GEP-ISFG mitochondrial DNA collaborative exercise 2004: assessing mtDNA male-female contribution in a semen/saliva mixture DNA sample.

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As part of the 2004 GEP-ISFG mtDNA proficiency exercise, the Quality Control Unit of the National Institute of Toxicology and Forensic Sciences (Madrid) provided a mixture stain (called M6) consisting of 100 μL of saliva from a supposed female victim and 50 μL of a 1:20 semen dilution from a supposed offender. Blood samples of both donors were also provided (namely, M4 and M5 from victim and suspect, respectively). HVS-1/2 haplotypes were 263G 315.1c for M4 and 16266T 263G 309.1C 315.1C for M5. MtDNA sequencing analysis of the saliva/semen mixture M6 produced an unexpected consensus result (13 out 19 labs): only the HVS1/2 saliva haplotype (M4) was detected, either after preferential lysis or after complete DNA digestion. This result paradoxically contradicted the autosomal STR profile obtained from a complete lysis, where the male component (M5) was predominant.

Several labs carried out additional experiments to clarify the puzzle: (i) two labs analysed HVS-1 with additional different primer sets which yielded the same unique M6 haplotype, thus allowing rejection of the hypothesis of a primer binding site mutation in the semen of the donor; (ii) other lab reproduced the same mixture experiment using different saliva and semen donors, an experiment that yielded the same pattern as in M6: only the saliva haplotype was detected; (iii) the same lab performed HVS-1 amplifications using decreasing nuclear DNA dilutions from semen and saliva and the results showed a loss of signal at 2 pg of nuclear DNA from semen, while at 0.2 pg of nuclear DNA from saliva amplicons were still detected; (iv) theoretical calculations of the relative number of mitochondrial DNA copies in spermatozoa and epithelial cells in the mixture was made by another lab, and (v) finally, one lab carried out coding region SNPs analysis using SNaPshot, a more sensitive method than sequencing that allowed the detection of the two components (M4 and M5) of the M6 sample.

In conclusion, the results pointed to the existence of different relative amounts of nuclear and mitochondrial DNAs in saliva and semen. This circumstance could deeply influence the detection of mtDNA in evidences with unbalanced mixtures of different fluids and could lead to false exclusions.

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