Copan and Puritan flocked swabs provide equivalent quality for epigenetic age prediction

A collaborative study between Tally Health, Tempus, and Mawi DNA Technologies

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Introduction

Epigenetic age prediction relies on accurate and consistent quantification of methylation as the predictions are sensitive to relatively small systematic biases between technologies [1]. Infinium EPIC arrays are a gold standard technology for assaying methylation from human samples collected using cheek swabs. Tally Health's cheek swab epigenetic age test analyzes specific DNA methylation patterns associated with lifestyle, health, and mortality risk [2,3]. Previous work by Mawi DNA Technologies has demonstrated specific DNA yields, integrity, and purity for the Copan and Puritan flocked swabs for buccal cell collection [4,5], however, the effect of swab choice on DNA methylation and age prediction has not been shown. Here we directly evaluate the effect of swab type on methylation quantification and epigenetic age prediction, using a small cohort of healthy adults, and demonstrate that there is no significant difference between Copan or Puritan flocked swabs for epigenetic age prediction.

Study Design

We collected 18 buccal samples from 9 healthy adults using iSWAB-Discovery collection device (Mawi DNA Technologies) with both swab types simultaneously swabbing one cheek per swab in accordance with swab instructions. The iSWAB buccal samples were preprocessed at Tempus using the Beckman DNAdvance kit, and the Biomek i7 instrument to extract the DNA. Once extracted, the Quant-iT™ PicoGreen™ dsDNA Assay Kits and dsDNA Reagents kit were used to quantify the DNA yield according to Illumina's protocols array preprocessing and loaded onto a MethylationEPIC v2.0 935K array. Raw methylation results were processed by Tally Health to yield epigenetic age predictions as described previously [2].

Results

18 buccal samples from 9 healthy adults were collected using iSWAB-Discovery (Mawi DNA Technologies) with both swab types according to instructions for use. DNA samples were preprocessed and quality tested (Picogreen for DNA quantity and Bioanalyzer for DNA quality) by Tempus according to Illumina's protocols for MethylationEPIC v2.0 935K array preprocessing. While some samples showed lower DNA concentration values (Supplemental Table 1) and DNA quality (Supplemental Figure 1), potentially caused by improper donor collection, all samples had sufficient DNA of acceptable quality to proceed and were loaded onto a MethylationEPIC array.
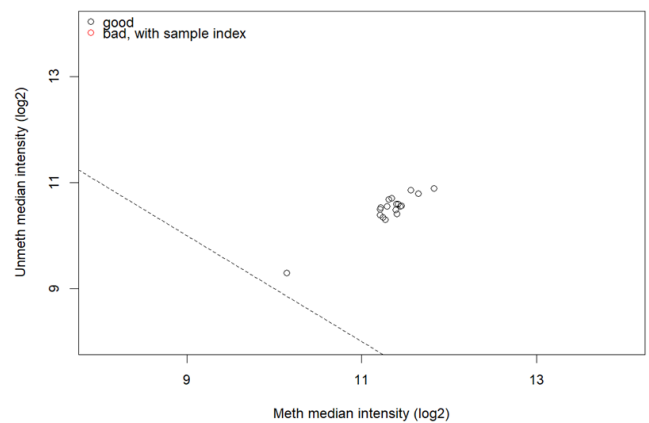

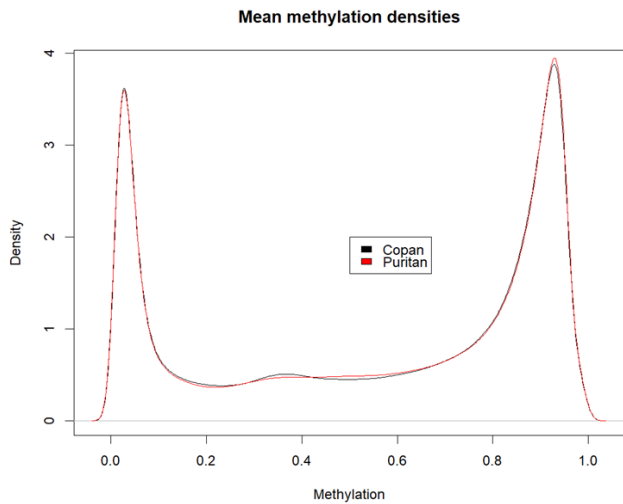**Figure 1**. log median methylation intensities
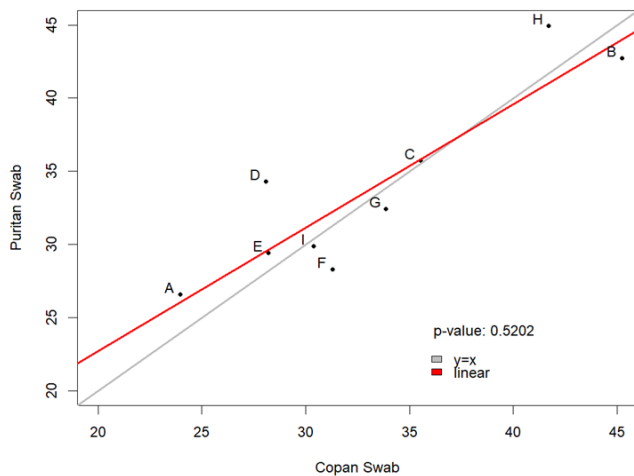
Figure 2: Methylation density plots

Figure 3: Epigenetic Age Prediction

Raw methylation values were preprocessed using the minfi analysis package as described previously [2]. In short, raw idat values were read in and normalized using the preprocessNoob function, and the average log methylation intensities were checked to ensure sufficient quality of the arrays (Figure 1). One sample had a relatively low mean log fluorescence intensity value of 9.72, but was still included in downstream processing. All samples showed the typical bimodal distribution of methylation values, and there wasn't a large difference in the methylation distribution between the two swab types (Figure 2).

Next, epigenetic age was predicted from the methylation values using the CheekAge clock [2]. We compared the predicted values from the Copan and Puritan swabs (Figure 3), and while there was a slight bias toward higher values in the Puritan swab as shown by the linear trend line, this trend was not statistically significant (p-value = 0.5202, paired t-test). Furthermore, removing the lowest quality sample from the analysis resulted in the two sets of epigenetic age predictions becoming virtually identical (p-value = 0.9776, paired t-test) as shown in Supplementary Figure 2. **We concluded that there was no statistically significant epigenetic age prediction bias for either swab type when collected and stabilized iSWAB-Discovery.**

References

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Supplementary Table 1: Sample DNA Concentrations

Sample ID	Swab Type	Concentration (ng/uL)
A	Copan	148.56
	Puritan	201.36
B	Copan	142.47
	Puritan	186.80
C	Copan	19.77
	Puritan	33.69
D	Copan	197.87
	Puritan	182.34
E	Copan	192.64
	Puritan	207.16
F	Copan	150.49
	Puritan	202.52
G	Copan	106.82
	Puritan	112.17
H	Copan	191.77
	Puritan	215.39
I	Copan	108.96
	Puritan	200.20
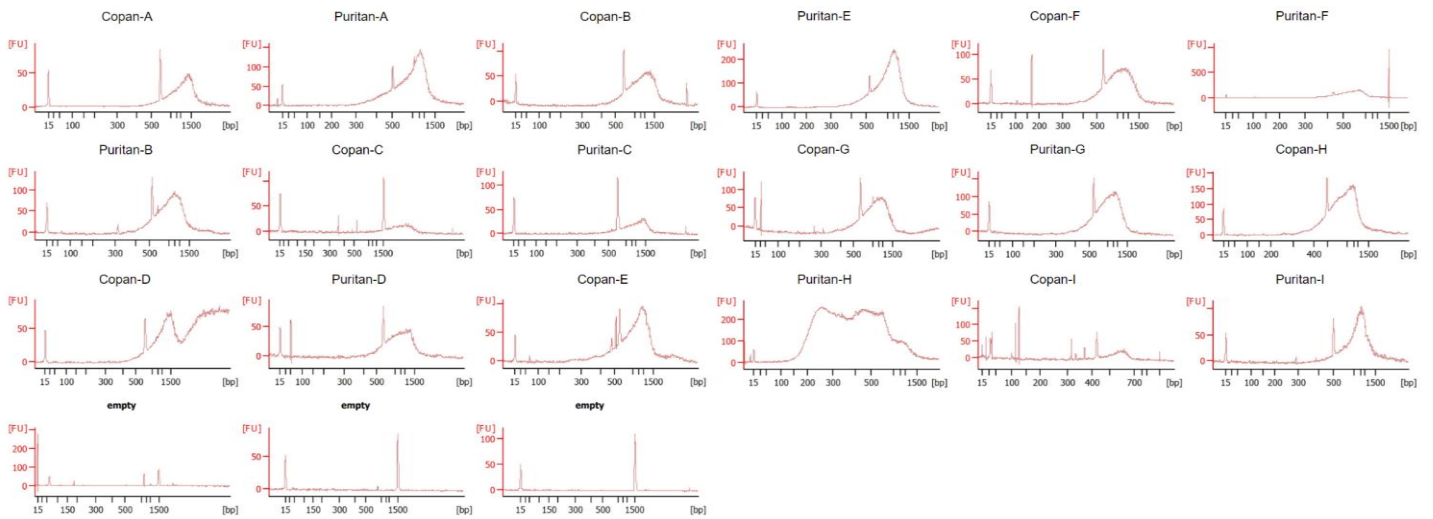
Supplementary Figure 1: BioAnalyzer quality control traces for all samples.
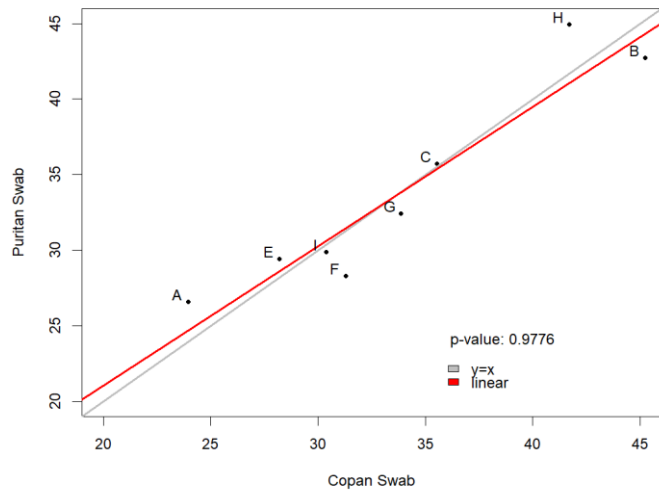
Supplementary Figure 2: Age prediction results after removing lowest quality pair of samples from figure 1