B2 A Powder-Free Approach to Extracting DNA From Environmentally Challenged Bone Samples

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After attending this presentation, attendees will appreciate some of the advantages and disadvantages of altering bone processing, tissue digestion, and DNA extraction methods in an attempt to increase DNA quantity and quality for more successful Short Tandem Repeat (STR) typing from environmentally challenged bone samples. This presentation proposes a method to efficiently extract DNA from bone samples without the need to pulverize the bone tissue into a fine powder prior to digestion.

This presentation will impact the forensic science community by providing insight into the benefits and limitations of demineralizing bone tissue without the traditional requirement for powdering. This sample-processing approach is coupled with a commercial DNA extraction kit performed with and without automation for downstream STR analysis.

Bone samples are often encountered in missing persons' cases and mass disasters for human identification purposes. In order to generate a high-quality STR profile, it is necessary to extract a sufficient quantity of high-quality DNA (>100pg) while simultaneously eliminating unwanted contaminants and Polymerase Chain Reaction (PCR) inhibitors. Traditional bone DNA extraction methods rely on cutting and crushing bone into a fine powder and a long demineralization step coupled with an organic or silica-based DNA purification method. An alternate approach may be to use a whole bone digestion buffer in combination with a commercial DNA extraction kit to avoid powdering bone samples prior to purification. This study compared the efficacy of both methods with and without automation.

Bone samples (N=6) from human cadavers exposed to various insults (fire, decomposition, sun exposure, burial, and embalming) were prepared, extracted, and STR-typed in triplicate. Compared to each of the other methods, the whole bone digestion buffer paired with the automated extraction consistently resulted in a significantly lower DNA yield per milligram of powdered bone (p<0.05). Regardless of the chemistry used, significantly less DNA (p<0.01) was obtained when the extraction process was automated, compared to performed manually. No statistical difference was observed in the average DNA yields when the commercial kit was performed with or without the whole bone digestion step. Although DNA yields were higher using the complete demineralization protocol, STR success rates and overall profile quality were comparable across all the manual methods tested.

The whole bone system is an alternative method to conventional complete demineralization methods which are more laborious and time consuming (~30hrs versus ~16hrs), require powdering of bone tissue, pose a higher risk of contamination, and consume the entire sample. Another advantage of the whole bone system is the provision for a second DNA extraction from the same sample. These multiple extracts may be used to perform additional analyses or combined and concentrated to increase the amount of input DNA for improved results.

Overall, this research has shown that eliminating the need to powder bone tissue can simplify the DNA extraction process without significantly reducing downstream STR success.

Bone, DNA Extraction, Short Tandem Repeats