E4 Extraction and Quantification of DNA From Buccal Cells of Peruvian Coca Leaf Users and Non-Users

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Learning Overview: After attending this presentation, attendees will be familiar with the practice of coca leaf use as a stimulant by a Peruvian population and the mechanism of studying the impact of coca leaf alkaloids on DNA samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the variability in DNA yields obtained from buccal samples and illustrating a study design to investigate the impact of alkaloids and other compounds on DNA yields from swabbing human subjects.

Background and Objective: The *Erythroxylon coca* plant, also known as the coca shrub, is a plant from the Andes mountain range in Western South America. Historically, Peruvian people have used coca leaves as a mild stimulant and some continue to use it today. Coca leaf users typically remove the stem or midrib from each leaf, fold the leaf in half twice, and place it in the cheek. Leaves are continuously added until a ball of plant material, approximately 2–3cm in diameter, is formed. The leaves are not masticated but moved around the cheek with the tongue while in contact with saliva for a period of time before being discarded. The primary alkaloid in the coca leaf is cocaine and the dried leaves contain approximately 0.6% cocaine; however, there are 18 other alkaloids present in the leaf, which may affect DNA yield from buccal cells or cause inhibition during DNA amplification. The purpose of this research was to collect buccal swabs from coca leaf users and non-users to determine if there are any differences in the quantity or quality of DNA recovered from the subjects.

Experimental Design: Buccal swab samples using 4N6FLOQSwabs™ Genetics were collected from 13 coca leaf users and 10 non-coca users in the village of Rosaspata in the Huamanga province of Peru after signing an informed consent form. A survey of the donors indicated that the age range for coca leaf users was 18 to 74 years. Three of the donors were male and ten were female. Three of the donors reported they use coca leaves once a day, four reported two or three times a month, and six said two to three times a week. Seven donors reported that they added a pinch of lime ($CaCO_3$) to the cheek when using coca leaves. The number of leaves used ranged between 10 and 30 with an average of 17, and the length of time in the mouth ranged between 15 to 60 minutes with an average of 30 minutes. The non-coca leaf users included three males and seven females ranging in age from 14 to 44 years. All samples were extracted with the PrepFiler® Express™ Forensic DNA Extraction Kit. The Quantifiler® Trio DNA Quantification Kit was used for DNA sample quantitation and the GlobalFiler® DNA Amplification Kit was used for typing. The AB3500 Genetic Analyzer was used for Capillary Electrophoresis (CE) and the data files were analyzed using the GeneMapper® ID-X v 1.4 software (AT=100RFU).

Results and Conclusions: DNA samples were obtained from all 23 buccal swabs with observed differences in the quantitation values that can be attributed to, and expected from, normal variance in sample collection. No significant differences in the quantitation value means were observed between the 13 coca leaf users (M=8.98ng/µl; SD +/- 4.199ng/µl) and 10 non-coca users (M=47.39ng/µl; SD +/- 23.69ng/µl). The differences observed between the two groups did not reveal a statistical significance using the unpaired, two-sided t-test statistic (p=0.372). All buccal swab samples yielded full DNA profiles with no apparent inhibition or degradation. All male subjects were coca leaf users (n=3). No appreciable differences were observed in DNA yields from males versus females. Furthermore, DNA yields do not appear to be affected by the frequency, duration, or recency of cocoa leaf use. Based on the donor survey, the most recent coca leaf use was one day prior to the DNA sample collection (n=7). It is possible that the lag in time of one day resulted in the metabolism of the alkaloids and/or the recovery of the mouth mucosa in which any impact on the DNA quantity and quality would have been attenuated to reveal any appreciable effect on the DNA sample.

DNA Yield, Coca Leaf, Buccal Swab