAX Stabilized Saliva Collection Kit provides comparable or better results in terms of specimen stability and full workflow.

Experimental design

Two commonly used, on-the-market saliva collection kits—kit A and kit B—were compared to the SpeciMAX Stabilized Saliva Collection Kit. Kit A and the SpeciMAX stabilized kit were used across 32 donors to test the sample-to-sample variability of RNA extracts spiked with a low or medium concentration of gamma-irradiated and inactivated SARS-CoV-2. Fresh, unfrozen saliva samples were collected from the donors using both the SpeciMAX stabilized kit and kit A. Low and medium concentrations of inactivated SARS-CoV-2 virus (BEI Resources) were spiked directly into the kits and mixed thoroughly. The samples were then left overnight at ambient temperature. Nucleic acid was extracted from the samples using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (RUO) and the saliva extraction workflow on the Thermo Scientific™ KingFisher™ Flex Purification System. Quantitative PCR (qPCR) was performed using Applied Biosystems™ TaqMan® reagents to determine C_t values for the SARS-CoV-2 N gene target and MS2 (Figure 1A).

To determine workflow compatibility, kit B was tested with three different extraction kits targeting RNA, genomic DNA (gDNA), mRNA, and microRNA (miRNA) after 7 days of storage at ambient temperature. Fresh, raw saliva samples were collected from 5 different donors using the SpeciMAX kit, kit B, and 15 mL conical tubes. The samples were then spiked with the BEI inactivated virus and ATCC™ VR-1™ human adenovirus at medium concentrations and allowed to sit for 7 days. Viral extraction was performed on days 1 and 7 of incubation using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (RUO) with the saliva extraction protocol on the KingFisher Flex Purification System. TaqMan reagents were utilized to detect the N gene of the RNA virus along with the MS2 internal extraction control and DNA adenovirus on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System in 384-well format (Figure 1B).

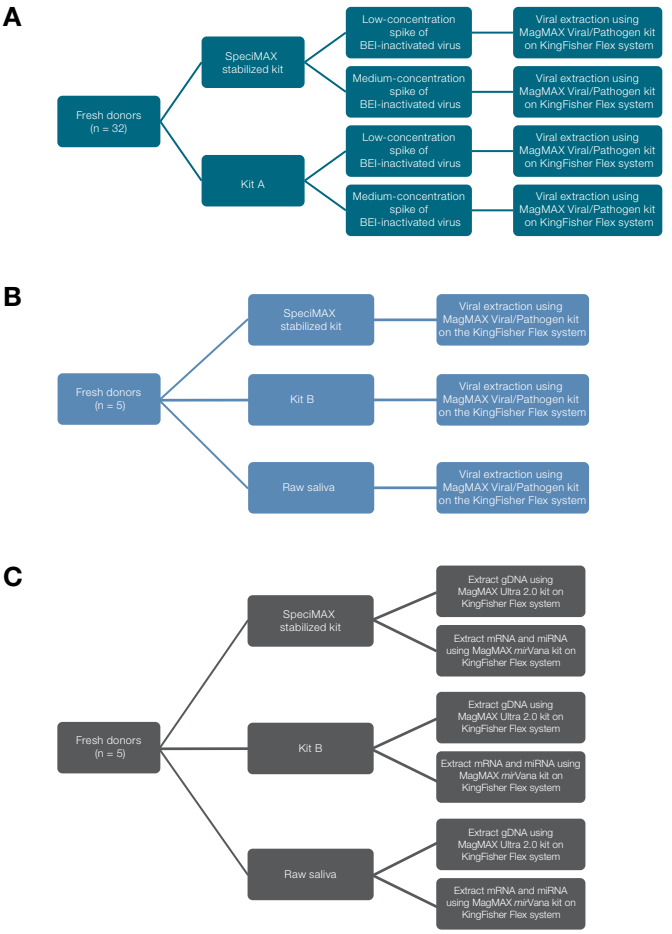

Figure 1. Experimental summary. (**A**) Comparison of RNA detection with saliva samples collected using the SpeciMAX stabilized kit and kit A. (**B**) Viral extraction with the MagMAX Viral/Pathogen II kit on the KingFisher Flex system and detection of RNA and DNA utilizing the SARS-CoV-2 N gene, adenovirus, and RNase P. (**C**) gDNA, mRNA, and miRNA extraction with the MagMAX DNA Multi-Sample Ultra 2.0 Kit and MagMAX *mir*Vana kit across saliva samples collected from 5 donors using the SpeciMAX kit, kit B, and raw saliva.

An Applied Biosystems™ TaqMan® RNase P assay was utilized as an internal control on the QuantStudio 5 PCR system in 384-well format. To determine gDNA extraction efficiency, gDNA was extracted from the same 5 samples collected with the SpeciMAX stabilized kit, kit B, and 15 mL conical tubes using the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit and saliva extraction protocol on the KingFisher system. TaqMan reagents were utilized for qPCR to target the *ACTB* and *GAPDH* genes on the QuantStudio 5 PCR system using a 384-well plate. The Applied Biosystems™ MagMAX™ *mir*Vana™ Total RNA Isolation Kit was used to extract mRNA and micro RNA (miRNA) from the same 5 donor samples (Figure 1C). TaqMan reagents were used to target *PKG1* and *B2M* for mRNA detection, and *let-7B* and *mir24* were targeted for miRNA detection.

Results and discussion

Among the 32 donors collected with SpeciMAX stabilized Kit and Device A, little not no variation was observed in the N gene target after spiking with a medium concentration of inactivated SARS-CoV-2. As the saliva specimens varied in consistency and content, some variation in the MS2 C_t values was observed. This was expected due to natural donor-to-donor variation. However, use of kit A resulted in higher MS2 C_t values across all samples. At the lower concentration of BEI inactivated virus, the N gene target indicated that kit A retained less virus than the SpeciMAX stabilized kit (Figure 2). The data showed that the SpeciMAX stabilized kit was comparable or better across 64 samples with low and medium viral RNA concentrations when extracted using the MagMAX Viral/Pathogen II kit.

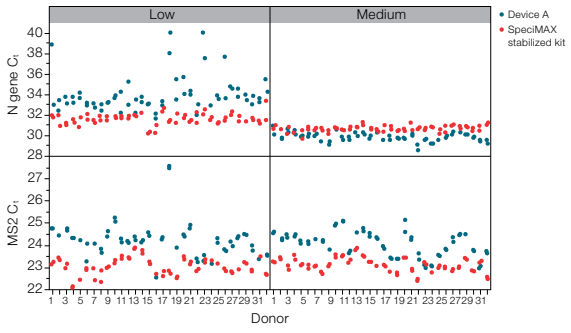

Figure 2. Comparison of C_t values for samples collected using the SpeciMAX stabilized kit and kit A. Nucleic acid was extracted from the samples using the MagMAX Viral/Pathogen II kit (RUO) and saliva extraction protocol on the KingFisher Flex system. TaqMan qPCR reagents were used to target the N gene and MS2 from 32 donor samples.

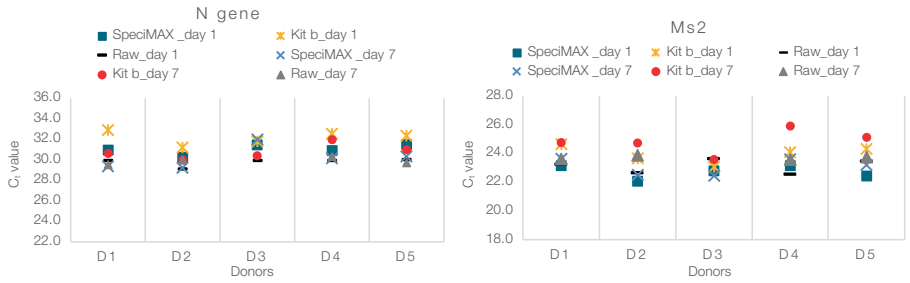

Figure 3. Comparison of C_t values for samples collected using the SpeciMAX stabilized kit, kit B, and raw saliva collected in 15 mL conical tubes across 7 days. Nucleic acid was extracted using the MagMAX Viral/Pathogen II kit and the 200 µL saliva protocol on the KingFisher Flex system. TaqMan qPCR reagents were used to target the N gene and MS2 from the samples.

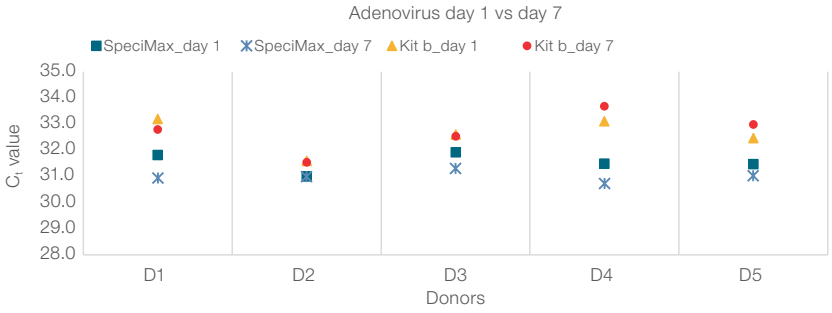

Figure 4. Comparison of C_t values for samples collected using the SpeciMAX stabilized kit, kit B, and raw saliva collected in 15 mL conical tubes across 7 days. Nucleic acid was extracted using the MagMAX Viral/Pathogen II kit and the saliva extraction protocol on the KingFisher Flex system. TaqMan qPCR reagents were used to target DNA adenovirus from the samples.

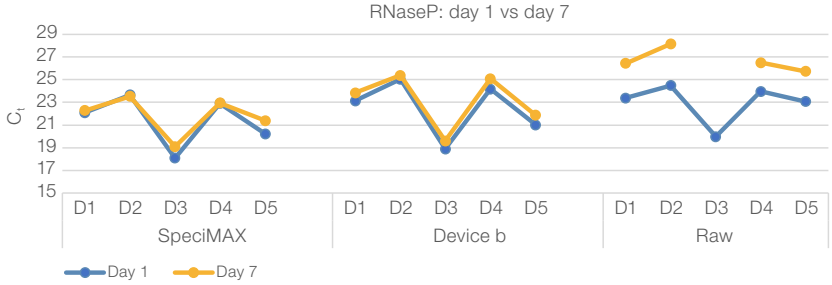

Figure 5. Comparison of C_t values for samples collected using the SpeciMAX stabilized kit, kit B, and raw saliva collected in 15 mL conical tubes across 7 days. Nucleic acid was extracted using the MagMAX Viral/Pathogen II kit and the saliva extraction protocol on the KingFisher Flex system. TaqMan qPCR reagents were used to target RNase P from the samples.

Viral extraction from saliva samples collected with the SpeciMAX stabilized kit, kit B, and raw saliva using the MagMAX Viral/Pathogen II kit (RUO) showed minimal variation in C_t values for the N gene and MS2 from day 1 to day 7 (Figure 3). At the medium concentration of BEI inactivated virus, the mean C_t values varied by less than 2 C_t. Samples containing adenovirus and viral DNA showed even less variation across the two collection kits and raw saliva samples (Figure 4). The level of RNase P remained stable over the course of a week in samples collected using the SpeciMAX stabilized kit and kit B. However, raw saliva samples showed a reduction in RNase P on day 7. Not enough sample was collected from donor 3, so analysis of the raw saliva could not be conducted on day 7 (Figure 5).

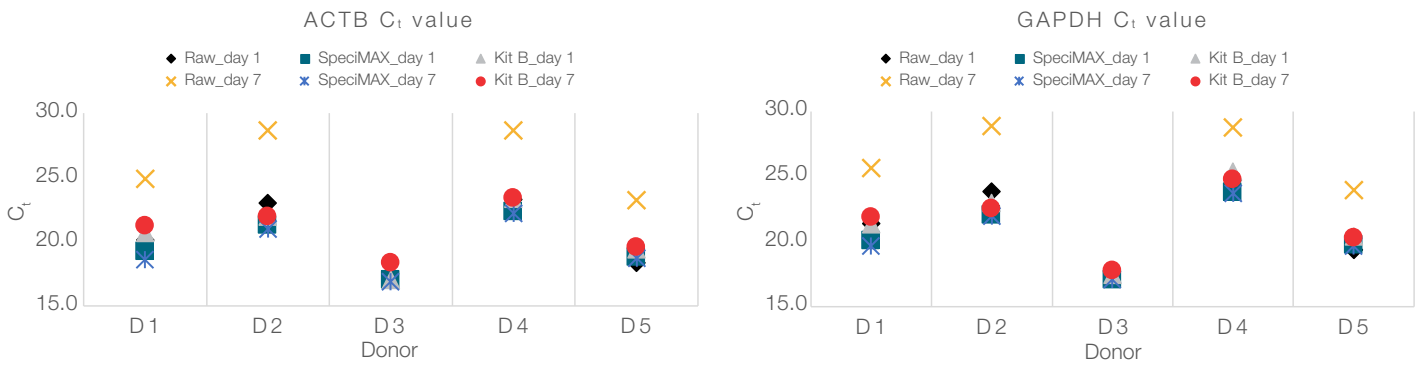

Figure 6. Comparison of C_t values for samples collected using the SpeciMAX stabilized kit, kit B, and raw saliva collected in 15 mL conical tubes across 7 days. Genomic DNA was extracted using the MagMAX DNA Multi-Sample Ultra 2.0 Kit and the saliva extraction protocol on the KingFisher Flex system. TaqMan qPCR reagents were used to target *ACTB* and *GAPDH* from the samples.

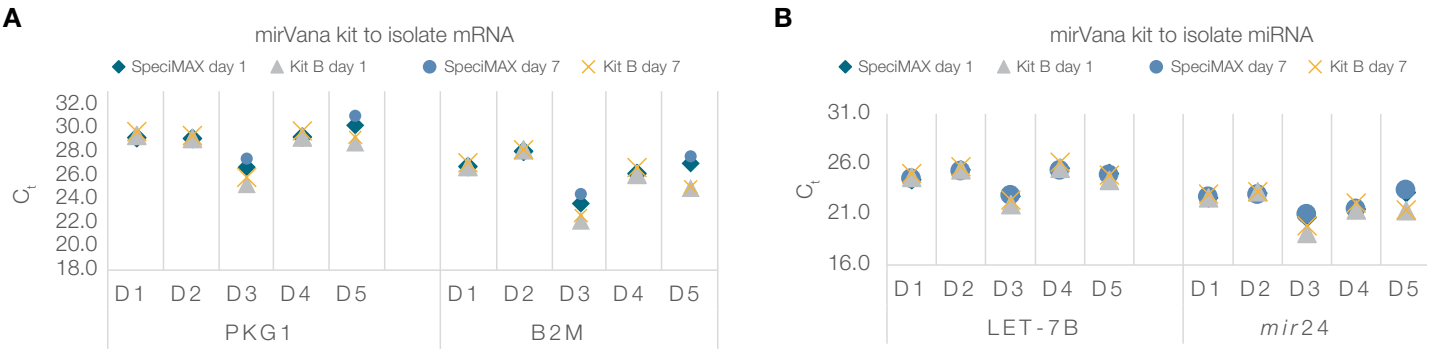

Figure 7. Comparison of C_t values for samples collected using the SpeciMAX stabilized kit, kit B, and raw saliva across 7 days. (**A**) mRNA and (**B**) miRNA were isolated using the MagMAX *mir*Vana Total RNA Isolation Kit and the saliva protocol on the KingFisher Flex system. qPCR reagents were used to target mRNAs *PKG1* and *B2M* and miRNAs *let-7B* and *mir24*.

gDNA was extracted from the same 5 donor samples after 7 days at ambient temperature with the MagMAX DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Flex system using the saliva protocol. TaqMan assays were used to detect the housekeeping genes *ACTB* and *GAPDH* on the QuantStudio 5 Real-Time PCR System. Figure 6 shows that gDNA extraction from raw saliva is far less efficient after 7 days of storage when compared to samples collected and stored using kit B and extracted using the MagMAX DNA extraction kit. The samples that were collected and stored using the kits showed minimal variation in C_t values from day 1 to day 7.

mRNA and miRNA were extracted from the same samples using the MagMAX *mir*Vana Total RNA Isolation Kit. All samples displayed minimal variation in mRNA and miRNA extraction efficiency, as shown in Figure 7. TaqMan assays were used to detect the *PKG1* and *B2M* mRNA targets and *let-7B* and *mir24* miRNA targets. The C_t values varied minimally from day 1 to day 7.

Conclusions

The SpeciMAX stabilized kit is capable of stabilizing RNA and DNA in saliva samples for up to a week at ambient temperature, and it is fully compatible with multiple bead-based extraction kits utilizing the KingFisher Flex system. The SpeciMAX stabilized kit was fully compatible with the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (RUO), the MagMAX Multi-Sample Ultra 2.0 Kit, and the MagMAX *mir*Vana Total RNA Isolation Kit. The SpeciMAX stabilized kit had comparable or better stabilizing capability than two other stabilizing saliva collection kits, kit A and kit B. Although there was natural donor-to-donor variation between the saliva samples, the SpeciMAX stabilized kit improved detection of RNA in samples from 32 donors that were spiked with low and medium concentrations of inactivated SARS-CoV-2 virus. The SpeciMAX stabilized kit was also more effective for preserving RNA, DNA, gDNA, mRNA, and miRNA for up to a week when compared to kit B and raw saliva samples collected and stored in conical tubes.

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Ordering Information

Description	Cat. No.
SpeciMAX Saliva Collection Kit	A50696
SpeciMAX Stabilized Saliva Collection Kit	A50697
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (RUO)	A48383R
KingFisher Flex Purification System with 96 Deep-Well Head	5400630
MagMAX *mir*Vana Total RNA Isolation Kit	A27828
MagMAX DNA Multi-Sample Ultra 2.0 Kit	A36570
TaqMan 2019-nCoV Assay Kit v1 (singleplex)	A47352
QuantStudio 5 Real-Time PCR System, 384-well	A428140
TaqMan Gene Expression Assay (FAM)	4331182

Reference

1. Williams E, Isles N, Chong B et al. (2021) Detection of SARS-CoV-2 in saliva: implications for specimen transport and storage. *J Med Microbiol* 70(2):001285, doi:10.1099/jmm.0.001285.

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