INCREASED DNA YIELD FROM SPECIMENS USING ALTERNATIVE REDUCING AND ALKYLATING AGENTS

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During forensic DNA analysis, dithiothreitol (DTT), a reducing agent, may be utilized to reduce and break disulfide bonds in proteins to facilitate the extraction and purification of DNA from a specimen. DTT reduces disulfides to dithiols, allowing release of the DNA from its protective proteins and further degradation of the proteins by proteinase K. This agent is often employed when extracting DNA from hair shafts which are largely composed of keratin, a structural protein replete with disulfide bonds. DTT is also typically used to lyse and extract DNA from spermatozoa, the outer membrane and chromatin of which contains disulfide bonds. However, there exists the possibility that disulfide bonds may reform once reducing conditions are removed later in the extraction process and that such reformation may in turn reduce DNA yield and/or purity. To prevent such reformation, an alkylating agent such as iodoacetamide (IAM) may be employed in conjunction with DTT. In addition, tris(2-carboxyethyl)phosphine (TCEP) may be used as an alternative to DTT, or DTT plus IAM, since it is both a reducing agent and an alkylating agent. This study aimed to examine the effect of IAM and TCEP on the quantity and quality of DNA extracted from hair, semen, and blood. DNA yields were assessed using nuclear and mtDNA-specific qPCR methods and quality was assessed following 16 loci STR analysis. The results showed that DTT plus IAM typically yielded more DNA over DTT alone, and that TCEP alone or in combination with IAM significantly and substantially yielded more DNA than DTT alone. The results also confirmed that TCEP and IAM did not interfere with downstream STR analysis. The use of alternative reducing and alkylating agents such as TCEP may enhance the recovery of DNA from forensic specimens and promote their successful forensic analyses, particularly for challenging specimens which contain low quantities of DNA and/or degraded DNA.