**Purpose**

To establish a policy relative to the use of RDO to achieve decalcification of tissue in Surgical Pathology

**Definitions**

*Decalcification* = the process of removing calcium from bone or tissue, thus allowing routine microtomy of paraffin embedded tissue

**Procedure**

1. **Safety Considerations**
   * Personal Protective Equipment (PPE) should be utilized.  This includes, but is not limited to gloves, protective clothing and protective eyewear.
   * Do not combine formalin and RDO.  Hydrochloric acid (active ingredient of RDO) and formaldehyde vapors have been reported to form a potent carcinogen, bischloromethyl ether.
   * It is necessary to fixate tissue formalin for at least 12-24 hours prior to decalcification. After formalin fixation, the specimen must be washed in running tap water for 10 minutes before being placed in RDO.
   * Immediately after decalcification is completed the specimen must be washed in running tap water for 10 minutes before being placed in the cassette rack (immersed in formalin) for tissue processing.
2. **Quality Control**
   * Overexposure to RDO can result in poor hematoxylin staining.  If this occurs, satisfactory results can be obtained by treating deparaffinized slides prior to hematoxylin with aqueous saturated lithium carbonate (1-2 minutes).
   * Poor histological detail and artifacts such as swelling and fragmentation can also occur from excess decalcification.
   * Hemosiderin is not removed by RDO.
   * Do not use metallic equipment for decalcification as RDO corrodes most metals after long periods of exposure.
3. **Storage and disposal**
   * Store at room temperature.  Keep container closed at all times.  The container must be properly labeled with patient/specimen information, RDO fixative, date and time. Store only in a glass or plastic container.  Do not use metal containers, as RDO will irreversibly corrode most metals.
   * After long periods of storage, some change of color or an increase of suspended precipitate may occur.  These are normal occurrences and do not affect the decalcifying potential of RDO.  The precipitate may be allowed to settle or removed by filtration; however, neither action is necessary.
   * Pour waste RDO into a properly labeled waste container.  (See UMHS Classified Hazardous Waste Table in the Waste Codes binder.)
4. **Specimen**
   * Specimen size must not exceed 2.5 cm x 2.0 cm x 0.5 cm to ensure adequate fixation and decalcification.
5. **Considerations**
   * **Do not over decalcify**.  **Check specimen each day in the am and pm.**RDO action is very rapid.
   * Most specimens can be decaled in four hours or less.
   * Use an adequate volume of RDO to tissue: a 20:1 volume is recommended.
   * Most mature bones of 1 cm size are decalcified in 4-6 hours; smaller-cancellous bone in 2-4 hours.  Bone biopsies are decalcified in 30-60 minutes.  Large compact bones may require overnight treatment.
   * If RDO action is too rapid, dilute with distilled or deionized water.

**DECAL PROCEDURE FOR GROSSING:**

1. **Specimen needs to be adequately fixed previous to using RDO.  RDO is not a fixative** Allow 12-24 hours of formalin fixation (10% neutral buffered).
2. Rinse cassette(s) in running tap water for 10 minutes.
3. Place cassette(s) in RDO. The container must be properly labeled with patient/specimen information, RDO fixative, date and time.
4. Remove specimen from RDO when it is appropriately decalcified. It should be easy to cut with a scalpel.
5. Rinse cassette(s) in running tap water for 10 minutes.
6. Place cassette(s) in container of formalin to be processed.
7. Pour RDO waste into a properly labeled waste container.  (See UMHS Classified Hazardous Waste Table in the Waste Codes binder.)