Stability of RNA in GeneFix RFX-01 Saliva Collectors over 60 days at Room Temperature

Salivary RNA's utility as a diagnostic medium has been steadily increasing, particularly for uses such as infectious disease surveillance (e.g., SARS-CoV-2), oncology, and systemic disease monitoring, via rt-qPCR and RNAseq transcriptomic analysis. Effective stabilisation protocols are essential to maximise data quality and comparability across studies. Proper RNA stabilisation enables the full potential of saliva as a viable, non-invasive alternative to blood for molecular diagnostics and biomarker discovery.

However, RNA species are a challenging biomolecule to collect and preserve, particularly in saliva samples, due to their inherent instability and the pervasiveness of RNAses and other contaminants, which rapidly cause degradation of samples if not properly stabilised. Isohelix's unique, non-toxic RNA saliva stabilization buffer system immediately preserves RNA in saliva upon collection at room temperature, and utilises the successful GeneFiX™ collection system for quick and easy sampling from donors.

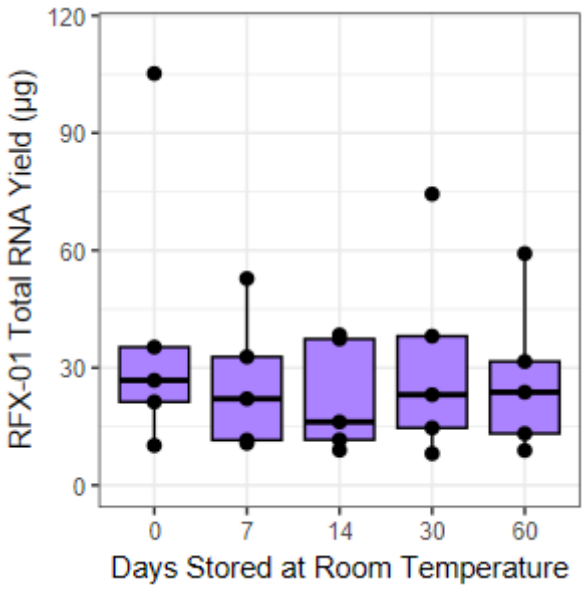
Methods & Materials

In this study, five donors each collected 1ml of saliva into GeneFix RFX-01 stabilisation kits which were then stored at Room Temperature (15-30°C), with sample aliquots taken for extraction and analysis at 7, 14, 30, & 60 days. An initial control aliquot was also taken upon return to the lab for comparison. Following incubation 250µl sample aliquots underwent RNA extraction and DNAse treatment before yield and purity assessment using Qubit™ RNA and UV spectroscopy.

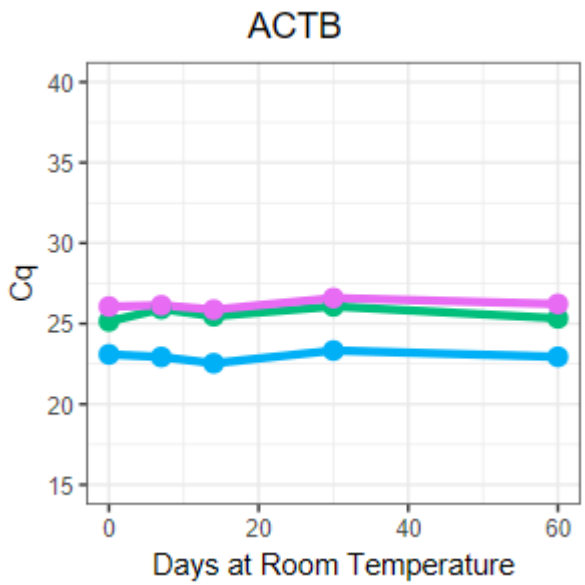
Once assessed, 10ng/µl RNA dilutions were prepared from a subset of 3 RFX donor samples, which were then used as template in rt-qPCR amplification using ACTB and GAPDH gene targets using a BioRad™ CFX-Connect thermal cycler. Appropriate no-template and no-reverse transcriptase controls were also included to rule out contamination. Cq cycle thresholds for each gene target were compared between time points to assess RNA stability at room temperature.

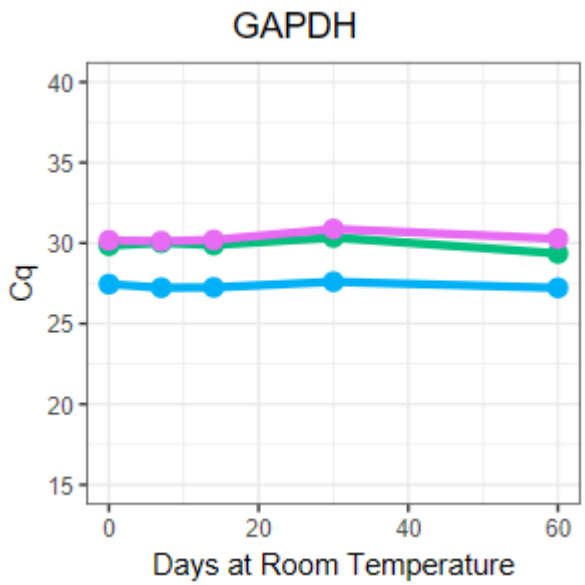
Results & Discussion

Median Results: RNA Yield & Purity					
	Fresh	**7 Days**	**14 Days**	**30 Days**	**60 Days**
RNA Concentration (ng/µl)	67.0	55.2	40.4	57.7	59.4
250µl Aliquot RNA Yield (µg)	3.35	2.76	2.02	2.89	2.97
Total RNA Yield from an RFX-01 kit (µg)	26.80	22.08	16.16	23.08	23.76
A260/280	2.10	2.07	2.02	2.03	2.04
A260/230	1.70	1.76	2.07	2.02	1.83



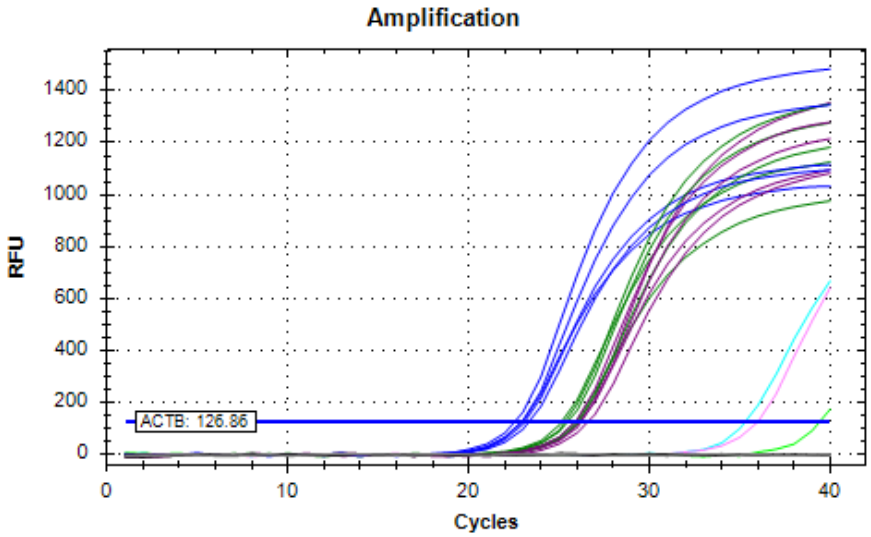
RNA yields of RFX-collected saliva samples remained consistent over the 60-day incubation period, with estimated total yields ranging from 8.04-105.2 µg per RFX-01, with a median yield of 23.08 µg. RNA purities also remained high, with a median A260/280 of 2.05 across all samples and time-points.





Subsequent rt-qPCR amplification and analysis of the collected RFX saliva samples following extraction demonstrated consistent amplification of mRNA from both ACTB & GAPDH gene targets.

Amplification signal remained stable across donors and time points up to 60 days post-collection when samples were stored at room temperature.



Key Takeaways:

- GeneFix RFX-01 effectively preserves salivary RNA samples for periods up to 60 days when stored at room temperature, generating high yields and purity ideal for various downstream applications such as rt-qPCR gene expression or RNAseq transcriptomics.
- Isohelix's tried and tested collection method allows for easy at-home or in-clinic collection, without the need for costly cold-chain transport of samples.
- RFX is highly versatile and is compatible with a wide range of extraction methods, such as Trizol purification and Isohelix's own XMR-50 Xtreme RNA column extraction kit.