COMPARISON OF DNA EXTRACTION PROCEDURES FOR HUMAN BONE IDENTIFICATION

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Recovery of DNA from bone samples exposed to adverse environmental conditions for prolonged periods has significant application in the identification of human remains. At times, information derived from DNA typing serves as the only tool for identification. Proper management and processing of human bone samples is important in countries like the Philippines which experiences a variety of natural, e.g. up to 20 typhoons per year; as well as man-made disasters. In addition, the tropical climate with relatively high humidity, promote rapid decomposition of any recoverable tissue from human remains thus preventing identification through standard pathological examinations. Hence, this study undertook to compare five DNA extraction procedures for handling fresh bone samples and bone samples that had been exhumed six months post burial. The criteria used for comparison includes DNA yield, DNA purity, e.g. absence of PCR inhibitors in solution and DNA quality, e.g. successful amplification of up to 500-base fragments routinely used in autosomal Short Tandem Repeat DNA profiling.

DNA from fresh and exhumed bone samples was isolated using QIAamp® Micro and QIAamp® DNA Investigator Kits (silica based), Promega DNA IQ™ Kit (paramagnetic resin-based), and standard organic extraction. In addition, the Promega Maxwell™16 LEV System, an automated liquid handling workstation using DNA IQ™ chemistry, was also evaluated. DNA yield and presence of PCR inhibitors were assessed by real time PCR using the Plexor® HY System (Promega) and amplified in a PowerPlex® 16 multiplex reaction.

DNA quantification results of exhumed bone showed comparable DNA yield between DNA IQ™ and organic based procedures with a concentration range of 1.6-6.0ng per 0.1g bone powder compared to a slightly lower yield observed from samples extracted with Maxwell™16 LEV system (1.0-2.5ng/0.1g bone powder) and silica based (0.16-0.84ng/0.1g bone powder) procedures. Inhibition was likewise observed in 75% of the extracts using silica based procedures.

Full DNA profiles with minimal peak height imbalance for heterozygous alleles were obtained from fresh bone samples using DNA extraction procedures tested in the study. In contrast, preliminary STR typing of DNA extracted from exhumed bone samples showed that the organic extraction procedure provided the best DNA in terms of yield, purity and quality compared to commercially available kits tested.