Forensic DNA Typing of Twenty Year Old Bloodstains Using Highly Discriminating Multiplexes

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This study presents the results of DNA analysis of twenty year old bloodstains. Experimental bloodstains were prepared on a piece of cotton fabric, clean and rusty iron, wood and glass, and stored at room temperature and 4°C for twenty years.

DNA was extracted in a buffer containing 10 mM TRIS, 100 mM NaCl, 10mM EDTA, 39 mM DTT, 2% SDS and 0.1 mg Proteinase K at 56°C for 20 hours, followed by the treatment with phenol/chloroform/isoamyl alcohol and the Microcon 100 concentration. DNA concentration was determined using the QuantiBlot Human DNA Quantitation Kit (Perkin Elmer). Quality of DNA was analysed by agarose gel electrophoresis.

Three highly discriminating multiplex systems have been used for DNA amplification and typing: *GenePrint*™ PowerPlex™ Fluorescent STR System which allows co-amplification and two-color detection of eight STR loci: vWA, TH01, TPOX, CSF1PO, and D5S818, D13S317, D7S820, D16S539, *GenePrint*™ FFFL multiplex from Promega (F13A01, FESFPS, F13B, LPL) and AmpFISTR Blue from Perkin Elmer (D3S1358, vWA and FGA). Amplified fragments were separated by electrophoresis in a 5% denaturing polyacrylamide gel using the ABI Prism 377 DNA Sequencer. Fluorescent ladder CXR 60-400 Bases (Promega) was included in each lane as an internal standard. Allele size determination and genotyping were performed using ABI Prism GeneScan Analysis 2.1 and ABI Prism Genotyper 2.0 software by comparison of amplified fragments with internal size standards and allelic ladders.

For the DNA isolated from twenty year old bloodstains on a rusty and clean iron, wood and glass stored at room temperature full profiles were obtained using all multiplex STR systems. Negative results were obtained only for twenty year old bloodstains stored at room temperature on a piece of cotton fabric. Full profiles were also obtained for the DNA isolated from twenty year old bloodstains stored at 4°C on all surfaces analyzed. Nevertheless, significant differences in peak height between different loci were observed. Due to the small amount and high level of degradation of the DNA extracted from twenty year old bloodstains, successful and highly confident genotyping requires individual amplifications in a wide range of template concentration.