Stability of RNA in GeneFix GFX DNA/RNA Collectors over 28 Days

In just over past year, the COVID-19 pandemic has seen an exponential growth in the need for RNA collection and stabilisation solutions for both rt-qPCR testing and variant sequencing. RNA is an inherently difficult biomolecule to preserve due to its inherent instability and its susceptibility to ubiquitous ribonucleases in biofluids.

The Isohelix GFX DNA/RNA collector solves this issue by combining an effective method for the stabilisation of RNA in saliva, with an easy to use, ergonomic design for sample collection which is non-toxic & guanidine-free. This paper demonstrates the stability of RNA in GeneFix DNA/RNA collectors over a 28-day period.

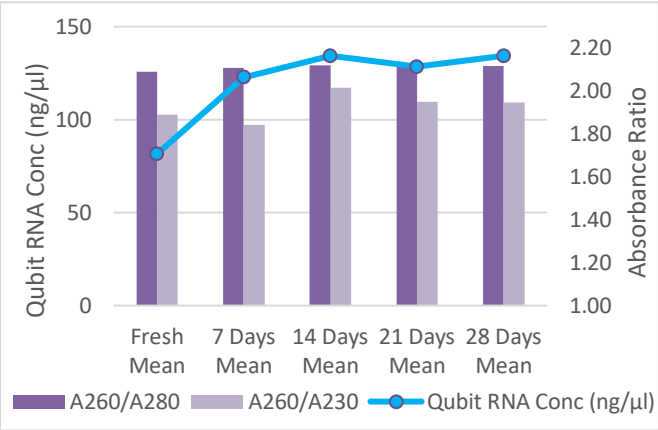
Methods:
Five GFX-02 saliva samples were collected from separate donors. These samples were then pooled together into a 50ml tube and homogenised by vortexing. The prepared bulk sample was then split into five sets of three 500µl aliquots.

Each set of three were stored at ambient temperature (23°C ± 5°C) for variable periods of time to determine the stability of the RNA, the time points being: Fresh samples (0 Day Control), 7 Days, 14 Days, 21 Days, & 28 Days. When each time point was reached samples were stored at -20°C, prior to extracting all samples simultaneously once the final time point had been reached. Samples were extracted using the Zymo Quick-RNA mini prep kit with an elution volume of 50µl.

Following extraction, samples were analysed for purity & yield by Nanodrop & Qubit RNA BR assays. In addition, 10ng of total RNA per sample were used for rt-qPCR analysis targeting the human ACTB gene to assess sample suitability for downstream applications.

Results:
Nanodrop & Qubit Assay:

- Yield and purities of all samples over the 28-day period remained consistently high and of good quality, with a mean yield of 120.3 ng/µl and A260/280 of 2.11.
- These purities and yields were maintained over 28 days.

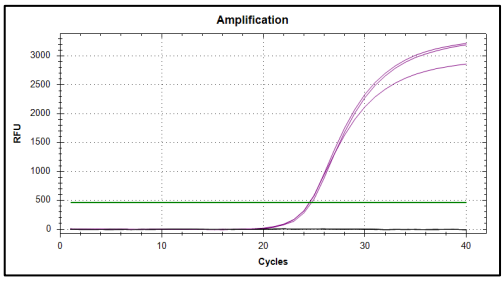


Sample ID	RNA Concentration (ng/µl)	A260/A280	A260/A230
Fresh A	77.9	2.10	2.01
Fresh B	80.0	2.09	1.88
Fresh C	86.8	2.08	1.78
Fresh Mean	**81.57**	**2.09**	**1.89**
7 Days A	131.0	2.10	1.95
7 Days B	122.0	2.12	1.82
7 Days C	115.0	2.11	1.76
7 Days Mean	**122.67**	**2.11**	**1.84**
14 Days A	138.0	2.12	2.09
14 Days B	134.0	2.11	1.97
14 Days C	131.0	2.13	1.98
14 Days Mean	**134.33**	**2.12**	**2.02**
21 Days A	124.0	2.12	2.10
21 Days B	139.0	2.11	1.99
21 Days C	122.0	2.12	1.76
21 Days Mean	**128.33**	**2.12**	**1.95**
28 Days A	143.0	2.12	2.07
28 Days B	135.0	2.11	1.92
28 Days C	125.0	2.12	1.85
28 Days Mean	**134.33**	**2.12**	**1.95**

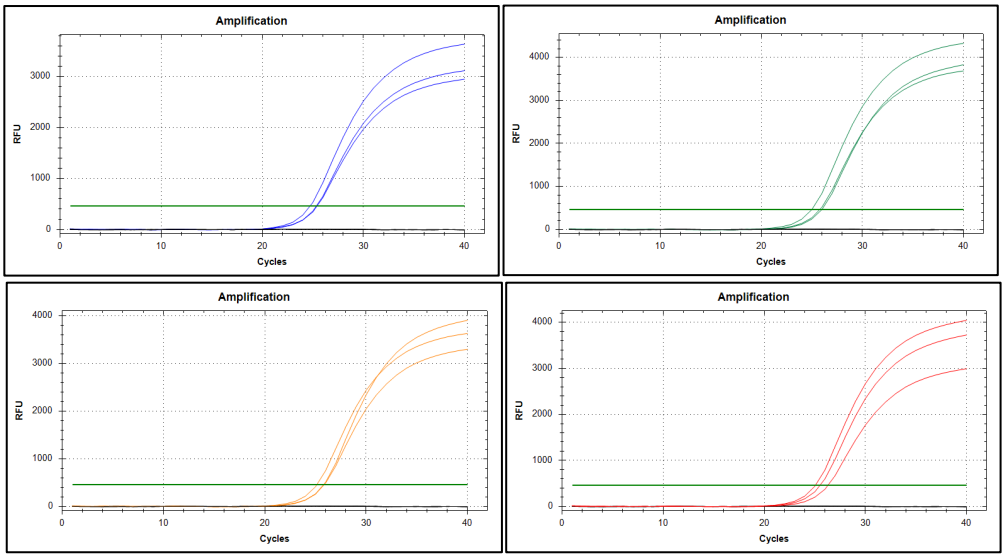
RT-qPCR Analysis:

ACTB Cq

Sample ID	A	B	C	Mean
Fresh	24.52	24.74	24.56	**24.61**
7 Days	25.41	25.34	24.72	**25.16**
14 Days	25.00	26.00	25.77	**25.59**
21 Days	25.89	25.81	25.15	**25.62**
28 Days	26.30	25.51	25.06	**25.63**



- All samples run through RT-qPCR analysis targeting the human ACTB gene successfully amplified, up to 28 days following sample collection.
- In that time Cq values remained stable and consistent, indicating that the yield of intact, amplifiable mRNA had not decreased.



Conclusions:

- Salivary RNA stabilised using Isohelix GeneFix GFX Saliva Collectors remained stable for up to 28 days following collection when stored at ambient temperatures, giving high yield and purity results.
- Subsequent RT-qPCR analysis demonstrated that sample integrity is maintained over this period, providing consistent, repeatable amplification of RNA samples.
- Collection of samples is intuitive, designed for ease of use, and ideal for mailing for at-home sample collection. Samples can also be grouped together for high-throughput processing.