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Forensic Science International  
94 (1998) 65–71

**Forensic  
Science  
International**

## Evaluation of a decontamination protocol for hair shafts before mtDNA sequencing

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Received 2 December 1997; received in revised form 3 March 1998; accepted 12 March 1998

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### Abstract

Mitochondrial DNA (mtDNA) sequencing is a powerful and sensitive method to identify the donor of shed hairs found at a crime scene. Because of the low amounts of DNA in shed hair and the sensitivity of PCR, contaminating cells (e.g. saliva, blood), sometimes present on these hairs, will be co-amplified. This will result in ambiguous sequencing results and might even lead to erroneous exclusions of suspects. We have evaluated a strategy for effectively removing saliva and blood contamination from hair samples. Unambiguous mtDNA results were obtained by incubating the hair samples in a differential lysis buffer (which contains no DTT) prior to DNA extraction. Since the nuclear DNA of the hair root is affected, this procedure should be restricted to hair shaft proportions. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords:* Forensic science; Decontamination; Hair shaft; Mitochondrial DNA typing

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### 1. Introduction

Human hair is one of the most frequently found biological samples at crime scenes. Previously, forensic hair analysis was based on the microscopic comparison of evidence hair with reference hair from victim or suspect. With the advent of the polymerase chain reaction (PCR), DNA typing of hairs has become feasible [1]. Most human hairs recovered from crime scenes, however, are naturally shed hairs and do not contain a root. Amplification of nuclear DNA from these hairs is problematic because most DNA is located in the root cells. However, by amplifying mtDNA, which is present in high

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copy numbers in each cell [2], DNA typing on evidentiary hair samples still remains possible [3], in particular by analysing the sequence variation of the non-coding region of mtDNA [4].

Because of the sensitivity of PCR and of the low amounts of DNA in shed hairs, co-amplification of contaminants can influence the interpretation of the results. Therefore, forensic hair samples should be thoroughly ‘cleaned’ prior to DNA extraction. This is usually done by an incubation overnight in a physiological salt solution followed by ethanol (100%) washing [5]. Nevertheless, we and others [6,7] observed that after this washing procedure a substantial number of hairs showed multiple ambiguous positions in the mtDNA sequences from these hairs. Wilson et al. [7] have demonstrated that using a commercial available detergent ‘Terg-a-zyme’ and ultrasonic cleaning prior DNA extraction resulted in correct mtDNA typing from hairs contaminated with blood, saliva or semen.

In the present report, we evaluated an alternative method that is fully effective in eliminating saliva and blood contamination from hair samples. The principle of the method is based on the differential lysis of sperm cells and vaginal or uterus cells from a vaginal swab of a rape victim [8]. Similar to the protein coat of the sperm head, the disulphide bridges of the keratins of the hair shaft are resistant to mild lysis conditions (in the absence of dithiotreitol (DTT)). By applying this differential lysis method, the hair shafts can be essentially decontaminated.

## 2. Materials and methods

Single hairs were plucked from ten individuals and were contaminated with saliva from another person who was known to have a different mtDNA sequence from the hair donor. The hair samples were contaminated by moving the single hairs through a cheekbrush that is normally used to scrape the cheek of a control individual. Similarly, single hair shafts from three different individuals were contaminated with blood. These hair samples were placed in a petri dish and dried at 60°C overnight or for several days. Each hair was separated into a ‘root’ (containing bulb, sheath, elongation zone and shaft) and shaft portions of about 1 cm. Because of the extreme experimental design where cheek and blood cells were ‘baked’ onto the hairs at 60°C for several days, the experiments were also repeated under more realistic conditions. Hair shafts of two individuals were contaminated with saliva and blood and left at room temperature for one week.

Prior to DNA extraction, the hairs were decontaminated by incubation in 0.5 ml differential lysis buffer containing 100 mM NaCl, 10 mM EDTA, 0.4% SDS and 250  $\mu\text{g ml}^{-1}$  Proteinase K. After incubation at 56°C for 2 h with sporadic agitation, two washings were performed, one in physiological salt solution (0.9% NaCl) and one in ethanol (100%). The hair segments were placed in physiological salt solution overnight followed by a rinse in the same solution, in ethanol (100%) and in sterile water.

DNA was extracted according to the procedure described by Higuchi et al. [1]. Concentration of the DNA extract was done by filtration on a Microcon 100 filter (Amicon, Beverly, MA) and the DNA was recovered from the filter in a volume of 90  $\mu\text{l}$  of TE buffer (10 mM Tris (pH 8.3), 0.1 mM EDTA).

In addition, DNA was extracted from contaminated hair using only the washing steps after the differential lysis (physiological salt solution, ethanol, water) and also without using any washing or decontamination procedure. To test for the presence of DNA contamination in the reagents, each set of DNA extractions included mock-extracted samples (no hair) that were processed in parallel with the samples.

The mtDNA sequence which was used for analysis, is a segment of the first hypervariable of the D-loop (position 15996 to 16196, according to Anderson et al. [9]). A quantity of 5  $\mu$ l of recovered hair DNA was subjected to semi-nested PCR and fluorescent DNA sequencing was performed directly on the amplified DNA as described by Decorte et al. [10]. Briefly, HVR1 was amplified for 25 cycles. The resulting PCR products were further subjected to 25 cycles with a semi-nested primer set resulting in two overlapping fragments which were used for solid-phase sequencing.

### 3. Results and discussion

The results of the effect of different decontamination procedures on hair samples contaminated with saliva or blood are summarized in Table 1. After direct DNA extraction, saliva contamination was identified in 85% of the hair samples by a heterozygous position in the sequence reflecting the difference between donor hair and the contaminating saliva (Fig. 1). For two samples, the saliva contamination was 100%:

Table 1  
Effect of different decontamination procedures on hair samples contaminated with saliva or blood

Individual	Contamination source	No treatment		Physiological salt solution and alcohol		Differential lysis buffer	
		Root	Shaft	Root	Shaft	Root	Shaft
1	Saliva	+	0	+	0	0	0
	Blood	ND	+	ND	+	ND	0
	Saliva <sup>a</sup>	ND	+	ND	+	ND	0
	Blood <sup>a</sup>	ND	+	ND	+	ND	0
2	Saliva	0	+	+	+	0	0
	Blood	ND	+	ND	+	ND	0
	Saliva <sup>a</sup>	ND	+	ND	+	ND	0
	Blood <sup>a</sup>	ND	+	ND	+	ND	0
3	Saliva	+	+	0	+	0	0
	Blood	ND	+	ND	+	ND	0
4	Saliva	+	+	0	+	0	0
5	Saliva	+	+	0	0	0	0
6	Saliva	+	+	ND	ND	0	0
7	Saliva	+	+	ND	ND	0	0
8	Saliva	+	+	ND	ND	0	0
9	Saliva	0	+	ND	ND	0	0
10	Saliva	+	+	ND	ND	0	0

Summary of the mtDNA sequencing results on hair shaft and root samples of ten individuals contaminated with saliva or blood after extraction without a decontamination procedure, after a standard procedure (physiological salt solution and alcohol) and after the lysis buffer procedure.

<sup>a</sup> Dried at room temperature.

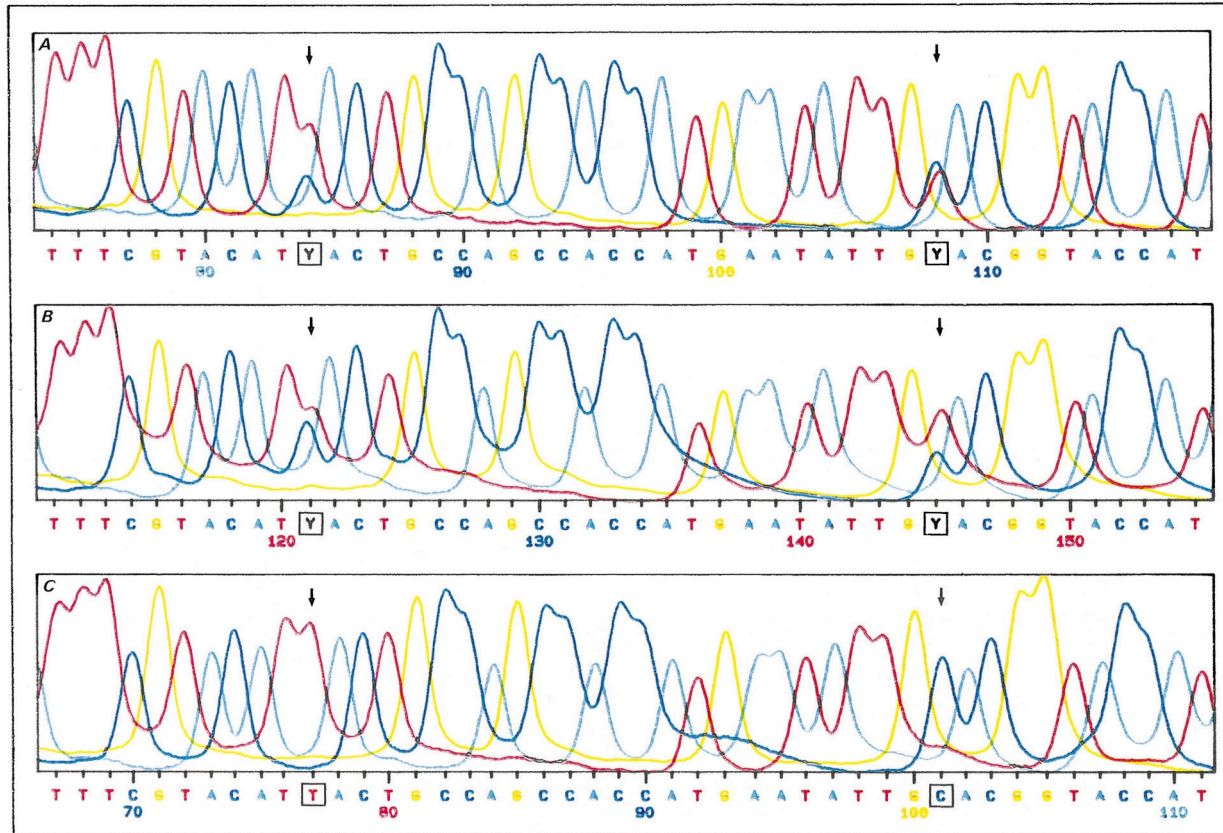


Fig. 1. Comparison of the mtDNA sequences (positions 16092 to 16136) of three different hair shaft portions of a single hair of one individual contaminated with saliva: (A) without any decontamination procedure; (B) standard procedure (physiological salt solution and alcohol); (C) differential lysis buffer procedure. The saliva donor has at position 16102 a cytosine and at position 16126 a thymine. The hair donor has at position 16102 a thymine and a cytosine at position 16126.

the mtDNA sequence of the donor hair could not be detected. Use of a differential lysis buffer allowed for complete decontamination of all the hair segments, even for those where 100% contamination was observed. Overnight incubation of the hairs in physiological salt solution was not sufficient for decontamination of the hair samples. Moreover, there was only a minor difference in the amount of contamination in the mtDNA sequences after direct DNA extraction compared to DNA extraction after incubation in physiological salt solution and ethanol (100%). There was no difference between saliva or blood contamination, even if the hairs were dried at room temperature. Only treatment of the hairs with the differential lysis buffer was effective in eliminating the saliva and blood contamination from the hairs. Differences in the proportion of the contaminating sequence on each of the hair samples (Fig. 1) can be attributed to the fact that a different portion of the hair shaft was taken for individual experiments and that these samples did not contain an equal amount of contaminating cells.

Nuclear DNA (ACES<sup>TM</sup>2.0+ Human Quantification System, Gibco BRL, Gaithersburg, MD) was also quantified and nuclear sequences amplified (Amelogenin gene [11] and STR's [12]) on root and shaft samples of five samples contaminated with saliva. Amplification of nuclear DNA in shaft portions is known to be problematic and usually without a result. As expected, in the root samples treated with the differential lysis buffer, no nuclear DNA was detected and no amplification products were obtained (data not shown) in contrast to hair samples without treatment where 5–400 ng DNA/root was detected. The differential lysis buffer thus released not only the DNA from the saliva cells but also from the root cells.

The decontamination method described by Wilson et al. [7] required the use of special equipment (ultrasonic bath) and a commercial proprietary reagent. In contrast, the procedure described here is a routine method used in the extraction of DNA from rape samples and, therefore, does not require any additional equipment or reagents. Moreover, it is known that ultrasonication results in degradation of DNA [13]. It is unclear if it would affect also DNA present in the hair shaft.

The alternative decontamination protocol evaluated in our study has now been applied in more than twenty forensic cases and resulted in unambiguous mtDNA sequences. Even in a forensic case where freshly plucked hairs were the only reference sample for a suspect, the mtDNA sequence showed several ambiguous positions, which is normally not expected from freshly prelevated samples. This case stresses the absolute need of a preliminary decontamination procedure in every forensic case where hairs are the evidence samples. Moreover, the differential lysis buffer, used to decontaminate the hair shaft, can be extracted also. This allows us to determine if there were contaminant cells present on the hair shaft and also to obtain its mtDNA sequence (data not shown). In case the hair still shows an ambiguous mtDNA sequence after the decontamination procedure, the hair DNA sequence would still be identifiable by comparison with the DNA sequence from the differential lysis buffer.

Investigations of hair samples are not only frequently used in forensics but also in population studies because of the simple acquisition, handling and stability of the samples [14]. Recently, somatic mosaicism has been observed in mtDNA isolated from single hairs of an individual [15–18]. Gill et al. [16] argued that a bottleneck in the number of mtDNA molecules together with the high rate of cell division in hair-forming

cells enables fixation of a mutation in individual hair shafts within the lifetime of an individual. This might explain the relative higher occurrence of heteroplasmy in hairs compared to other tissues. However, heterozygous positions in mtDNA sequences which could be due to heteroplasmy, might in some cases originate from sample contaminants. Decontamination with a differential lysis buffer performed prior to DNA extraction allows one to obtain unambiguous results and should be applied to exclude contamination.

In conclusion, we have shown that correct mtDNA typing results on hair shafts and root parts can be obtained if contaminated hair samples are 'cleaned' with a differential lysis buffer prior to DNA extraction. A simple wash procedure with physiological salt solution and alcohol was not sufficient to eliminate these contaminations in all the samples. An important restriction in the use of a differential lysis buffer is that the roots of the hairs must be removed before differential lysis, otherwise nuclear DNA analysis will not be possible anymore.

## Acknowledgements

The help of the donors of the hair samples is gratefully acknowledged. This work was supported by a grant (nr G.0241.98) from the Fund for Scientific Research-Flanders (1998–1999).

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