

DECALCIFICATION USING RDO

Adapted from the Standard Operating Procedures manual of the Histology Laboratory
Department of Anatomic Pathology at EMORY UNIVERSITY HOSPITAL, Atlanta, GA

PRINCIPLE:

Before any bone or other calcified tissue can be processed and sectioned conventionally, the calcium must be removed. This process is called “decalcification”. Failure to decalcify results in torn, ragged sections and damage to the cutting edge of the microtome knife. The principle underlying the action of acid decalcifying agents involves the solubility of metallic salts. Calcium in bones is mainly carbonate and phosphate salts which are only slightly soluble in water. An acid acts to release calcium from its combination with these anions and effects an ion exchange to give a soluble calcium salt. The calcium ions are effectively removed from the bone and remain in the decalcification solution.

REAGENT:

RDO Rapid Decalcifier (Apex Engineering Products Corporation)

PRECAUTIONS:

- * Wear gloves and protective clothing during decalcification procedures.
- * Perform procedures in a well ventilated area.
- * Take caution not to spill on stainless or formica counters.

***DO NOT MIX RDO WITH SOLUTIONS CONTAINING FORMALDEHYDE.
POTENTIAL FOR THE RELEASE OF TOXIC GAS EXISTS.***

PROCEDURE:

1. Specimens should be well fixed prior to **RDO** decalcification.
2. Specimens should be trimmed to less than 1/8 inch thickness for efficient, expedient and complete decalcification.
3. Wash specimens thoroughly in tap water to remove as much fixative as possible.
4. Place specimens in **RDO** and check periodically for complete decalcification by bending to check flexibility.
5. After decalcification, rinse specimens to remove excess **RDO**.
6. Process specimens with routine workload.

DISPOSAL:

RDO as a whole is considered a non-hazardous and biodegradable material and may be disposed of down regular city sewers with a water flush according to federal, state and local regulations.

REFERENCES:

Sheehan, D., Hrapchak, B., “Theory and Practice of Histotechnology, 2nd Ed.”,
Mosby, St. Louis, pp.89-96, 1980
Apex Engineering Products Supplemental Instruction Sheet.

BONE MARROW BIOPSIES
By Becky Scholes, HTL,MT (ASCP)

H.I.S.T.O.

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Tec Tips

I acquired this decalcification procedure after the pathologists complained of poor detail with nitric acid and various commercial products. Dr. Mahoney must like it-he kissed me on the cheek! Thanks to Jan Graham in Fort Dodge for the procedure.

ASPIRATE:

1. Allow aspirate to clot in petri dish.
2. Transfer clot to specimen container filled with 10% buffered formalin.
3. Process as usual.

BONE CORE FIXATION:

1. Immediately fix in acetic formalin for a minimum of one hour.
2. Fix overnight if brought in later than 2 hours before the end of the work day. Decalcify first thing in the morning.

Acetic Formalin
80% ethanol.....900cc
conc. formalin...100cc
acetic acid.....50cc

Dilute RDO
Distilled water.....1 part
RDO.....3 parts

BONE CORE DECALCIFICATION:

1. Place fixed core and label in a plastic cassette (**RDO** discolors metal). Decalcify in dilute RDO* for one hour. Do not leave overnight.
2. Test for decalcification by gently checking for pliability.
3. Rinse in running tap water for approximately 2 minutes.

PROCESSING:

1. If biopsy came in the afternoon, process with the rest of the specimens.
2. If the biopsy is decalcified in the morning, hand process in 120cc plastic specimen cups, 15 minutes in each container, starting with 70% ethanol or speed process through processor, 15 minutes in each station with heat and vacuum, starting with the first dehydrant station.

MICROTOMING:

1. Cut both aspirate and bone cores at 2 microns.
2. Place 3 levels on one slide. Cut an extra slide of the last level and set aside on the back of the water bath in case a special stain is requested.

STAINING:

1. Hematoxylin approximately 5 minutes. Bone core 1 minute (check under the microscope before counter staining).
2. Eosin for 10 dips, dehydrate, clear and coverslip.

DISCUSSION:

Over decalcification will result in poor or indifferent histological detail and staining characteristics. Less

than one hour is usually not sufficient.

ANIMAL TISSUE TECHNIQUES

Fourth Edition

Gretchen L. Humason

DECALCIFICATION

Calcium deposits may be so heavily concentrated in the tissue that they may interfere with sectioning and result in torn sections and nicks on the knife edge. If deposits are sparse, overnight soaking of blocked tissue in water will soften the deposits sufficiently for sectioning. Heavy deposits may be removed in any of several methods, but do not leave tissue in any of the fluids longer than necessary.

If any doubt arises about the completion of decalcification, check for calcium by the following method:

To 5 ml of the solution containing the tissue, add 1ml of 5% sodium or ammonium oxalate. Allow to stand for 5 minutes. If precipitate forms, decalcification is not complete. A clear solution indicates it is complete. Sticking needles in the tissue to check hardness is a sloppy technique that can damage cells.

An excellent decalcifying fluid, ***RDO***, can be purchased by the gallon. After using ***RDO*** for several years, I recommend it as superior to other solutions. Its rapidity of action is remarkable and the quality of staining and histological detail following its use is excellent. Old bones cut down to 1 cm in thickness, if possible, require a 6 hour treatment: small and young pieces, only 1-2 hours. Teeth will require overnight and up to 18-24 hours. Do not over decalcify: this detracts from the staining quality. Decalcifying may be followed by a brief washing in water, but this is not necessary. Fixation and decalcification may be combined in a mixture of 1 part undiluted formalin with 9 parts ***RDO***.

MANUFACTURER'S NOTE

The combination of ***RDO*** and formalin is discouraged, but should always be done under a fume hood to ensure the removal of potentially harmful vapors. Always follow the suggested directions for use. Please contact Karen Kohout at APEX ENGINEERING PRODUCTS CORPORATION with any questions you may have about the use of ***RDO***.