

**A COMPARISON OF THE RECOVERY OF MITOCHONDRIAL AND NUCLEAR DNA FROM BIOLOGICAL STAINS SUBMERGED IN SALT WATER**

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This study examines the probative value of biological stains that have been submerged in saltwater. The particular focus was to evaluate the recovery of nuclear and mitochondrial DNA from blood and semen stains submerged for up to 60 days. Prior to DNA analysis, presumptive color tests were performed on each biological stain. It was found that blood tested KM positive up to 60 days submerged whereas semen tested AP positive up to 5 days submerged. Stain extracts were quantified in order to render a comparison of the mitochondrial and nuclear DNA yield. For mtDNA analysis, the extracts were amplified using the LINEAR ARRAY<sup>®</sup> duplex PCR system and the amplified product was analyzed by agarose gel electrophoresis. Using this approach, the estimated yield from the bloodstains ranged from 25ng/mL (1 day submersion) to <2.5ng/mL (60 day submersion). However, no mtDNA was recovered from semen stains deposited on the cotton or denim substrates. Nuclear DNA was quantified by Real-Time PCR with the ABI Quantifiler™ Kit and the ABI Prism® 7000 Sequence Detection System. With the exception of one sample (bloodstained denim submerged for 1 day, 0.710ng/mL), the bloodstains gave low yields that ranged from 0.073ng/mL – 0.002ng/mL. Also of note, more than fifty percent of the bloodstains gave a zero yield. In contrast, the semen stains gave high yields, ranging from 182.140ng/mL to 0.238ng/mL. Only one semen stain fell below the target input for genotyping (semen stain on cotton submerged for 6 days, 0.071ng/mL). In general, the quantitation results obtained for nuclear and mitochondrial DNA reflect a decrease in yield as the submersion period increases. Mitochondrial DNA polymorphisms were observed using the LINEAR ARRAY<sup>®</sup> mtDNA HVI/HVII Region-Sequence Typing kit. A full LINEAR ARRAY<sup>®</sup> mtDNA profile was obtained from bloodstained cotton and denim substrates following a submersion period of up to 60 and 25 days, respectively. These results will be compared to the STR profiles obtained following genotyping with the AmpFISTR® Identifiler® PCR Amplification Kit and the ABI Prism® 310 Genetic Analyzer. This study demonstrates that, in principle, human mitochondrial and nuclear DNA can be recovered from biological stains that have been submerged in salt-water. An important consideration that must be evaluated is the type of the biological stain (i.e., semen v. blood), which may dictate the most appropriate analytical approach.