H44 An Experimental Test of the Accuracy of Human Forensic Identification Techniques for Analysis of BurnDamaged Bone and Tissue

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The goal of this presentation is to provide the forensic community with information about the potential for errors in DNA-based forensic identification of hard and soft tissues with increasing burn damage.

While technical methods continue to improve the potential for the analysis of degraded DNA samples, forensic analysts should be cautioned that, especially in cases involving highly degraded skeletal material where the amount of DNA extracted is very small, the potential for problems may be of concern. Further study and improved coordination between DNA analysts and forensic scientists will impact the forensic community and/or humanity by becoming useful in the development of guidelines for forensic identification of different types of highly degraded human remains.

Forensic scientists have been challenged by recent events, such as the tragedies of 9/11 and genocide incidents abroad, which have highlighted the difficulties of DNA analysis from fragmented and thermally degraded remains. Previous studies have investigated the effectiveness of alternate methods for extraction of highly degraded DNA. Here, the authors use an experimental framework to investigate the accuracy, relative to results from unburned tissue, of the commonly used molecular forensic identification methods. Specifically, the authors address how results vary with increasing temperature and duration of the thermal event, document the consequences for identification methods as human DNA is exposed to heat of varying intensity, and discuss the limitations of current technologies in cases involving burned tissue and bone.

Microsatellite (STR) loci, mitochondrial and nuclear DNA have been amplified with varying degrees of success from burn-damaged tissues. Thermally induced deamination with increasing DNA chain fragmentation in burned samples has been implicated as the mechanism of DNA damage that has made molecular analysis difficult in some cases. This study tested samples from fleshed, human remains, not embalmed, without burning, and with increasing levels of burn damage. Prior to burning, unburned control samples for muscle, skin, bone, and teeth were collected from seven individuals, then the remains were burned in simulated forensic fire environments fueled by wood without accelerants. Scorch-damaged soft tissue and tooth samples were collected following charring of the muscle and skin (after 42-45 minutes of burning at a fire temperature of 600-700^{O}F). With increasing time and temperature, superficial soft tissues retract and burn away, leaving *charred bone* from carbonization of organic materials, and prolonged heat exposure results in brittle grayish calcined bone *(*samples taken after 90-100 minutes of burning at a fire temperature of 600-800^{O}F*)*.

DNA was extracted from hard and soft tissues with increasing burn damage using three extraction methods: CTAB/QIAquick, DNeasy, and CTAB/phenol-chloroform/QIAquick. Amplification of three types of genetic markers (mitochondrial control region HV1, microsatellite and a sex chromosome marker) was attempted for each tissue type and burn damage level. For testing potential variability in the effect of burn-induced DNA degradation, five microsatellite loci representing small to large amplicons were analyzed in this experiment. A series of control region primers covering the HV1 region were used to determine the maximum amplicon size associated with different tissue types and burn damage levels. Amelogenin primers flanking a sex-specific indel were used to assess variability in the effectiveness of sex determination.

All extraction methods were successful; however the CTAB/phenolchloroform /QIAquick combination produced the highest DNA yield (consistent with results reported by Ye *et al*., 2004). As expected, amplification of larger control regions segments was more difficult with higher levels of burn damage. Potential difficulties associated with increasing burn damage include: (1) DNA so highly fragmented that amplification of some loci (especially larger amplicons) is difficult or impossible; (2) inhibition of amplification by chemical byproducts that persist despite purification procedures during extraction; (3) PCR artifacts resulting in altered control region sequences and microsatellite allele sizes, resulting from chemical byproducts in burned tissues; and (4) bias in microsatellite amplifications due to fragmentation damage leading to a higher percentage of null alleles. While technical methods continue to improve the potential for the analysis of degraded DNA samples, forensic analysts should be cautioned that, especially in cases involving highly degraded skeletal material where the amount of DNA extracted is very small, the potential for problems as listed above may be high.

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Experimental verification of which molecular markers and analysis methods are robust to progressive stages of thermal degradation in various tissue types, and when such methods may be inaccurate, biased, or misleading, is needed to produce guidelines for effective and appropriate analysis of burn-damaged human remains. The preliminary results suggest that further study and improved coordination between DNA analysts and forensic scientists are needed to clarify if and when DNA analysis techniques can effectively be used in forensic identification.

Forensic Identification, Burned Bone, DNA Degradation

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