PHOSPHATE BUFFER EXTRACTION OF DNA FROM CALCIFIED TISSUE

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DNA extraction from bones and teeth is a laborious process mainly due to the densely calcified matrix combined with often relatively low levels of DNA. The predominant component of the calcified matrix is the inorganic mineral hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Extraction procedures for calcified tissue (CT) specimens commonly use an EDTA-based extraction buffer to demineralize the matrix to recover endogenous DNA. However, demineralization is often incomplete, possibly resulting in reduced DNA yields.

This study investigated phosphate buffer (PB) extraction of CT specimens toward improving DNA yields. This concept was derived from hydroxyapatite chromatography separation and elution of nucleic acids with PB. In theory, the addition of PB to powdered CT might likewise disrupt the interaction between DNA and hydroxyapatite, thus potentially improving DNA recovery.

This study evaluated three approaches for the incorporation of PB into a standard operating protocol (SOP) for CT specimens, which specifies the incubation of CT powder in an EDTA-based demineralization (demin) buffer, PCIA extraction, Amicon concentration, and MinElute purification. The first approach evaluated the use of a PB extraction of CT samples *in lieu* of the SOP demin buffer. On average, the PB (0.4 M) yielded 78% nuclear DNA (nDNA) and 72% mitochondrial DNA (mtDNA) compared to the SOP. A second approach investigated whether PB may complement the demin buffer, i.e. a dual buffer combining both PB and demin buffer. On average, the dual buffer yielded 86% of nDNA and mtDNA compared to the SOP. Lastly, tandem buffer extraction methods, i.e. demin buffer extraction followed by PB extraction (demin-PB), PB-demin, demin-demin, and PB-PB, were tested to determine if DNA could be salvaged in a second extraction of residual powder from an initial extraction. However, the percent yields varied not only between CT specimens but also among the various tandem methods tested, from 31% to 109% that of the SOP.

Given these results, a modification to the SOP using PB could not be recommended since the PB extraction methods generally yielded less DNA compared to the SOP method. However, PB extraction methods were often equivalent to, even slightly exceeded, the SOP yields. Overall, this study supports the continued use of EDTA-based demineralization buffers for DNA extraction of CT.