



## Decalcification

**Decalcification** describes the technique for removing minerals from bone or other calcified tissue so that good-quality paraffin sections can be prepared

**Bone decalcification** is the softening of bones due to the removal of calcium ions

### Step of decalcification of bone to preparation histopathology

#### Biopsies

Biopsies used or diagnosis of several diseases such as cancer , hemopoitic disorder and infections. Large specimens can be bisected or reduced in size by sawing into multiple slabs and immersed into fixative immediately , no longer than 48 hours after initial fixation.

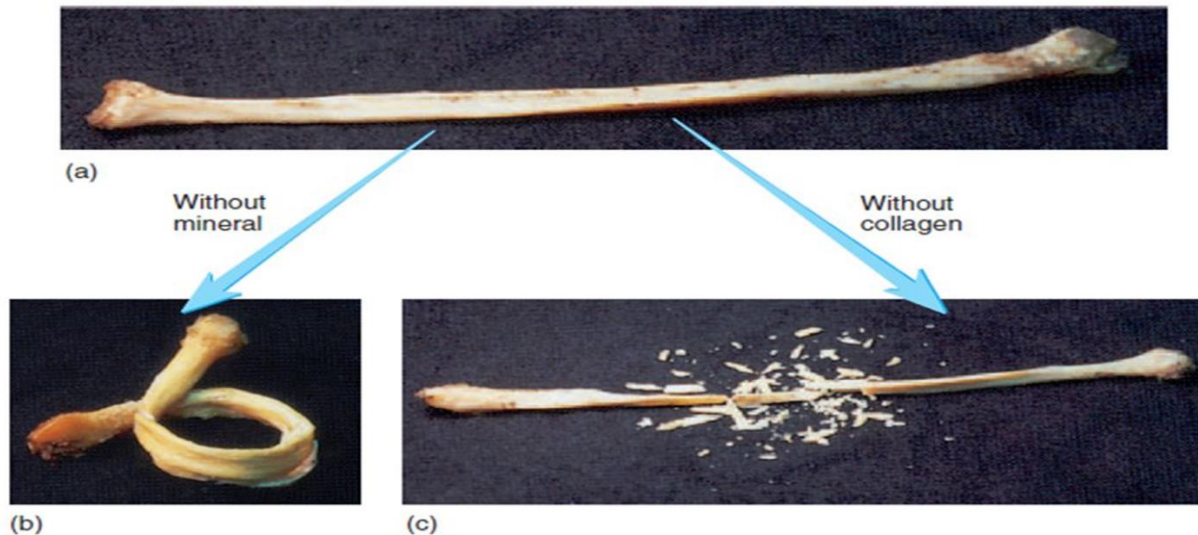
#### Fixation

Biopsy should be totally fixed . 10% neutral buffered formalin is suitable . complete fixation helps protect bone and surrounding soft tissue from damaging effects of acid decalcification

#### Decalcification

In order to obtain paraffin section of bone , inorganic matrix , calcified cartilage and surrounding tissue , this is called decalcification. It is carried out by chemical agents , either by acids to form soluble calcium salts , or chelating agents that bind to calcium ions .

Effects of Changing the Bone Matrix (a) Normal bone. (b) Demineralized bone, in which collagen is the primary remaining component, can be bent without breaking. (c) When collagen is removed, mineral is the primary remaining component, thus making the bone so brittle it's easily shattered.



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## 1. Acid decalcification

There are two groups :

1- Strong (inorganic ) acids.

Most laboratories keep an acid from each group available for either rapid or slower diagnostic routine work.

Strong inorganic acids e.g. nitric , hydrochloric acid ,these used as a simple aqueous (diluted ) solutions with concentrations 5-10%

These acids decalcify rapidly causes tissue swelling and damaging the bone if used longer than 24- 48 hours .



- \* Old nitric acid damaging bone should be replaced with fresh stock.
- \* Strong acid tend more damaging the bone antigens for immunohistochemical staining , and enzyme

### **The formula and preparations**

- aqueous (diluted) nitric acid 5-10% rapid decalcification .

-Perenyi's fluid : decalcification more slowly than diluted nitric acid .

10% nitric acid	40 ml
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0.5 % chromic acid	30 ml
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Absolute alcohol ( ethanol )	30 ml
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-hydrochloric acid diluted with distal water (5-10%). Formalin should be washed from specimens before placing in Hcl .

-Van Ebner's solution : rapid action .

Sodium chloride saturated solution	50 ml .
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Distilled water	42ml.
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Hydrochloric acid	8ml.
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### **2- Weak ( organic ) acids.**

Weak organic , e.g. formic acid , acetic , picric acids

Formic is the only weak acid used as primary decalcification . Acetic and picric acids cause tissue swelling and are not used alone but are found as a component Bouin's and Zenker's fixative .



Formic acid		10% in distilled water.
Evans and krajan	formic acid	25 ml .
An effective formic sodium citrate		10 gm
Acid decalcification	distilled water	75 ml

## 2.Chelating agent such as ethanediaminetetractic acid (EDTA):

This process is very slow but gentle (week may be required depending on the size of the specimens ). This reagent not suitable for urgent specimens but more appropriate for research applications

Neutral EDTA 250gm

pH 7 by adding sodium hydroxide about 25 gm

Distilled water 1750ml .

This solution act slowly but cause little bone damaging .stains are unaffected .

## Factors influencing the rate of decalcification :

Concentration

Temperature

Agitation

Time .