

Analysis of mtDNA mixtures from different fluids: An inter-laboratory study

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Abstract. The mitochondrial DNA (mtDNA) working group of the GEP-ISFG carried out an inter-laboratory exercise consisting of the study of mixture stains (saliva/semen and blood/semen) in order to investigate the behaviour of these common forensic samples when analysing their mtDNA using standard sequencing methodology. All labs extracted the DNA by preferential lysis and amplified and sequenced the first hypervariable region I (HVS-I). The results showed high consensus between labs for the first fraction of the lysis but not for the second one. We also observed differences between mixtures

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prepared from different donors and different body fluids. The present study has important consequences for the analysis and interpretation of mtDNA mixtures. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

The analysis of mixed stains is a routine practice in forensic casework, mainly related to sexual assault cases. These analyses are commonly performed using preferential lyses followed by nuclear STRs typing. However, in a number of cases, it could be interesting to know the mtDNA haplotypes that contributed to the mixture (e.g. degraded reference samples, exclusion of a maternal relationship between the victim and suspect in rape cases). Theoretically, when a preferential lysis is performed, the sperm mtDNA remain in the first fraction while the sperm nuclei are expected to remain in the second one [1]. On the other hand, the number of mtDNA copies varies depending on the type of tissue [2]. This fact could deeply influence the detection of minor components in unbalanced mixtures. In order to evaluate the influence of some parameters that may affect the study of a mixture by mtDNA sequencing analysis, the mtDNA working group of the GEP-ISFG carried out an inter-laboratory exercise consisting of the analysis of saliva/semen and blood/semen mixtures.

2. Materials and methods

We have studied mixtures from three semen donors and three female saliva/blood donors. The stains were prepared as shown in Table 1 and subsequently sent to the fourteen participating labs. No a priori information was provided concerning either the mitochondrial haplotypes of contributors or the dilutions of semen. Each lab used their routine methodologies to carry out preferential lyses, cell count, nuclear or mtDNA quantification, HVS1-PCR and automated sequencing.

3. Results

3.1. Comparing the first and second fractions

First fraction: most of the participants reported a mixture of male and female mtDNA haplotypes for the undiluted semen stains. The male component became less obvious in proportion to the degree of the semen dilutions, although the loss of signal was not uniform throughout all the nucleotide positions. Most labs detected only the female component in mixtures where the semen was diluted 1:10 or 1:20 (Fig. 1).

Second fraction: results were more diverse and ambiguous. Some labs did not obtain any amplicon or obtained partial or blurred electropherograms (inconclusive in Fig. 1); moreover, contamination

Table 1
Samples analysed in this inter-laboratory study

Mixture	Haplogroups	Female saliva+semen mixtures (set 1)	Female blood+semen mixtures (set 2)
1	Female T2	50 µl saliva+50 µl pure semen	50 µl blood+50 µl pure semen
	Male H	50 µl saliva+50 µl semen 1:10	50 µl blood+50 µl semen 1:10
		50 µl saliva+50 µl semen 1:20	50 µl blood+50 µl semen 1:20
2	Female K	50 µl saliva+50 µl pure semen	50 µl blood+50 µl pure semen
	Male H	50 µl saliva+50 µl semen 1:10	50 µl blood+50 µl semen 1:10
		50 µl saliva+50 µl semen 1:20	50 µl blood+50 µl semen 1:20
3	Female H	50 µl saliva+50 µl pure semen	50 µl blood+50 µl pure semen
	Male H	50 µl saliva+50 µl semen 1:10	50 µl blood+50 µl semen 1:10
		50 µl saliva+50 µl semen 1:20	50 µl blood+50 µl semen 1:20



Fig. 1. Haplotype results in first and second fractions.

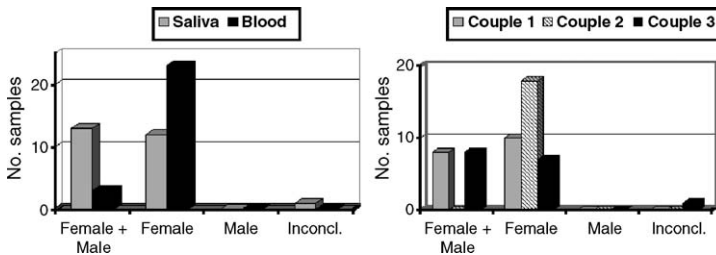


Fig. 2. First fraction results in 1:10 semen dilution samples: comparing fluids (left) and donors (right).

problems were also reported by several labs (inconclusive in Fig. 1). This could be because very small amounts of mtDNA remain in this fraction, and contamination is more likely to occur.

3.2. Comparing tissues

We did not find differences between saliva/undiluted semen and blood/undiluted semen mixtures, since most labs reported the female/male mixture. However, when comparing the samples prepared with 1:10 diluted semen, the female haplotype was mostly detected in blood/semen stains whereas a mixture of haplotypes was detected in half of the saliva/semen stains (Fig. 2, left). Therefore, it seems that the number of mtDNA copies (per volume) in the blood could be higher than in saliva.

3.3. Comparing the donors

The results obtained in mixture-1 and -3 (Table 1) were similar, but very different to the ones in mixture-2. In the 1:10 semen dilution samples, most labs detected both male and female haplotypes in mixture-1 and -3, but all labs reported only the female haplotype in mixture-2 (Fig. 2, right). Therefore, our results suggest that different donors contribute with different mtDNA content.

4. Conclusion

The analysis of mixtures of body fluids by mtDNA-sequencing technology should be performed with special care. Several variables should be taken into account for interpretation: the types of body fluids involved in the mixture, the risk of contamination mainly in the second fractions, the loss of signal in some nucleotide positions (but not in others), and the fact that differences in mtDNA content between donors are also possible. We also advance that phylogenetic interpretation of the DNA mixtures could play an important role to detect loss of particular diagnostic variants in the mtDNA profile of the contributors.

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