B119 A Faster, Easier, and More Effective Bone Processing Method for DNA Analysis

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Learning Overview: After attending this presentation, attendees will understand the potential for using a commercial DNA extraction kit and automated platform to screen and process samples from contemporary environmentally challenged bone samples to improve sample throughput and possibly reduce the need to outsource some casework. This paper proposes a method to quickly and efficiently extract DNA from bone samples without the need to pulverize bone tissue into a fine powder.

Impact on the Forensic Science Community: 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 short tandem repeats (STR) analysis.

In missing persons' cases, fire fatalities, mass disasters, and some forensic casework, skeletal samples are commonly used for human identification (HID) purposes. Bone and tooth samples are not routinely processed by all forensic laboratories, as the laboratory may not have the resources required such as bone grinding equipment, adequate lab facilities, or experienced analysts. Alternatively, specialized DNA analyses (e.g., mitochondrial analysis) may also be required. Due to the more complicated nature of these samples, skeletal remains may be sent to regional "hub" laboratories for processing. Traditional DNA extraction protocols involve the powdering of bone followed by a lengthy digestion (e.g., total demineralization) and DNA purification (e.g., organic or silica-based). While many laboratories that process skeletal remains prefer to process bone samples manually using their own in-house protocols, several commercial DNA extraction kits are available to standardize the process and improve sample throughput. However, these kits still require bone to be ground into a fine powder. This study explored the efficacy of a commercial DNA extraction kit and automated platform to purify DNA from small bone fragments to eliminate the need to crush the bone into a powder. This option has the potential to save time, reduce the risk of contamination, conserve evidence, and more effectively triage samples while also retaining the ability to automate the process (if desired).

In a pilot study, two main variables were evaluated to determine the most efficient protocol for whole bone chips using a commercial DNA extraction kit: chip number/size and incubation time. Three variations of each variable were tested in tandem for a total of nine combinations with five replicates each (N=45). Bone fragments (50mg chips) were collected from two cadavers (buried and fire exposure). For controls, powdered bone (50mg) was tested in the same manner as the bone chips. Neither an increase in incubation time (2, 4, or 16 hrs) nor bone chip mass (50, 100, 150mg) and/or number (1 – 3 bone chips) significantly improved results compared to the current manufacturer's recommended protocol (one 50mg bone sample for 2 hrs). Therefore, results suggest that no further optimization of conditions would be required to successfully process bone chips (in lieu of bone powder).

To test the efficiency of an automated platform as a potential screening and/or processing tool for crime labs, twenty bones and five tooth fragments were collected from nine sets of skeletal remains that have been environmentally challenged (fire exposure, embalming, burial, and advanced decomposition). The results of this study show that although slightly less DNA was recovered from the whole bone chips and tooth fragments, STR success rates were comparable to the powdered samples.

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. The processing of bone chips also offers the unique possibility for further testing, as a second round of extraction can be performed on the remaining partially digested bone chips. Additionally, an automated extraction of bone chips could provide less-specialized labs a simple and affordable means of screening (or processing) skeletal remains in-house with their existing chemistry.

Bone, Automation, Short Tandem Repeats