**Purpose**

To remove calcium salts from bone or other calcified tissue while preserving the cellular and organic components.  Without the removal of these salts microtomy is virtually impossible using standard microtomy procedures resulting in torn, compressed, and ragged sections.  Decalcification does not remove bone rather the process removes the inorganic components that make up bone.  The majority of inorganic components are calcium salts, which consist mainly of calcium phosphate (85%) and calcium carbonate (10%).  Although other forms of decalcification are available we use simple acid methods to dissolve these calcium salts.

**Procedure**

**Sections submitted for histology:**

3 to 5 mm in thickness, overall size and volume not as critical as thickness of the sample. However, the bone or tissue in the cassette should not touch more than one side.

**Types of Bone and Tissue for Decalcifiation:**

Cortical Bone: Dense compact bone sections appear solid except for microscopic spaces (ex. skull, pelvis, mandible).  Takes a very long time to decalcify.

Cancellous Bone: Spongy bone which consists of slender irregular trabeculae, which branch and unite to form a meshwork creating spaces which are filled with bone marrow (ex. tibia, femur, etc.)

Bone Marrow: May or may not need decalcification.

Tissue: Certain tissues tend to calcify (i.e. Thyroid nodules, Leiomyomas, etc.) requring them to undero the decalcification process for proper processing.

**Fixation:**

Prevent autolysis and putrefaction, basically to stop the decomposition process.  Most important step in preparing any specimen for the RDO process is fixation.  Fixation prior to decalcification is critical since the acid in the decal solution will destroy the cellular morphology of unfixed tissue. Fixation for bone or tissue for the Cal-ex process is not needed as Cal-ex already contains formalin.

**Types of Decal Solutions:**

Formic acid (Cal-Ex): Used on both compact and cancellous bone including soft tissue containing calcifications.

     Advantages:

* Allows crisp staining including immunohistochemistry
* Contains formalin so that tissue is fixed and decaled simultaneously
* Safer to use than RDO
* Does not impair nuclear staining

     Disadvantages:

* Slower than RDO

*Formic acid and EDTA* *(Formical 2000): Used exclusively on bone marrow biopsies*

Advantages:

* Contains formalin so that tissue is fixed and decaled simultaneously
* Works especially well for tissue needing immunohistochemistry
* Will not destroy antigen sites on tissue
* Safer to use than RDO

Disadvantages:

* Slower than RDO

Hydrochloric acid (RDO): Used on mainly compact bone

     Advantages:

* Acts rapidly

     Disadvantages:

* Tissues should be **thoroughly** fixed at least 12-24 hours in formalin before using RDO.
* Thorough washing of the tissue between formalin containing fixatives and RDO treatment is critical since formalin and hydrochloric acid combined together give off a carcinogenic gas, namely bischloromethyl ether.
* Overexposure can result in poor histological detail/artifacts (swelling, fragmentation, and poor nuclear staining).
* Irreversibly corrodes aluminum, nickel and some stainless steel equipment (metal forceps, countertops).

**Decalcification Process:**

**Amount of fluid needed for proper decalcification (Cal-ex, Formical 2000 and RDO):**

 \*10 to 20 times the volume of tissue.

The large amount of volume is necessary since the mineral content of a good sized piece of bone will soon neutralize the small amount of acid present in the solution.  As the pH of the solution rises becoming saturated with calcium ions, it almost forms a barrier to further decalcification the transport of calcium into the solution is decreased so agitation and the frequent changing of the decal solution is necessary.  Fresh solution contacting the specimen surface promotes faster penetration of the decalcifying fluid.

Decalcification time for Formical 2000 is standard at 2 hours and 15 min. Decalcification time for Cal-ex and RDO will vary significantly based on bone type, tumor, site of resection, etc. Specimens in Cal-ex will generally be ready anywhere from 12-24 hours. Specimens in RDO should be checked every 1-2 hours.

For specimens being taken out of **all** decalcifiers, rinse thoroughly for 10 minutes under running water before placing in formalin processing rack.