

Effect of decalcification protocols on immunohistochemistry and molecular analyses of bone samples

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Abstract

Diagnosis of osteocartilaginous pathologies depends on morphological examination and immunohistochemical and molecular biology analyses. Decalcification is required before tissue processing, but available protocols often lead to altered proteins and nucleic acids, and thus compromise the diagnosis. The objective of this study was to compare the effect of different methods of decalcification on histomolecular analyses required for diagnosis and to recommend an optimal protocol for processing these samples in routine practice. We prospectively submitted 35 tissue samples to different decalcification procedures with hydrochloric acid, formic acid, and EDTA, in short, overnight and long cycles for 1 to >10 cycles. Preservation of protein integrity was examined by immunohistochemistry, and quality of nucleic acids was estimated after extraction (DNA and RNA concentrations, 260/280 ratios, PCR cycle thresholds), analysis of DNA mutations (high-resolution melting) or amplifications (PCR, in situ hybridization), and detection of fusion transcripts (RT-PCR, in situ hybridization). Hydrochloric acid- and long-term formic acid-based decalcification induced false-negative results on immunohistochemistry and molecular analysis. EDTA and short-term formic acid-based decalcification (<5 cycles of 6 h each) did not alter antigenicity and allowed for detection of gene mutations, amplifications or even fusion transcripts. EDTA showed superiority for in situ hybridization techniques. According to these results and our institutional experience, we propose recommendations for decalcification of bone samples, from biopsies to surgical specimens.