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

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The analysis of mixed stains is a routine practice in forensic casework, mainly related to sexual assault cases. These analyses are commonly performed using differential lysis that allows the separation of epithelial cells DNA from that at of spermatozoa, followed by nuclear STR typing. In a number of cases, however, it could be interesting to know the mitochondrial DNA (mtDNA) haplotypes that contributed to the mixture (e.g. degraded or low-copy number reference samples, exclusion of a maternal relationship between victim and suspect in rape cases, etc). In the last GEP-ISFG mtDNA proficiency exercise (2003'04), the mtDNA analysis of a mixture stain (saliva from a female plus 1:20 diluted semen) yielded an unexpected consensus result: only the mtDNA hypervariable I and II saliva haplotype was detected, in contrast to the predominant presence of the male autosomal STR profile. Hence, the use of only mtDNA typing for this mixture sample could in this case lead to a false exclusion. Several additional experiments carried out by some laboratories pointed to the existence of different relative amounts of nuclear and mtDNA in saliva and semen (Crespillo et al. 2005, in press). In order to disentangle this puzzle, the mtDNA GEP-ISFG working group decided to carry out an inter-laboratory study.

We have studied mixtures from three semen donors and three saliva/blood female donors. Three semen dilutions (pure, 1:10 and 1:20) from each donor were mixed with saliva or alternatively, blood taken from each female donor (see Table 1). No *a priori* information was provided to the participating laboratories concerning either the mitochondrial haplotypes of contributors or the dilutions of semen. Each laboratory used their routine methodologies in order to carry out differential lysis, cell count, nuclear or mtDNA quantification, PCR and sequencing. There was a high consensus between labs for the epithelial fractions. In contrast, results concerning the seminal fractions were more ambiguous. In addition, some laboratories reported contamination problems in the male fraction. The most plausible explanation to this finding is that, after differential lysis, female and male mitochondria remain in the epithelial fraction and, theoretically, no mtDNA should be found in the male fraction (assuming effective differential lysis). Nevertheless, the first lysis is not always completely effective, so that mtDNA is also detected in the seminal fraction. The detection level of the male component decreased in accordance with the degree of semen dilutions, although the loss of signal was not uniform throughout all the nucleotide positions. There were clear differences between the mixtures prepared from different donors and body fluids. In some cases the male component was not detected. This may indicate that there are differences in the number of mitochondria (or cellular content) contributed by different donors and body fluids.

In conclusion, we can tentatively say that special care should be taken when analysing mtDNA in mixtures. There are several variables that we should bear in mind: the types of body fluids involved in the mixture, the possibility of contamination mainly in male fractions, the loss of signal in some nucleotide positions (but not in others), and the fact that differences in cellular content between donors are also possible. In addition, unlike the autosomal STR mixtures, the interpretation of mtDNA mixtures can be supported by using a phylogenetic approach. (contact: lourditasmt@ya.com).

Female/male pair number	Haplogroups	Female saliva / semen mixtures	Female blood / semen mixtures
1	Female T2	50 µl of saliva + 50 µl of pure semen	50 µl of blood + 50 µl of pure semen
	Male H	50 µl of saliva + 50 µl of semen 1:10	50 µl of blood + 50 µl of semen 1:10
		50 µl of saliva + 50 µl of semen 1:20	50 µl of blood + 50 µl of semen 1:20
2	Female K	50 µl of saliva + 50 µl of pure semen	50 µl of blood + 50 µl of pure semen
	Male H	50 µl of saliva + 50 µl of semen 1:10	50 µl of blood + 50 µl of semen 1:10
		50 µl of saliva + 50 µl of semen 1:20	50 µl of blood + 50 µl of semen 1:20
3	Female H	50 µl of saliva + 50 µl of pure semen	50 µl of blood + 50 µl of pure semen
	Male J2	50 µl of saliva + 50 µl of semen 1:10	50 µl of blood + 50 µl of semen 1:10
		50 µl of saliva + 50 µl of semen 1:20	50 µl of blood + 50 µl of semen 1:20

Table1. Composition of the mixture stains analysed in the inter-laboratory study.