B114 Enhanced DNA Extraction Via the Reduction and Alkylation of Disulfide Bonds by Iodoacetamide (IAM) and Tris(2-carboxyethyl)phosphine (TCEP)

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After attending this presentation, attendees will understand the importance of reducing and alkylating reagents during forensic DNA extraction, how the selection of these agents can increase DNA yield, and how to incorporate them into a typical casework workflow.

This presentation will impact the forensic science community by providing methods to significantly increase DNA yield from various forensic specimen types (i.e., blood, hair, semen, and calcified tissue) through the use of novel reducing and alkylating reagents which facilitate the breakage of and/or prevention of disulfide bond reformation in cellular proteins during DNA extraction.

Dithiothreitol (DTT) is a key reducing agent used in forensic DNA extraction that facilitates the isolation and purification of DNA from proteins in a biological specimen. DTT reduces protein disulfide bonds to thiols, thereby facilitating protein digestion by proteinase K and permitting the release of DNA from protective and/or contaminating proteins. For example, the unique membrane of spermatozoa, which is rich in disulfide bonds, requires a particularly strong reducing agent to lyse these cells and extract their DNA. Reducing agents are also employed when extracting DNA from hair shafts which are largely composed of keratin, a structural protein which contains myriad disulfide bonds.[1-3]

When using a reducing agent such as DTT, which produces a disulfide exchange, disulfide bonds may reform and may, in turn, reduce DNA yield and/or purity. To prevent such reformation, an alkylating agent, IAM, may be employed in conjunction with DTT. IAM works as an irreversible alkylator by binding to the thiol group of the cysteine residues, thereby preventing the reduced disulfide from reforming. Alternatively, TCEP may be used in lieu of DTT, since it functions as both a reducing agent and an alkylating agent. TCEP also provides many advantages over DTT, such as being odorless and more stable at room temperature.[3,4]

This study examined the effect of IAM and TCEP on the quantity and quality of DNA extracted from hair, calcified tissue, semen, and blood. The following conditions were compared: (1) DTT alone (Standard Operating Protocol (SOP)); (2) DTT followed by IAM alkylation; (3) TCEP; and, (4) TCEP followed by IAM. DNA yields were assessed using nuclear and mitochondrial DNA (mtDNA) -specific quantitative Polymerase Chain Reaction (qPCR) methods and DNA quality was assessed following 16-locus Short Tandem Repeat (STR) analysis.

Both IAM and TCEP are water-soluble and were easily incorporated into a semi-automated SOP. Briefly, the SOP entails lysis of a specimen in buffer G2 from QIAGEN®, proteinase K, and DTT followed by purification on QIAGEN's® EZ1 platform. TCEP treatment simply involved replacement of DTT during the lysis step, at a concentration of 30mM with no further change in the lysis conditions. IAM treatment involved a separate incubation step with 150mM at 22°C for 30 minutes in the dark following the lysis step which included *either* DTT *or* TCEP.

The results revealed that for blood, TCEP treatment significantly increased DNA yield over the SOP by an average of 270%, whereas for semen, TCEP/IAM treatment increased yield over the SOP by an average of 350%. The results also confirmed that TCEP and IAM did not interfere with downstream STR analysis. The benefits of IAM and TCEP were similarly observed for calcified tissue and shed hair. Optimization experiments revealed that TCEP was optimal at 30mM.

In conclusion, the use of IAM, an alternative reducing agent, and TCEP, a reducing/alkylating agent, enhances DNA recovery from hair, calcified tissue, semen, and blood, thus promoting their successful forensic analyses. These enhancements should be particularly valuable for those challenging specimens which contain low copy DNA and/or degraded DNA.

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