Assessment of DNA Yields from Swabbing Different Areas of the Human Body in a Peruvian *Population*

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BACKGROUND

The determination of genetic identity in criminal and civil forensic investigation relies on the successful collection of reference DNA samples from human subjects. Historically, liquid blood and dried blood stains have served as the most reliable source of biological material for the purpose of developing the known DNA profile from a donor. Buccal samples are currently viewed as the most reliable source for high quality DNA from living subjects. The collection of buccal swabs is minimally invasive and carries almost no risk of injury to the donor. However, in instances where a buccal sample cannot be obtained from the subject, an alternate, minimally invasive, and reliable source of biological sample should be available to the collector. The objective of this project was to evaluate the DNA quantity and quality obtained from swabs collected from various superficial areas of the human body< and to identify whether one area can serve as a reasonable alternative to the buccal swab.

EXPERIMENTAL SETUP

A cohort of 23 volunteers from the village of Rosaspata in the Huamanga province of Peru were enrolled in this study. Area residents traveled to a nurse's station in the village to donate the samples. After signing an informed consent form, each volunteer was swabbed using a Genetics 4N6FLOQSwabs® with dry active system (Copan Italia). Six swabs were collected from each volunteer (n=138). In addition to obtaining a buccal swab from each donor, the additional areas sampled were 1) nose sill and alar rim, 2) alar flare, 3) temporal scalp, 4) back of auricle lobule, and 5) axillary fossa. To prevent contamination, the researchers wore surgical gloves and mask during the collection process. All samples were extracted with the PrepFiler Express™ Forensic DNA Extraction Kit (Thermo Fisher Scientific) according to the manufacturer suggested protocol, with elution volume of 50 µl. The Quantifiler® Trio DNA Quantification Kit (Thermo Fisher Scientific) was used for DNA sample quantitation; the GlobalFiler® DNA Amplification Kit (Thermo Fisher Scientific) was used for typing at 30 cycles on Veriti™ 96-well Thermal Cycler (Thermo Fisher Scientific), following the suggested cycling parameters by the manufacturer (target template was 0.5 ng or the maximum allowed volume for PCR reaction). The AB3500 Genetic Analyzer (Thermo Fisher Scientific) was used for capillary electrophoresis. Each CE was repeated twice on the same PCR sample to highlight possible artifacts. Data files were analyzed with the GeneMapper™ ID-X v1.4 software (Thermo Fisher Scientific), following the suggested parameters by typing kit (except for the analytical threshold, set at 100 RFU in the Lab).

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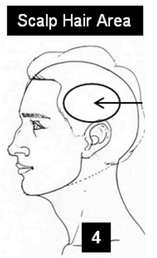
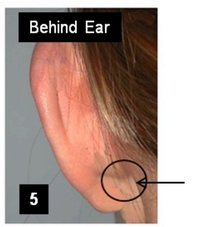
Six Collection Sites

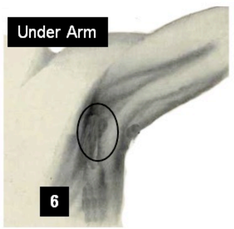
1-Inside cheek (Buccal area)
2-Rim of nose (Sill and alar rim)
3-Beside nose (Alar flare)
4-Scalp area (Temporal region)
5-Behind ear (Back of lobule)
6-Under arm (Axillary fossa)

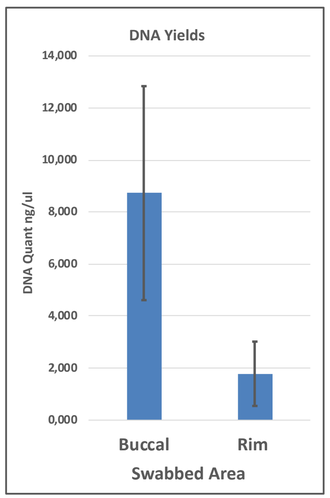
Results and DISCUSSION

DNA samples and full profile data were obtained from all 23 buccal swabs (mean quant = 8.72 ng/µl; SD +/- 4.11 ng/µl). The observed differences in the quantitation values can be attributed to normal variance in sample collection. One sample (quant = 164.31 ng/ul) was considered an outlier and removed from the dataset. DNA samples were obtained from all 23 swabs collected from the nose sill and alar rim (mean = 1.78 ng/µl; SD +/- 2.47 ng/µl). Of those, 21 samples (91%) revealed full, single source DNA profiles (**Graph 1**, note scale). Significantly lower quantitation values were obtained from swabbing the alar flare (mean = 0.035 ng/µl; SD +/- 0.041 ng/µl), with one sample yielding quantitation value less than 0.001 ng/µl. The swabs collected from the temporal scalp region (mean = 0.031 ng/µl; SD +/- 0.037 ng/µl) showed similar DNA yields to the ones obtained from the alar flare. However, three samples yielded quantitation values less than 0.001 ng/µl. Similarly, the swabs collected from the back of auricle lobule revealed low yields (mean = 0.027 ng/µl; SD +/- 0.025 ng/µl) and two samples with less than 0.001 ng/µl. Interestingly, the swabs from the axillary fossa (mean = 0.024 ng/µl; SD +/- 1.12 ng/µl) revealed seven samples with quantitation value less than 0.0005 ng/µl (**Graph 2**: note scale). One sample in the set (median = 0.001 ng/µl) yielded 5.53 ng/µl and appears to be an outlier. Full, single source DNA profiles were obtained from approximately 25 % of each group of the three groups: the alar flare, the temporal scalp region, and the auricle lobule. Full, single source DNA profiles were obtained from approximately 8% of the axillary fossa samples. The remaining samples were not profiled due to low quantitation data. Mixed DNA profiles were observed in one sample from the alar flare and in one sample from the temporal scalp. Those areas might not be suitable as collection sites for reference DNA sample due to possible exposure to exogenous DNA in the donor's environment. The results from this study suggest that the nose sill and alar rim is a viable alternate site for the collection of reference human DNA samples. No appreciable degradation was observed in any of the sample groups. However, the low yields seen in samples from the axillary fossa were unexpected given the anatomical and physiological nature of that body area.

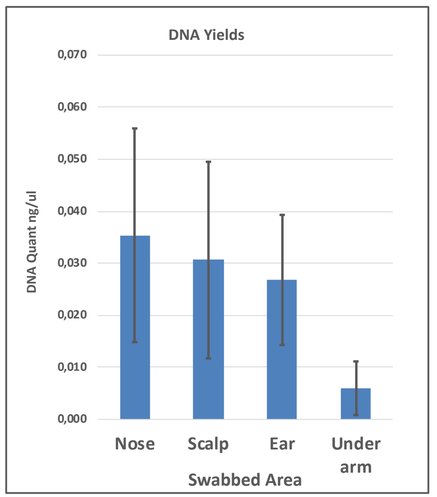








Graph 1



Graph 2

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