



Contents lists available at SciVerse ScienceDirect

## Annals of Diagnostic Pathology



# Analysis of the effect of various decalcification agents on the quantity and quality of nucleic acid (DNA and RNA) recovered from bone biopsies

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### ARTICLE INFO

#### Keywords:

Decalcification agents  
Bone biopsies  
Molecular analysis  
Metastatic cancer

### ABSTRACT

Molecular studies are part of standard care for cancer patients. Bone, a common and sometimes sole site of metastasis, requires decalcification for morphological examination. Many commonly used decalcification agents contain strong acids that degrade nucleic acids. The paradigm shift in oncology, with biomarker targeted therapy and gene expression profiling analysis, requires sufficient nucleic acid recovery from bone biopsy specimens. We systematically studied the effects of a spectrum of decalcification agents on the quantity and quality of RNA and DNA recovered from bone biopsies. Multiple bone biopsies of similar size and cellularity were fixed in 10% neutral-buffered formalin, randomized to various decalcification agents for 2 hours then processed, and embedded. Tissue lysates were obtained from unstained sections and nucleic acid isolated. DNA and RNA were quantified. Assessment of DNA and RNA integrity was accomplished by comparison of the average cycle threshold by polymerase chain reaction of selected housekeeping genes for each agent. Results were then analyzed by 2-sample *t* test. There was a significant decrease in both DNA and RNA yield and integrity with strong acids (hydrochloric, nitric) vs 14% EDTA and formic acid. DNA yield was (mean nanograms) 6.15 vs 68.68 ( $P < .001$ ) and RNA was (mean nanograms) 121.53 vs 288.89 ( $P = .003$ ), respectively. DNA integrity (mean cycle threshold) was 35.79 vs 30.16 ( $P < .001$ ), and RNA was 33.03 vs 26.5 ( $P < .001$ ), respectively. Decalcification of bone biopsies with EDTA or formic acid agents was associated with a significant improvement in recovered nucleic acid quantity and quality.

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