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Analysis of body fluid mixtures by mtDNA sequencing: An inter-laboratory study of the GEP-ISFG working group

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Abstract

The mitochondrial DNA (mtDNA) working group of the GEP-ISFG (Spanish and Portuguese Group of the International Society for Forensic Genetics) carried out an inter-laboratory exercise consisting of the analysis of mtDNA sequencing patterns in mixed stains (saliva/semen and blood/semen). Mixtures were prepared with saliva or blood from a female donor and three different semen dilutions (pure, 1:10 and 1:20) in order to simulate forensic casework. All labs extracted the DNA by preferential lysis and amplified and sequenced the first mtDNA hypervariable region (HVS-I). Autosomal and Y-STR markers were also analysed in order to compare nuclear and mitochondrial results from the same DNA extracts. A mixed stain prepared using semen from a vasectomized individual was also analysed. The results were reasonably consistent among labs for the first fractions but not for the second ones, for which some laboratories reported contamination problems. In the first fractions, both the female and male haplotypes were generally detected in those samples prepared with undiluted semen. In contrast, most of the mixtures prepared with diluted semen only yielded the female haplotype, suggesting that the mtDNA copy number per cell is smaller in semen than in saliva or blood. Although the detection level of the male component decreased in accordance with the degree of semen dilution, it was found that the loss of signal was not consistently uniform throughout each electropherogram. Moreover, differences between mixtures prepared from different donors and different body fluids were also observed. We conclude that the particular characteristics of each mixed stain can deeply influence the interpretation of the mtDNA evidence in forensic mixtures (leading in some cases to false exclusions). In this sense, the implementation of preliminary tests with the

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aim of identifying the fluids involved in the mixture is an essential tool. In addition, in order to prevent incorrect conclusions in the interpretation of electropherograms we strongly recommend: (i) the use of additional sequencing primers to confirm the sequencing results and (ii) interpreting the results to the light of the phylogenetic perspective.
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1. Introduction

The analysis of mixtures of fluids is routine practise in forensic casework usually related to sexual assault cases. These analyses are generally performed using preferential lyses followed by STR genotyping. In a number of cases, however, the mtDNA analyses can be the unique strategy (e.g. when analyzing degraded or low copy number samples). In other cases, establishing the mtDNA haplotypes of the contributors can add information to the legal investigation (e.g. to exclude a maternal relationship between victim and suspect in rape cases, or when trying to obtain some information about the (geographical) origin of the mtDNA carried by the unknown offender [1]).

Theoretically, when a preferential lysis is performed on semen mixed with other fluids (e.g. saliva, blood, or vaginal fluid), DNA from the non-spermatic cells remains in the first fraction, while the nuclear DNA (nDNA) from the spermatozoa remains in the second one. This is due to the fact that in the spermatic nuclei there are rich disulfide bond proteins [2], which give relative resilience (compared to epithelial and other cells) against the enzymatic treatment employed during DNA extraction [3]. Therefore, if the preferential lysis is effective, the first fraction should contain a mixture of male (from non-spermatic cells and the mid-pieces of spermatozoa) and female mtDNAs, whereas the second fraction should not retain any mtDNA from mitochondria (note however that some mtDNA inserts from the nuclear genome [4] could be interpreted as real mtDNA).

On the other hand, the number of mtDNA copies varies depending on the cell type [5,6]. It is unknown to what extent this fact could affect the detection of minor components in unbalanced mixtures. During the 2004 GEP-ISFG mtDNA proficiency exercise [7], a mixture stain (saliva from a female and semen diluted 1:20) was studied and the mtDNA sequencing analysis yielded an unexpected consensus result: only the HVS-I/HVS-II saliva haplotype was detected, while the male autosomal STR profile was predominant. Hence, the use (exclusively) of mtDNA analysis could in this case lead to a false exclusion. Several additional experiments were performed in order to clarify these apparent contradictory results. The results of these experiments pointed to the existence of different relative amounts of nuclear and mitochondrial DNAs in saliva and semen [7].

Forensic labs have demonstrated to have a great deal of experience in analysing nDNA when performing preferential lysis, but very little in mtDNA [8]. In order to shed light on the mtDNA patterns originated when analyzing mixtures of semen with other body fluids, the mtDNA-working group of the GEP-ISFG carried out the present inter-laboratory study.

2. Materials and methods

The stains were prepared using mixtures of fluids from three healthy couples (which hereafter will be referred to as couples 1, 2 and 3), each one made-up with samples from a male and a female donor. For each couple, the males donated their semen while the female provided the saliva and blood. Samples were prepared in the Policía Científica DNA lab in Madrid (Spain) by mixing saliva or blood with the same volume of semen. In order to simulate forensic casework, in each case the samples were prepared using three semen dilutions in saline buffer: pure, 1:10 and 1:20 (see Table 1). The fresh fluids were mixed in a laminar-flow hood, shaken, and subsequently, 100 μl of the mixture were

Table 1
Samples analysed in this inter-laboratory study

Female/male number pair	Female saliva/semen mixtures	Female blood/semen mixtures
Samples analysed by participating labs		
1	50 μl of saliva + 50 μl of pure semen	50 μl of blood + 50 μl of pure semen
	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	50 μl of saliva + 50 μl of pure semen	50 μl of blood + 50 μl of pure semen
	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	50 μl of saliva + 50 μl of pure semen	50 μl of blood + 50 μl of pure semen
	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
Samples analysed by coordinating lab		
4 (Female 3 + vasectomized male)	50 μl of saliva + 50 μl of pure semen	50 μl of blood + 50 μl of pure semen
	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

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