H46 Nuclear DNA Preservation in Soft and Osseous Tissues

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The goals of this presentation are to further elucidate the relationship between gross tissue condition and nuclear DNA preservation. Participants will learn about the utility of organic vs. inorganic DNA extraction techniques as well as troubleshooting failed PCR amplification attempts when dealing with degraded DNA. Furthermore, environmental and laboratory contamination prevention and detection will be addressed.

During the previous decade, forensic investigators have witnessed a paradigm shift to an increased reliance on DNA based decedent identification techniques. The benefits of DNA evidence include relatively high discriminatory powers as well as the capability of providing a match probability and likelihood estimate for each comparison. However, DNA identification has several limitations. Of primary concern is the ability to obtain authentic DNA from the decedent remains. This limitation has prompted research on biotic and abiotic variables affecting postmortem DNA degradation. In the laboratory, investigators have subjected isolated teeth, ribs, and bloodstains to a range of temperatures, pH, UV irradiation, soil types, and humidity levels for varying amounts of time [1,2,3,4]. Relatively few investigators have explored the possible correlation between tissue preservation and DNA survival under actual rather than laboratory conditions [5,6,7]. This study will refine the current understanding of postmortem nuclear DNA degradation by sampling a range of tissues under natural conditions from individuals with documented taphonornic histories. The following questions will be addressed:
(a) Which DNA extraction method (phenol chloroform vs. silica) appears to be most effective for each tissue and postmortem sampling interval?
(b) Is there a positive correlation between locus size and postmortem interval as measured by total body score and accumulated degree-days?
(c) Are there variations in DNA quantity and quality when comparing a range of soft and osseous tissues? What are some potential reasons for these variations in DNA preservation? (d) Can DNA degradation and PCR inhibition be distinguished when presented with a failed amplification? Which PCR inhibitor prevention strategies work best given the taphonornic history of the samples?

Weighing between 45 and 60 pounds, 28 pigs (Sus scrofa) were stunned using a captive-bolt gun and exsanguinated on June 8, 2001. The pigs were then deposited, 14 in each of two environments: tree cover surface and pond shore. These environments were located adjacent to each other within the Cornell University Experimental Ponds facility in Ithaca, New York. Tissues were collected on a scheduled basis beginning several hours postmortem, continuing on a biweekly, monthly, and bimonthly basis for 20 months. During this time, tissues were harvested from each pig twice, once in the first 10 months and once in the second half of the experiment. This schedule minimized anomalous tissue exposure in the early postmortem period, which would compromise the "natural" degradation process. When present, lung, liver, spleen, psoas muscle, adipocere, skin, and hair samples were collected. Additionally, ribs, lumbar vertebrae, femoral shafts, and parietal bones were collected at each sampling period. All samples were placed in a -20°C freezer upon procurement to minimize further DNA degradation. At each sampling event, gross decomposition was quantified and accumulated degree days were calculated as described by Megyesi [8]. This method results in a representation of decomposition state that will potentially reduce the subjective nature of qualitative descriptions, allowing a proper assessment of the relationship between gross preservation of sampled tissues and DNA preservation.

For comparative purposes, each tissue sample was extracted using an organic (phenol-chloroform) and inorganic (silica) protocol. Following extraction, the DNA was quantified using slot-blot hybridization with a porcine specific radioactive probe. PCR amplification of four short tandem repeat (STR) loci varying in size was then utilized to assess DNA degradation by measuring allele dropout. Five percent of the samples were then sequenced to assure amplification of the target loci. In situations where repeated amplification attempts failed, several methods were employed to overcome PCR inhibition. These include the addition of more DNA polymerase to the amplification reaction, which might overcome a potential inhibitor, the addition of bovine serum albumin (BSA), and the addition of sodium hydroxide. Each of these methods is compared for their effectiveness in resolving PCR inhibition. Contamination issues, which are of primary concern when dealing with degraded samples, are also addressed through the use of positive and negative controls throughout the study.

The ability to use a rapid, non-invasive screening process to assess potential DNA yield from various tissues allows for the optimization of sampling protocols in cases where limited sample is available. Optimizing tissue sampling for DNA analysis is also of keen interest to the mass fatality incident investigator who is charged with the identification of numerous decedents whose remains are often highly fragmented and degraded due to thermal and/or decomposition processes.

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