

ANALYSIS OF THE DIFFERENT SUPPORTS IN ALTERNATIVE SAMPLES USED IN THE IDENTIFICATION OF POSSIBLE MISSING PERSONS DESCENDANTS

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The Banco Nacional de Datos Geneticos (BNDG) in Argentina was created by National Law No. 23.511. This law establishes our laboratory as the official expert in causes related to civil state suppression, during the military government between 1976 and 1983. Sometimes, Argentine Justice can't obtain blood samples or bucal swabs from possible descendants of missing persons as they refuse to be DNA analyzed to go on with the research for each of the cases where we investigate the possible suppression of identity. In those cases, there is a National Law that allows the use of alternative samples such as toothbrushes, shavers, underwear, shirts, socks, pants and other elements related to daily use as they might contain genetic material suitable for obtaining a genetic profile.

Aim: To present the effort of the BNDG for the identification of possible missing persons' descendants through the obtention of the genetic profile in alternative samples and to show the relevance of using different supports (plastic and porous), taking into account the characteristics of these materials in the preservation of cells, product of body's natural desquamation.

We received 160 samples, obtained from 23 searches, corresponding to 11 male and 12 female individuals. The samples were: toothbrushes, razors, stubs of cigarettes, clothes used by the individual at the moment of the search such as trousers, T-shirts, female protectors and towels. DNA was isolated by phenol-chloroform method or QIAamp® DNA Investigator Kit by QIAcube. They were extracted by court of the material and in case of the clothes, by scrapping and swabbing the regions in contact with the individual.

Materials and methods: AmpFISTR1 Identifiler, AmpFSTR® MiniFiler™ PCR Amplification Kit, GenePrint PowerPlex® 16 System and AmpFLSTR1 Yfiler kit (Applied Biosystems) were used. Electrophoresis of the amplification products was performed on an ABI PRISM1 3100 and 3130 Genetic Analyzer (Applied Biosystems). Data Collection, GeneScan Analysis v3.1 and Genotyper Analysis v2.5.2 Data Collection V 3.0, GeneMapper® Software V 3.2.1 softwares were used. Mitochondrial DNA: HV1 and HV2 fragments were amplified with: L-15997/H-16255; L-16209/H- 16401 and L-00030/H-00412 primers. Sequencing was performed with BigDye1 Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). PCR products were purified and then analyzed in an ABI PRISM1 3100 Genetic Analyzer (Applied Biosystems).

Results: When we could not exclude the haplotypes of possible descendants of missing persons neither by autosomal STRs analysis nor by comparison of mitochondrial DNA sequences (HV1 + HV2) with the haplotypes of family groups held in the database of the Banco Nacional de Datos Geneticos, "Familias" software was used. Toothbrushes and razors (plastic source) resulted as a good source for DNA recovery. Porous supports (socks, intimate apparel, t-shirts and stubs of cigarette) turned out to be suitable for DNA obtaining. In all cases, we used both plastic holders and porous supports to reproduce genetic profiles necessary for comparison with the family groups mentioned above. We considered the toothbrushes and stubs of cigarette as the alternative samples of preference to be analyzed for genetic identification. We recovered DNA from 126 processed samples. Nine individuals were restituted to their biological families by using alternative samples. All the samples were first analyzed by autosomal STRs

and Y Chromosome STRs. According to the results obtained, we verified the quality and quantity of the extracted DNA, we corroborated the sex with the amelogenin locus and then the samples were transferred to DNA Mitochondrial Area in order to obtain the DNA mitochondrial haplotype that constitute the screening method in Crimes of Lesa Humanity. From the 23 individuals analyzed, five were not transferred to DNA Mitochondrial area because the amelogenin locus didn't coincide with the sex of the individual to identify. From the other 18 individuals sent to the DNA Mitochondrial area, 17 were identified with the use of toothbrushes and 1 with the use of razors.