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# Elemental and DNA analysis of constituents obtained from extracting bone samples

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This presentation will elucidate the separation of sample constituents as a consequence of DNA extraction from human skeletal remains. This work aims to increase the number of individuals identified from skeletal remains by improving the development of techniques for extracting and analyzing DNA from bone samples. These are considered one of the most challenging sample types in the laboratory and require laborious processing that frequently yields incomplete genetic results or amplification failure. Possible causes of poor results may include DNA damage, PCR inhibitors and/or inefficient extraction. Pulverized bone samples from adjudicated cases were obtained from the University of North Texas (UNT) Center for Human Identification. Separate, homogenized aliquots of each bone sample were used for chemical and DNA testing. Baseline elemental content was determined by inductively coupled plasma-mass spectrometry (ICPMS). Elemental analysis was performed in solution mode ICP-MS. Acid digestion was used for sample preparation; approximately 100 mg of each sample was dissolved in ultra-pure concentrated nitric acid (HNO3), and then diluted with 1% HNO3. For major and trace metal analysis, dilution factors vary between 20 and 100. All samples were measured in triplicate for statistical analyses. DNA extraction was performed on the second aliquot of each bone sample using the UNT Center for Human Identification "Demineralization Extraction of Skeletal Remains" protocol. Samples were weighed and placed into polypropylene test tubes. Demineralization buffer and proteinase K were added to each sample and reagent blank and incubated overnight. An equal volume of phenol-chloroform-isoamyl alcohol (PCIA) was added to each sample, vortexed and centrifuged. Aliquots for subsequent testing were removed from the aqueous phase, the waste fraction from the PCIA step could not be tested due to the material hazards present, and the remaining aqueous phase was transferred to a centrifugal filtration device. Samples were centrifuged through the device and aliquots were taken from the filtrate that had passed through the device and the retentate. The remainder of the retentate was additionally purified using a chaotropic salt solution and a silica-containing centrifugation column. Aliquots were also taken from the filtrate and retentate from this step for additional testing. A total of five fractions were examined: The aqueous phase following PCIA purification, the filtrate and retentate from the centrifugal filtration, and the filtrate and retentate from the additional purification. Analyses targeting DNA content were conducted, including: DNA sizing and quantity on a microfluidics-based platform, UVV is spectrophotometry, PCR-based DNA quantification, STR amplification, and genetic analysis on a capillary electrophoresis instrument. Elemental analysis of aliquots from each fraction was performed using ICP-MS. The microfluidics-based platform was unable to produce sizing results for many of the sample sets, believed to be a consequence of the regents present in those fractions; however, gel images were obtained for each fraction tested. The results of the UV-Vis spectrophotometric analyses demonstrate an increase in A260:A280 ratio that corresponds with a significant decrease in estimated quantity of nucleic acids through each step of the extraction process. PCR-based DNA quantification produced some results and indicated the presence of PCR inhibitors in many sample fractions.

## Biography

Laura Gaydosh is a PhD candidate and Graduate teaching assistant at University of North Texas Health Science Center, Texas. She is a graduate Research Fellow at NIJ. Her research project involves metal ion inhibition of PCR-based testing systems (quantification and STR amplification) and determination of the efficacy of DNA extraction from human skeletal remains.

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