B131 DNA Recovery From Waterlogged Bone: A Test of Three Methods

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After attending this presentation, attendees will better understand the effect of water on the quality and quantity of DNA retrievable from skeletal remains, as well as which extraction method, organic phenol-chloroform or solid-phase, and bone type is best for the isolation of DNA from skeletal remains found in water.

This presentation will impact the forensic science community by providing information concerning a neglected area of research: the quantity and quality of DNA retrievable from waterlogged skeletal remains, and the most successful type of bone (compact or spongy) for DNA extraction.

Following water-related accidents and mass disasters occurring over or in water (e.g. ferry disasters and commercial plane crashes), soft tissue on victims' remains could be entirely absent after a temperature-dependent period of time. Due to the physical and chemical components of bones that protect them from environmental deterioration and biological attack, the ability to identify the victims may rely on the examination of skeletal remains.[1,2] Ordinarily, DNA recovery from skeletal remains is difficult and results in a low yield of DNA due to degradation and Polymerase Chain Reaction (PCR) inhibitors.[3] Few case studies discuss remains found submerged in water. For example, a Short Tandem Repeat (STR) profile was successfully extracted from a 67-year-old bone found in a fresh water lake by creating and using a unique extraction protocol; however, a later study using the same protocol on remains found in seawater was unsuccessful.[4,5]

In this experiment, samples were derived from 12 pig (*Sus scrofa*) humeri and 12 pig ribs, divided into a total of 24 humerus and 24 rib samples. Cut bones and waterproof dataloggers were submersed in water and left outdoors. Water temperature was recorded hourly. Data from the loggers were monitored. Approximately every 500 Accumulated Degree Days (ADD), three rib and three humerus samples were collected and stored in a -20°C freezer. After collection, the DNA was extracted in triplicate using these different methods: organic phenol-chloroform, DNeasy Blood and Tissue Kit, and ChargeSwitch® gDNA Plant Kit. The quality and quantity of the DNA was determined using the NanoDrop™ Spectrophotometer, Qubit® 2.0 Fluorometer, and real-time quantitative PCR (qPCR), respectively.

In assessing the quality of DNA extracted from samples across 3,000 ADD, Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) analyses indicated a significant difference among methods (F=9.83, p=0.0004); the ChargeSwitch® gDNA Plant Kit had a decreased mean 260/280 ratio compared to DNeasy Blood and Tissue Kit. Qubit® quantitation values were highest using the ChargeSwitch® gDNA method and rib bone type. ANOVA analysis did not indicate a significant difference between bone type (F=200, p=0.1666) or among methods (F=0.87, p=0.4278). ANOVA analysis of Cycle threshold (C_t) from qPCR indicated a significant difference among the methods (F=5.36, p=0.0096). The ChargeSwitch® gDNA Plant Kit had the lowest mean C_t value (28.88) compared to DNeasy Blood and Tissue Kit (34.34) and organic phenol-chloroform (33.48). Analysis also indicated a significant difference between bone types with respect to DNA yield. The mean C_t value for humeri (33.81) was greater than the C_t value for ribs (30.83), indicating that the ChargeSwitch® gDNA Plant Kit method and rib bone type produced higher quantities of DNA.

Overall, this study suggests that magnetic bead technology is the most beneficial method for recovery of DNA from waterlogged bone, and ribs may be better suited for recovery of host DNA, contrary to common practice and belief.

Reference(s):

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Waterlogged, DNA Analysis, Skeletal Remains