



Memorial Sloan Kettering  
Cancer Center

## EDTA-Based Decalcification of Bone and Bone Marrow- Ideal Tool for Protein and Nucleic Acid Preservation - A Pilot Study

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### Background

Bone and bone marrow biopsies are performed to diagnose diseases such as hematologic malignancies, primary bone tumors and metastatic tumors. In the era of increasing molecular testing for diagnosis and targeted therapeutics, preservation of nucleic acids and proteins are necessary and is required for the best patient care. For routine processing of bone specimens decalcification with inorganic acids (nitric acid or HCl) is used. Acid based methods allow fast turnaround times, but, the procedure restricts use of the tissue for molecular diagnosis by damaging DNA, RNA and is not suitable for some protein assays. To address this we undertook a pilot study using 10% EDTA decalcification solution, pH 7.2, as an alternate source for decalcification.

### Materials and Methods

Twenty formalin-fixed bone resections, bone marrows, and corresponding aspirate specimens were selected. For each specimen, one half was decalcified using acid-based method; the other half with 10% EDTA (MoL-Decalcification) using Milestone's KOS Histostation at 37-45°C. Decalcification time ranged from 8-24 hours, depending on the type of tissue and thickness. After routine processing, cases were subjected to immunohistochemistry, DNA, RNA extraction, FISH analysis and molecular testing including next generation sequencing.

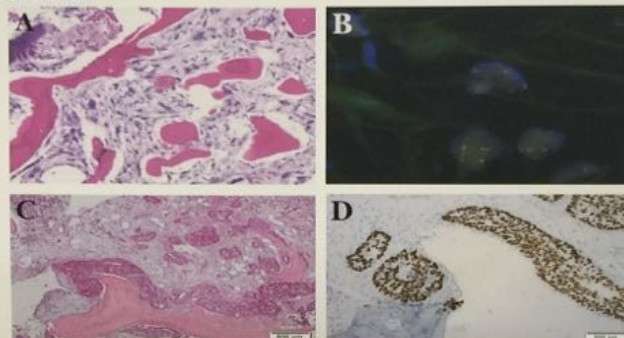
#### Procedure

- Fix specimen (2-3 mm) in 10% Formalin for a minimum of 6 hours
- Rinse sections with running water for 5 minutes
- Place bone sections/cores (in cassettes) in Milestone Mol-Decalcifier solution.
- Select Decalcification (37 °C - 45 °C; EDTA pH 7.2) protocol on KOS Histostation.
- Decalcify for 8 - 24 hours, size-dependent.
- Rinse sections with cold running water for 2-5 minutes in a serrated basket
- Place cassettes containing sections into tissue processors for processing



KOS Histostation,  
Milestone

### Results



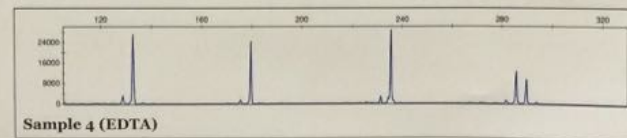
#### EDTA Treated Samples:

Sample 1: Top: A—H&E stain; B—FISH SYT break apart probe

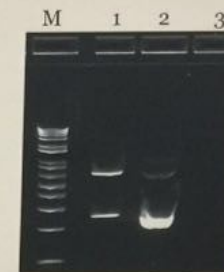
Sample 2: Bottom: C—H&E from a Bone Resection; D—TTF-1 by IHC

#### DNA and RNA concentration by Nanodrop Spectrophotometer

Bone resection ID	DNA		RNA	
	(ng/ul)	Ratio 260/280	(ng/ul)	Ratio 260/280
Sample 3 (EDTA)	8.625	1.28	1660	2.22
Sample 4 (EDTA)	27.44	1.99	766.7	1.75



Capillary Electrophoresis of four STR loci (D5S818, D13S317, D7S820, and D16S539) PCR product



Agarose Gel Electrophoresis of PGK RT-PCR product

M: Size standard, 1: Sample 4 (EDTA) 2: HL60 RNA control, 3: No Template Control (water)

#### Next Generation Sequencing analysis-Comparison of acid and EDTA decalcification aspirate and bone marrow samples

#	Sample	Read pairs examined	Mean target coverage	% target bases 30x
Case 1	Aspirate-fresh	29,174,336	1,299	99.00%
Case 2	Aspirate-fresh	37,296,917	1,633	99.09%
Case 3	Aspirate-fresh	34,443,717	1,420	99.10%
Case 4	Aspirate-fresh	30,188,471	1,310	99.07%
Case 1	Biopsy-acid	11,641,221	12	0.65%
Case 2	Biopsy-acid	4,940,352	3	0.10%
Case 3	Biopsy-acid	10,075,059	14	1.12%
Case 4	Biopsy-acid	12,924,007	19	5.55%
Case 1	Biopsy-EDTA	22,978,623	907	98.94%
Case 2	Biopsy- EDTA	15,900,564	601	98.55%

### Conclusion

- EDTA bone decalcification is practical and does not compromise turnaround time.
- There is good preservation of DNA, RNA and proteins
- Morphology is not compromised and routine immunohistochemistry can be performed with comparable results as formalin fixed tissue
- Molecular techniques including NGS and FISH assays can be successfully employed in bone and bone marrow biopsies and resection specimens