EVALUATION OF TCEP AND IAM ON LOW LEVEL SAMPLES

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The reducing agent dithiothreitol (DTT) is often employed during forensic DNA extraction to facilitate the extraction and purification of DNA from a specimen by reducing and breaking disulfide protein bonds. We previously demonstrated that, compared to the standard operating protocol (SOP) which utilizes DTT, significantly improved DNA yields from blood and semen where obtained without a reduction in DNA quality when using the alternative reducing reagent tris(2-carboxyethyl)phosphine (TCEP) either alone or in tandem with the alkylating agent iodoacetamide (IAM), which served to prevent disulfide bond reformation. In contrast, this study was directed at examining forensically relevant, low-level samples, and based on prior results, the study was limited to a comparison of three different extraction methods: (1) the SOP which utilized DTT, (2) TCEP (190 mM final) followed by IAM (250 mM final), and (3) TCEP (30 mM final) followed by IAM (150 mM final). The following three low level specimen types were prepared from three donors each, spotted onto cotton swabs, dried, extracted via the three methods described, and evaluated with respect to DNA yield: (1) a 1:250 dilution of blood, (2) a saliva rinse, and (3) a 1:220 dilution of semen with a 1:24 dilution of blood which served to mimic a post-coital swab. Incubation conditions were also varied with respect to time and temperature for the two TCEP/IAM methods. DNA extracts were quantified using the Quantifiler® Duo qPCR Quantification Kit and compared. The results demonstrated that both extraction methods which utilized TCEP followed by IAM yielded results which were comparable to that of the SOP (DTT) under most extraction conditions. Taken together, these results suggest that TCEP followed by IAM may be a suitable alternative for forensic DNA analysis of evidentiary items which contain low levels of biological material.

Key words: DNA extraction, Iodoacetamide (IAM), Tris(2-carboxyethyl)phosphine (TCEP)