FORENSIC IDENTIFICATION USING DNA RECOVERED FROM SALIVA ON HUMAN SKIN

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INTRODUCTION

Bites, suck marks or kisses occur often in crimes of aggression, in self defense and during sexual interactions. Saliva is presumed to be deposited on the skin under these circumstances.

Bite mark analysis using pshysical matching techniques is prone to distortion and subjective interpretation [Rothwell, 1994]. Comparisons depend on measurements of the size and shape of an injury on the skin surface wich is elastic and may be unstable.

Saliva is an adequate source of forensically useful DNA evidence since it contains cells sloughed from the oral epithelium and leukocytes [Raeste and Calonius, 1971; Watanabe et al, 1981; Hochmeister et al, 1991]. Whole saliva mixed body fluid stains containing saliva have been typed and successfully discriminated to their source [Comey and Budowle, 1991]. Salivary evidence is stable over a moderately long postmortem interval [Adams et al, 1991; Walsh et al, 1992].

An experimental protocol was designed to evaluate the amount of DNA wich can be extracted from saliva collected from human skin (of cadavers) and typed using PCR analysis. Stains with a volume of whole saliva equivalent to the amount deposited during a typical bite were studied. The most effective protocol for collecting salivary DNA from skin and avoiding contamination of the sample by DNA from the cadaver were tested.

MATERIALS AND METHODS

Thirty-three sites on the skin of 27 cadavers were divided into 4 quadrants with a surface area of approximately equal to an average adult bite mark (quadrant area = 10.7 cm^2). Expectorated saliva was collected from one male donor and aliquots containing 40 ul of whole saliva were deposited on three out of the four quadrants on the skin of each cadaver. The fourth quadrant remained undisturbed.

Recovery methods were studied to improve the number of nucleated salivary cells wich can be collected from the surface of the skin. A modification of the classical single swab technique was developed. In this method, referred to as the *double swab technique* [Sweet, 1995], a wet swab is used to wash the saliva from the skin. This is followed by a dry swab wich is used to collect the water left on the skin containing rehydrated salivary cells.

A sample was collected from one quadrant after 5 minutes of elapsed time. This was the positive DNA control. Other samples were collected from two other quadrants. one after 24 hours and one after 48 hours. Swabbing was completed on the fourth quadrant where no saliva had been deposited to determine if contamination of the samples occurred. An additional sample was collected from the cadaver as a negative control (blood or tissue).

DNA was extracted using the Chelex-100 extraction method [Walsh et al, 1991] with several modifications. These included: a) washing the cotton swab tips with a solution of distilled water and proteinase K (1 mg/ml); b) incubation at 56°C and at 100°C (8 minutes) prior to addition of Chelex-100; c) micro-concentration using Microcon-100™ tubes. DNA yield was quantified using a slot-blot apparatus and amplified using the short tandem repeats (STR) loci HUMTH01 and HUMVWA.

RESULTS

Using the classic single wet swab recovery technique, 33.5 ± 4.8% of the saliva deposited on the skin was recovered. Using the double swab method saliva recovery was improved to 44.6 ± 6.4% of the total amount of saliva deposited.

Following the classic Chelex-100 extraction method, 31.9 ± 4.2% of the DNA present in a solution with a known concentration was extracted. After incubation in a wash solution containing proteinase K, incubation at 56°C and at 100°C, and micro-concentration with Microcon-100 tubes, DNA yield was increased to 47.7 ± 6.9%

Positive PCR amplification results were obtained at locus HUMTH01 for 78.8% of the samples collected after 5 minutes, 75.8% of those recovered after 24 hours, and 69.7% of those recovered at 48 hours. Results were similar for the HUMVWA locus since 78.8% positive amplifications resulted after 5 minutes, 66.7% after 24 hours, and 57.6% after 48 hours.

DISCUSSION & CONCLUSSIONS

Extraction of DNA from saliva using Chelex-100 resin is improved if the samples ares ubmitted to pre-extraction treatment consisting on incubation in proteinase K and filtration with Microcon-100. The concentration of DNA in saliva recovered from skin varies as a function of time since deposition. There is a significant decrease in concentration in the first 24 hours, but the concentration remains stable from 24 to 48 hours.

In the majority of cases, positive PCR amplifications were obtained for STR loci HUMTH01 and HUMVWA from saliva deposited on the skin of cadavers. Amplification success is independent of time since deposition or concentration of DNA in the saliva sample. Contamination from the skin of the cadaver was not found in any of the cases studied.

These results indicate that forensic analysis of DNA from saliva recovered from human skin may be a valuable identification tool in cases involving bite marks or suck-marks, or in any case where saliva trace evidence is discovered.

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