Effects of buccal swab storage conditions on host and microbial nucleic acid stability and quality

Jennifer Whiting, Madhu Jasti, Angie Cheng Thermo Fisher Scientific, 2130 Woodward St, Austin, TX 78744

ABSTRACT

Buccal swabs offer a convenient, non-invasive, and low-cost method for collection of genetic material. This study observed the effects of swab storage on DNA yield and quality for host (human) and microbial DNA from buccal swabs. We compared swabs that were processed fresh (day of collection), versus swabs stored for two weeks at either room temperature or -20°C prior to isolation. Results of this study provide guidance for buccal swab storage practices to improve sample quality.

MATERIALS AND METHODS

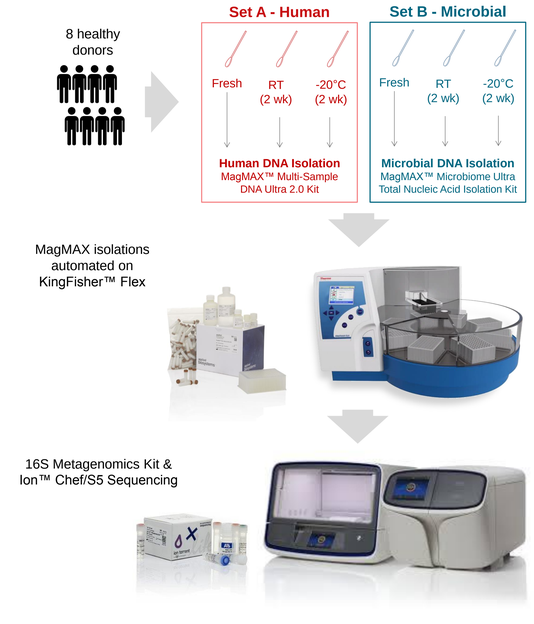
For this study, two sets of matched buccal swabs were collected on consecutive days from eight healthy adult donors (FLOQSwabs™, COPAN). Three swab were collected on the first day (Set A), and three on the second day (Set B). For both sets, a single swab was processed immediately ("Fresh" condition), and the remaining two swabs were returned to their original pouch and stored for two weeks at either at room temperature (RT) or -20°C prior to DNA isolation. Written informed consent for sample collection and isolation was obtained from all participants.

To observe the effects of swab storage on human DNA yield and quality, the first set of swabs (Set A) was processed with the MagMAX™ Multi-Sample DNA Ultra 2.0 Kit to isolate host genomic DNA. To observe effects on microbial yield and/or changes in microbial populations, the second set of swabs (Set B) was processed with the MagMAX™ Ultra Microbiome Total Nucleic Acid Isolation Kit (with bead plate) to isolate microbial nucleic acids. For each set, one swab was processed on the day of collection ("Fresh" condition), and the remaining two swabs were processed after 2 weeks storage. All isolations were automated on the KingFisher™ Flex Purification System, with 50µl elution volume for all.

Nucleic acid yield was measured by Nanodrop, and qPCR with TaqMan assays against RNaseP (human DNA) or pan-bacterial 16S (microbial DNA). Purity was measured by absorbance ratio A260/A280 on Nanodrop™. Nucleic acid size and quality was visualized on a 2% agarose gel with ethidium bromide.

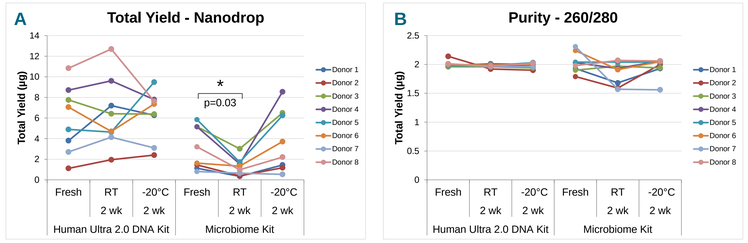
To compare changes in microbial population between storage conditions, samples from Set B were further analyzed by 16S sequencing using the Ion™ Torrent platform. Libraries were prepared with the Ion 16S™ Metagenomics Kit and processed on the Ion Chef™ & Ion S5™ Sequencing Systems. Automated analysis, annotation, and taxonomic assignments were performed with Ion Reporter™.

Figure 1 – Schematic representation of workflow



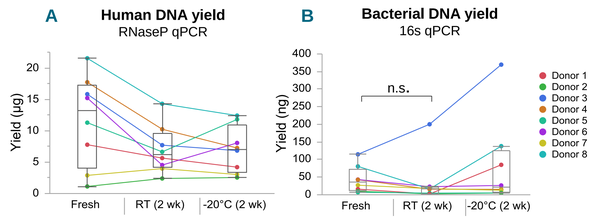
RESULTS

Figure 2 – Total DNA yield and purity by Nanodrop



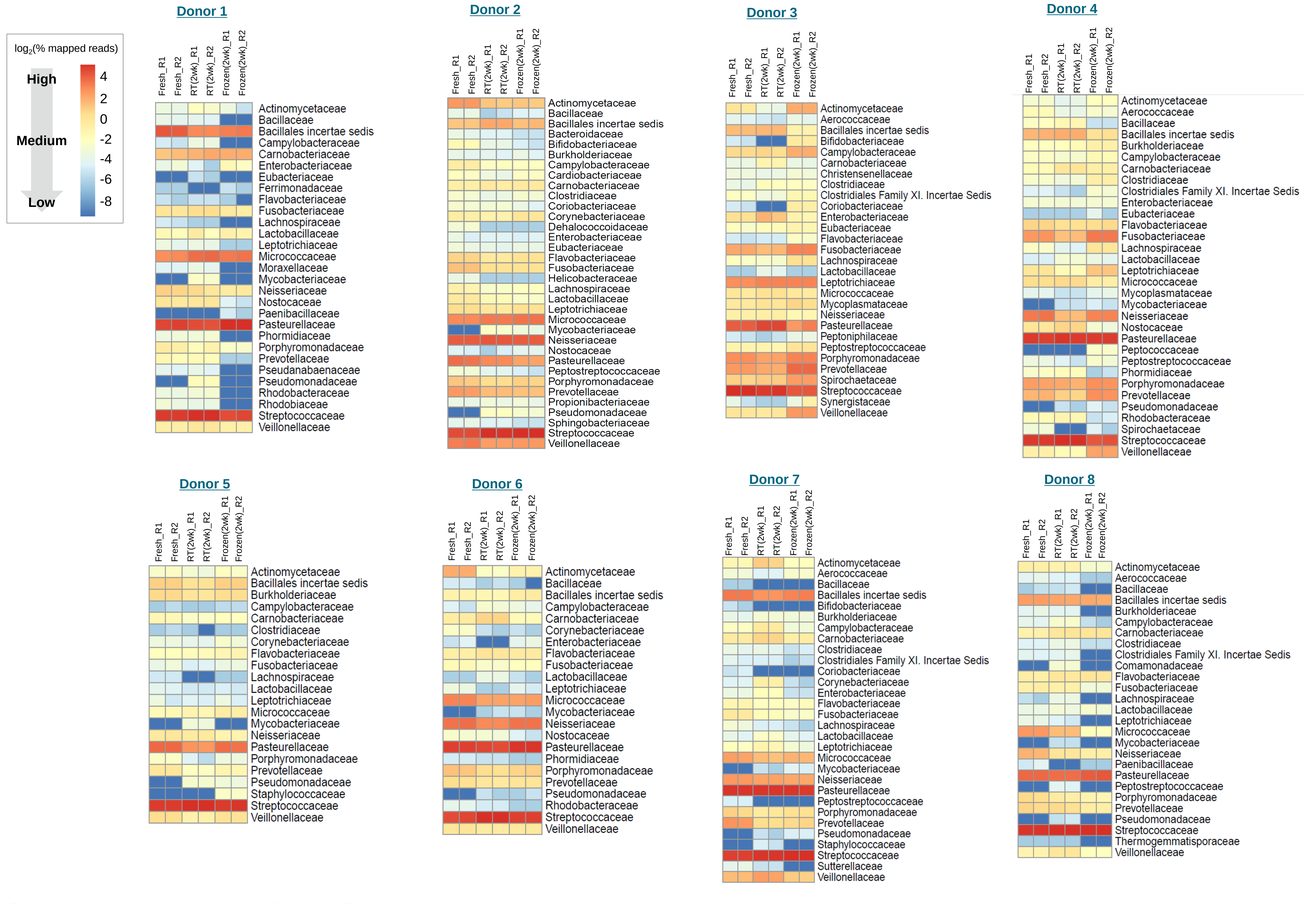
Total DNA yield and purity was measured by Nanodrop for all samples. A) Human DNA yield (Human Ultra 2.0 DNA Kit) varied by donor, but there were no significant differences observed between storage conditions. However, microbial DNA yield (Microbiome Kit) was significantly decreased when swabs were stored at room temperature for 2 weeks compared to fresh (p=0.03, Wilcoxon signed rank test). While there was an upward trend in microbial DNA yield when swabs were stored at -20°C, this difference did not achieve statistical significance. No correlation was observed between human DNA yield and microbial DNA yield for each individual donor. B) Nucleic acid purity was assessed by A260/280 ratio. All Human DNA samples had a ratio of >1.6, with no difference between storage conditions. Microbial nucleic acid isolations resulted in more variable purity ratios, but there were no significant differences between storage conditions.

Figure 3 – Human and microbial yield by qPCR



A) Human DNA yield was measured by qPCR with the human-specific RNaseP TaqMan assay against a standard curve of Human Control DNA. No significant difference was observed between storage conditions. B) Bacterial DNA yield was measured using a pan-bacterial 16s TaqMan assay, with a standard curve of *E. coli* genomic DNA. This data is consistent with Nanodrop measurements showing a downward trend in bacterial DNA yield for swabs stored at -20°C for 2 weeks, but it did not achieve statistical significance in this assay. Trends were consistent between Nanodrop and qPCR, although the absolute yields differed.

Figure 4 – 16S Sequencing Results

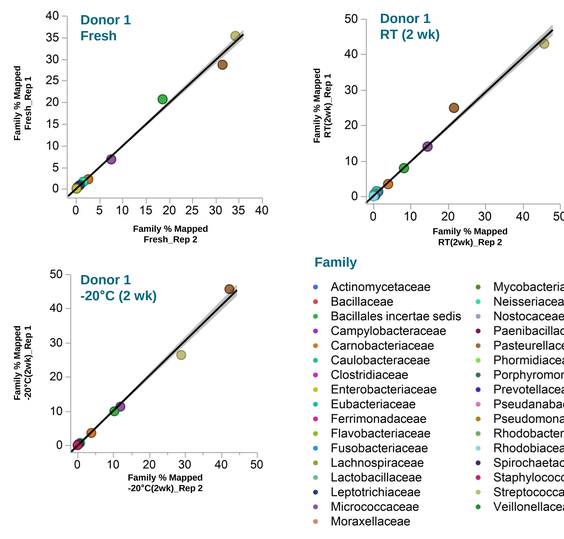


DNA extracted with the microbiome kit (Swab Set B) was further analyzed by 16S sequencing to compare changes in bacterial populations between swab storage conditions. Heat maps represent the percentage of mapped reads assigned to each family (log_2 transformed). Duplicate libraries for each sample are shown (Rep1, Rep2) to illustrate sequencing reproducibility. Donor-to-donor variation in bacterial communities was observed, but within each donor the overall profiles were maintained between Fresh swabs, and swabs that were stored at either room temperature (RT) or frozen (-20°C) for 2 weeks. However, two donors (Donors 1 & 8) had altered community profiles when swabs were stored at -20°C for 2 weeks, with a decrease in percent mapped reads in several families. However, the affected families were not consistent between the donors. Variation may have been due to storage, or may have resulted from swab-to-swab variation during sample collection.

Figure 5 – 16S Sequencing Reproducibility

To compare run-to-run variability in 16S sequencing, the percentage of mapped reads (by Family) were compared between duplicate libraries prepared and sequenced from each microbiome sample (Rep 1, Rep 2). Sequencing profiles were highly reproducible for all duplicates ($R^2 \geq 0.990$ for all pairs, $p<0.0001$ Pearson's r Test). A) R^2 values for duplicate libraries. B) Representative correlation plots for Donor 1, illustrating reproducibility of sequencing results from duplicate libraries by storage condition. Percentage of mapped reads by Family plotted for Rep1 vs Rep2 for each sample. Marker color indicates Family.

Donor ID	% Mapped Reads by Family (R^2 Value)		
	Fresh	RT (2 wk)	-20°C (2 wk)
Donor 1	0.9968	0.9962	0.9965
Donor 2	0.9980	0.9999	0.9997
Donor 3	0.9999	0.9976	0.9900
Donor 4	0.9960	0.9994	0.9995
Donor 5	0.9999	1	0.9987
Donor 6	0.9990	0.9989	0.9999
Donor 7	0.9995	0.9994	0.9996
Donor 8	0.9999	1	0.9998



CONCLUSIONS

- Storing buccal swabs at room temperature or -20°C for 2 weeks did not affect human DNA yield for quality.
- Bacterial DNA yield and quality were maintained when swabs were stored at -20°C for 2 weeks, but yield reduced when swabs were stored for 2 weeks at room temperature.
- Bacterial families detected by 16S sequencing were maintained across storage conditions, however two donors had altered profiles when swabs were stored at -20°C for 2 weeks.
- 16S library preparation and sequencing results are highly reproducible ($R^2 \geq 0.99$ for all duplicate libraries).

In conclusion, we recommend processing buccal swabs fresh when possible. For human DNA isolation, swabs may also be stored either at room temperature or -20°C for 2 weeks with no loss of sample yield or quality. For microbial nucleic acid isolations, we recommend processing fresh or storing at -20°C for up to 2 weeks, as storage at room temperature reduced total yield.

TRADEMARKS/LICENSING