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Evaluating the Reliability of DNA Profiling of Bite Mark Samples from Living Participants

Noboru Ishikawa[1*#], Yasutaka Nakamura[1#], Tetsu Naitou[2], Yuna Miura[1], Hidetaka Kuroda[3] and Masatsugu Hashimoto[1]

[1]Department of Forensic Odontology and Anthropology, Tokyo Dental College, Tokyo, Japan

[2]Department of Anatomy, Tokyo Dental College, Tokyo, Japan

[3]Department of Dental Anesthesiology, Kanagawa Dental University, Kanagawa, Japan

[#]These authors contributed equally to this manuscript

***Corresponding author:** Noboru Ishikawa, DDS, PhD, Department of Forensic Odontology and Anthropology, Tokyo Dental College, 2-9-18 kanda-Misakicho, Chiyoda-ku, Tokyo 101-0061, Japan, Tel: +81-3-6380-9276, E-mail: ishikawanoboru@tdc.ac.jp

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Abstract

One of the most common evaluations in forensic odontology involves bite mark analysis, which includes morphological evaluation of the indentations left on a victim to identify the suspect via the DNA profile left on the victim. DNA profiling is an extremely effective tool, but its reliability is expected to decrease with increasing time following injury. However, the majority of literature discussing bite mark analysis comprises case reports, and the number of primary studies exploring this technique is reasonably small. Here, we evaluated the impact of these evaluations on a living victim by assaying the effect of increasing exocrine fluid and contamination at the bite site over time. In this study, we compared the effectiveness of conventional DNA typing methods on saliva from living participants under various conditions. The results of these evaluations were shown to be most dependent on the absolute amount of saliva at the bite site or the absolute amount of saliva that could be collected from the victim. We also noted that the suspect could be clearly identified even 9 h after injury. In addition, we revealed that the accuracy of the results of these analyses were not affected by increased perspiration in the victim. These results indicate that DNA profiling of bite mark samples can be performed with extremely high accuracy, without any significant concerns around the environment of the victim after injury. This study provides valuable insight into non-fatal forensic investigations because it is the first study to report results based on experiments on living participants.

Keywords: Forensic Odontology; Bite Mark; DNA Profiling; Personal Identification; Saliva

Introduction

Forensic odontology is primarily associated with identification of bodies using dental findings, not only for mass casualty events but also for missing persons, crime victims, and suspects [1-6]. However, forensic odontology is also applied in a wide range of other fields, including age and gender estimations of skeletal remains of the head and neck, abuse-related research, trauma assessment including bite mark injury, and recently, research around estimating time of death [7-15]. This means that bite mark analysis is an extremely important area of research allowing for the identification of victims and suspects of heinous crimes. Bite mark analysis involves the careful evaluation of any bite marks left on the victim to identify the suspect, using morphology and DNA profiling [16, 17]. Morphological evaluation is challenging in the absence of open injuries as various factors, including the loss of indentation with time after injury and soft tissue distortion, reduce the reliability of these evaluations [18-21]. The reliability of bite mark analysis decreases with elapsed time not only in terms of the morphological evaluation methods but also in terms of the DNA profiling results. However, most of the articles evaluating these methods are case reports with very little primary literature, making these observations less reliable [22]. In addition, most of these reports focus on DNA from the suspect, and there are almost no reports that consider the effects of the victim's DNA. If the victim is an inanimate object or a dead body with no vital reaction, it is unlikely to have a significant effect, but if there is a vital reaction, it is necessary to consider the state of the victim. This means that as the collection of DNA from skin is different from that of inanimate objects, it is necessary to investigate the extent to which exocrine secretions from victims affect DNA profiling over increasing time intervals post incident. The significance of this study is that it has the potential to contribute toward the elimination of the need for controversial decision making, with respect to the reliability of expert testimony, during the time between injury and DNA typing and due to the environment in which the injury site is kept until a DNA sample is collected for typing. In this study, we applied the saliva of a hypothetical suspect to the human body of a hypothetical victim and examined the changes in DNA profiling accuracy over time.

Materials and Methods

Sample preparation

Saliva collected from the person assumed to be the suspect of a bite mark was collected into a tube and applied to the antebrachial region of the person assumed to be the victim with a forensic swab (Sarstedt, Germany). This application was performed across a 4 cm × 4 cm area and following the application, the "victim" was placed in two different environments: a naturally dry environment and an environment designed to increase sweat production, in which perspiration was promoted by wearing a polyethylene arm cover (AS ONE Co., Japan). Samples were then collected at different time points (0, 1, 3, 6, and 9 h). These samples were collected from the bite mark site by rubbing the skin 20 times with a forensic swab moistened with a sterile saline solution. A total of five samples from each condition were prepared for each time point (control (0 h): 5 cases, natural dry environment: 20 cases (5 samples × each time point), sweat promoting environment: 20 cases (5 samples × each time point)].

DNA extraction and PCR amplification

The DNA was extracted using a NucleoSpin DNA Forensic (Macherey-Nagel, Germany) kit according to the manufacturer's protocol. The fluorescent primers were amplified by PCR using the AmpFLSTA Identifiler Plus PCR Amplification Kit (Thermo Fisher Scientific, USA), and the thermal cycling conditions were as follows: heating at 95 °C for 11 min as the initial incubation step, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 3 min, and a final extension reaction at 60 °C for 60 min.

DNA analysis

We first determined the STR typing for each of the participants in the experiment. Our analysis focused on the STR typing of 12 unique loci (D8S1179, D7S820, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, D18S51, D5S818, and FGA) from the 15 autosomal loci labeled with this kit. Analysis was completed using a Genetic Analyzer 3100 (Thermo Fisher Co., USA), and evaluated using Gene Mapper Software 5 (Thermo Fisher Co., USA). The `height` value at each locus was used in the case of homozygotes, and the average value of each of the two `height` values was calculated for heterozygotes. The proportion of loci the suspect or victim was then used for evaluation. However, if the allele was common to both samples, it was excluded and only the `height` of the other allele was used.

Statistical analysis

Statistical analysis was performed using Student's *t*-test and Kruskal-Wallis test. We used Microsoft Excel 2018 (Mic-

rosoft Corporation) and the statistical software EZR (R version 3.2.2) and set the level of significance in the statistical tests at $P<0.05$.

Ethical approval

This study was completed in compliance with the code of ethical practice outlined by the Tokyo Dental College and the Japanese Society of Legal Medicine. All protocols were approved by the ethics committee at the Tokyo Dental College (Approval Number:866).

Results

We first isolated the STR profile for each DNA donor, victim, and suspect, using oral epithelium samples from each donor and then applied these profiles to our downstream identifications (Table 1). The suspect STR profile was shown to be easily identified for at least 9 h irrespective of the environmental conditions (dry or sweat inducing) and was clearly distinguishable from that of the victim (Figure 1). Although there were some significant differences in detection over time in each condition, there were no consistent trends in these deviations and no regular change was observed (Table 2). Figure 2 shows an electropherogram of the STR typing result for analyses completed using mixed samples as a template. This clearly shows that it was possible to detect a clear peak, comparable to that of the 0 h sample, at each timepoint (1, 3, 6, and 9 h post application). Finally, we compared the differences in the sample values for both the dry and sweat inducing environments at each time point. The results of these evaluations showed that the values for D7S820, D13S317, D2S1338, D19S433, D18S51, D5S818, and FGA were significantly higher in the dry environment samples at the 1-h time point. While D13S317 was shown to be significantly higher in the sweat inducing environment samples at the 6-h time point. No significant differences were observed in the other loci (Table 3).

Locus	Allele	
	Victim	Suspect
D8S1179	10	11
	13	12
D7S820	8	11
	13	
D3S1358	17	15
	18	16
TH01	6	7
	7	9
D13S317	8	9
	10	11
D16S539	10	11
	12	13
D2S1338	19	22
	20	24
D19S433	13	14.2
vWA	16	14
	19	17
D18S51	15	17
		18
D5S818	10	10
	11	13
FGA	23	22
	24	23

Table 1: STR polymorphisms in each of the victim and suspect

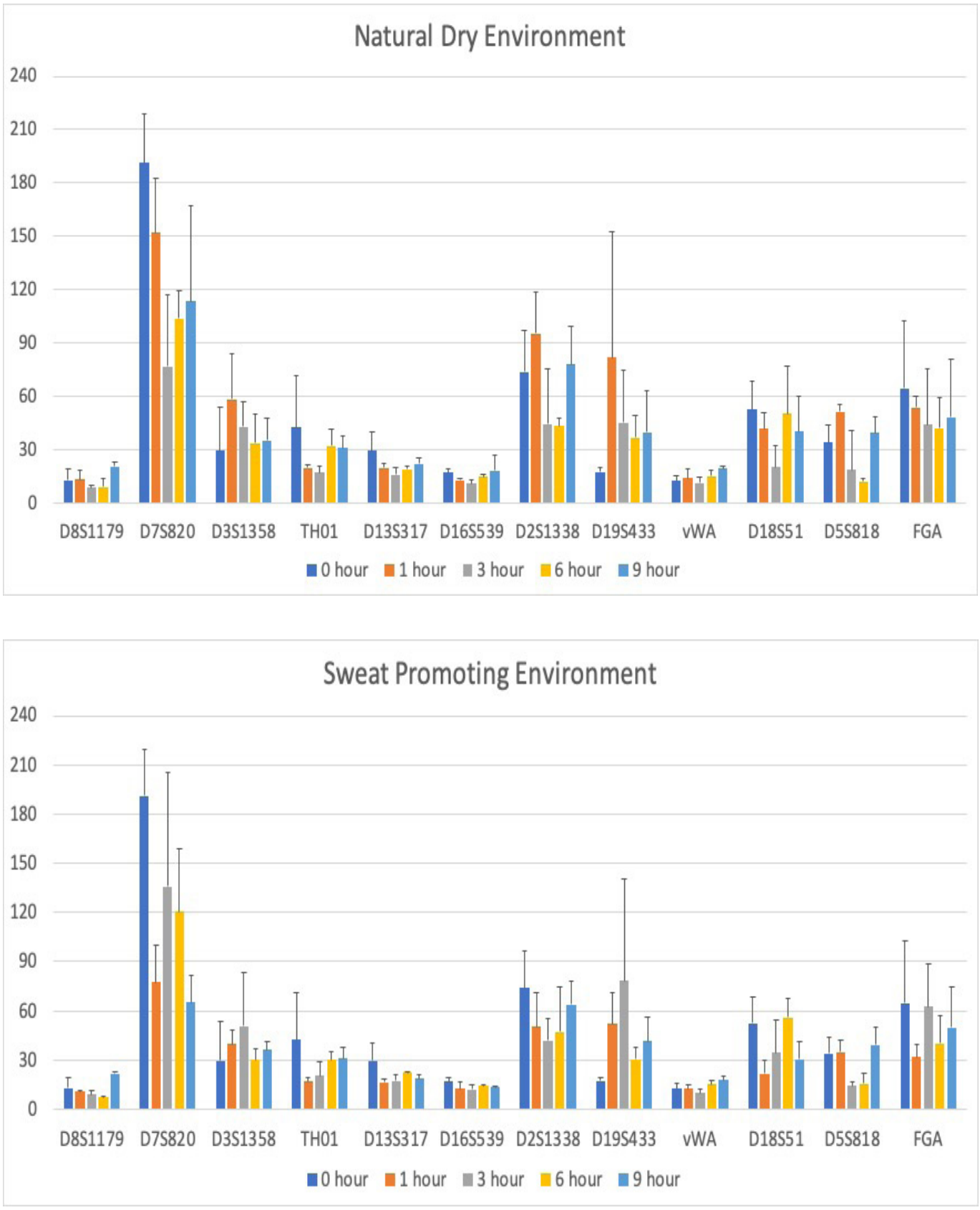

Figure 1: Change in elapsed time for each locus. Most loci did not demonstrate any constant trend

Natural Dry Environment				
	0-hour vs 1-hour	1-hour vs 3-hour	3-hour vs 6-hour	6-hour vs 9-hour
D8S1179	N. S	N. S	N. S	*
D7S820	N. S	N. S	N. S	N. S
D3S1358	N. S	N. S	N. S	N. S
TH01	N. S	N. S	N. S	N. S
D13S317	N. S	N. S	N. S	N. S
D16S539	N. S	N. S	N. S	N. S
D2S1338	N. S	N. S	N. S	N. S
D19S433	N. S	N. S	N. S	N. S
vWA	N. S	N. S	N. S	N. S
D18S51	N. S	N. S	N. S	N. S
D5S818	N. S	N. S	N. S	N. S
FGA	N. S	N. S	N. S	N. S

Sweat Promoting Environment				
	0-hour vs 1-hour	1-hour vs 3-hour	3-hour vs 6-hour	6-hour vs 9-hour
D8S1179	N. S	N. S	N. S	**
D7S820	N. S	N. S	N. S	N. S
D3S1358	N. S	N. S	N. S	N. S
TH01	N. S	N. S	N. S	N. S
D13S317	**	N. S	N. S	N. S
D16S539	N. S	N. S	N. S	N. S
D2S1338	N. S	N. S	N. S	N. S
D19S433	*	N. S	N. S	N. S
vWA	N. S	N. S	N. S	N. S
D18S51	N. S	N. S	N. S	N. S
D5S818	N. S	N. S	N. S	N. S
FGA	N. S	N. S	N. S	N. S

(*$p < 0.05$, **$p < 0.01$, N. S: No Significant)

Table 2: Significant differences for different intervals at each locus

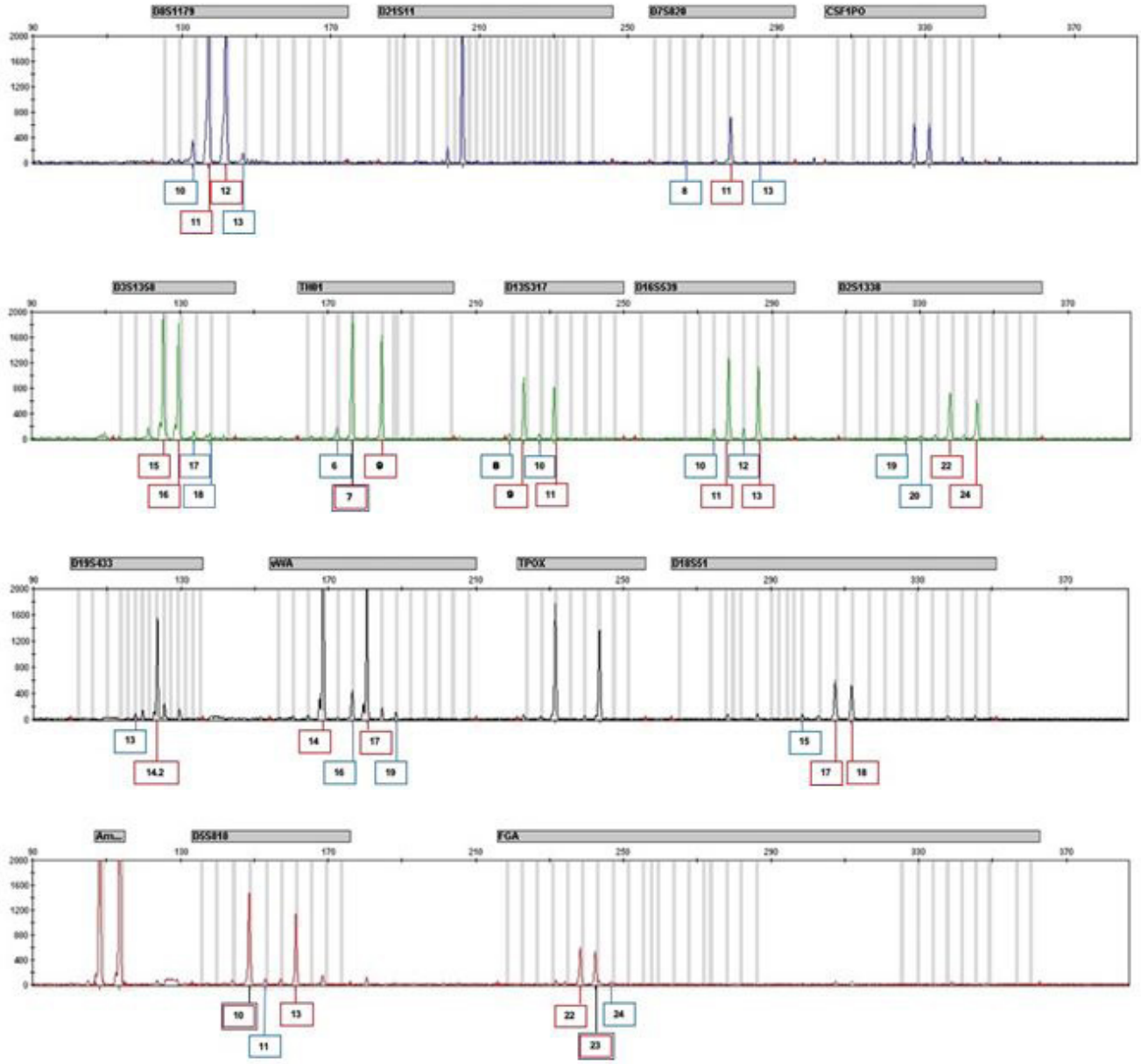

Figure 2: STR polymorphisms in a mixed sample. STR typing of the suspect was clearly distinguishable at all time points

Dry vs **Sweat**	D8S1179	D7S820	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	D18S51	D5S818	FGA
1-hour vs 1-hour	N. S	**	N. S	N. S	*	N. S	*	**	N. S	**	**	**
3-hour vs 3-hour	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S
6-hour vs 9-hour	N. S	N. S	N. S	N. S	*	N. S	N. S	N. S	N. S	N. S	N. S	N. S
9-hour vs 9-hour	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S

**: 0.01>p, *: 0.05>p, N. S: No Significant) (Dry; Natural Dry Environment, Sweat; Sweat Promoting Environment)

Table 3: Significant differences in detection at the same timepoint for each locus in each environment

Discussion

Several case reports describing bite mark analysis have been published recently. Most of these methods are classified into two categories: those that use 3D scanners or plaster to create models from the bite mark marks left on the victim to evaluate morphological features, and those that use STR typing of the saliva within the bite mark to identify suspects [23-26]. Morphological evaluations include measuring the distance between feature points on the victim's skin and the suspect's plaster cast, and often use superimposition of the suspect's plaster cast and the victim's bite marks to facilitate identification. However, bite marks heal with time, and it is often difficult to determine whether a mark is a bite mark. In contract, DNA profiling can identify the suspect without being affected by the clarity of the bite mark. There have been many reports describing bite mark DNA profiling, including various cases with bite marks on different parts of the body and analysis of human DNA from food. Therefore, investigations of methods to efficiently recover nuclear DNA and mitochondrial DNA from various foods [27] and evaluations of the degree of degradation of DNA attached to objects over time [28] have been reported. In addition, there have been reports on methods for collecting saliva of suspects from human skin [29]. However, most of these reports focus on the DNA of the suspect, with very little evaluation of the DNA from the victim (or object). Bite marks on living victims are often subjected to delay in DNA collection, during which time, sweat and other secretions from the victim may increase and interact with the suspect's sample. These secretions increase with time and may become contaminated, which may affect the DNA profiling of the suspect. However, here we show that it is possible to detect the DNA of the suspect without any effect from the victim's DNA, even 9 h after injury (Figure 1). The difference between the calculated values for the suspect and victim peaks in the electropherogram of STR polymorphisms were shown to be seven times higher than those of the victim, even when the detection volume was the closest to the victim, making it easy to differentiate between the suspect and victim DNA profiles (Figure 2). In addition, we were able to show that it is possible to determine these values for up to 9 h post injury. Our data demonstrated that there were no significant decreases in the STR profile values in response to extended collection delays confirming the efficacy of same day DNA profiling (Table 2). Despite this, there were many areas where the detection value was not consistent over time, although there was also no consistency in these changes. This may be due to differences in the absolute amount of attached saliva or the absolute amount of saliva that could be collected. We also conducted a series of experiments using a sweat inducing environment to examine the effects of different seasons and environments on these analyses. However, our data revealed that there were no significant differences in the STR profile values in these conditions when compared to the results from the dry environment. This suggests that it is unlikely that the amount of exocrine fluid excreted by the victim has any effect on the results of these analyses. However, our data did suggest that these values were significantly higher when the samples were left to dry naturally (Table 3). Therefore, we suggest that it is still more effective to collect DNA profiling samples rapidly and in less humid conditions, although this is not critical for success. These results indicate that contamination from air pollution, increased sweating, and prolonged collection times, had very little effect on the reliability of bite mark DNA profiling. The absolute amount of saliva on the victim or the absolute amount of saliva that could be collected had a significant impact on these results, but these parameters did not limit the efficacy of these evaluations under these conditions. It is possible that the reliability of DNA profiling may gradually decrease when time lapses, after injury exceeds 9 h, however it is rare for an injury to be left for a long time without washing or disinfection. However, if the victim is not alive, it is possible that the victim may be left for a longer period of time, making it necessary to consider the various environments in which the victim may have been in. In addition, it is necessary to examine various fibers and the extent to which clothing abrasion affects DNA profiling in living participants. However, the results of this study resolved much of the uncertainty, as encountered in numerous previous cases, associated with the reliability of DNA typing for bite marks. The possibility that different loci from bite marks have different levels of durability requires further study.

Conclusion

DNA profiling is extremely useful for bite mark analysis. This method was found to have a high capacity for specific identification without any demonstrable decrease in reliability in response to increased time from incident even on a living body, which has been a concern.

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