EXPEDITED BONE THROUGHPUT USING MICROWAVE DECALCIFICATION

Shelley L. Gruntz HT (ASCP), Vivian English HT (ASCP)
Experimental Pathology Laboratories Inc., 45600 Terminal Dr., Sterling VA 20166

Abstract

Histology laboratories supporting toxicology testing are routinely tasked with production of very large numbers of histologic specimens from standard laboratory animal species. Because of this, minimizing slide preparation time is an ongoing challenge.

One step that is especially time consuming is the traditional decalcification of bones by manual immersion in decalcifying agents at room temperature, and this is particularly true for large laboratory animals such as dogs and primates. Among other factors, the rate of bone decalcification is dependent upon size of the specimen, age of the animal, the type of decalcifying agent and methodology employed.

One potential method for decreasing the overall processing time for bone specimens involves the use of microwave decalcification. The goal of this study was to determine the shortest microwave times that would provide adequate decalcification without comprising tissue quality. Bones of various types (sternum, rib, femur, femorotibial joint and nasal turbinate) from eight animal species were decalcified in a microwave processor for variable time periods. Adequacy of bone decalcification was evaluated on an hourly basis for small animals, e.g., mice and rats, and every two hours for larger animals, e.g., dogs and monkeys, until decalcification was considered to be complete. Depending on the size and type of specimen, the time for complete decalcification of small animal bones was reduced to 1-16 hours as compared with the 1-6 days required for standard immersion decalcification. For larger animal bones, the amount of time for decalcification was reduced to 8-26 hours from 3-12 days.

Since microwave processing significantly reduced the time for bone decalcification, this method is suggested as the standard for larger animal bones, and on a case by case basis, for small animal species. Using this methodology, slide submission by the histology laboratory to the pathologist can be expedited significantly.

Introduction

As the need to expedite preparation of slides for pathology review increased in our laboratory, methods for optimizing the efficiency of various steps for microscopic slide preparation were considered. Since the traditional manual method of decalcifying bones is one of the most time consuming steps in slide preparation, an evaluation of microwave decalcification was conducted. The goal of this study was to determine if microwave conditions would significantly shorten the time required to produce adequate bone decalcification without comprising tissue quality. Traditionally, decalcification of bones from research animals, most notably large animals, can take from several days to a week or longer. In this study, various bones from several animal species were decalcified using both a conventional procedure and the newer microwave methodology. The same decalcification solution was utilized for both methods. The times required to achieve adequate decalcification were compared for the traditional versus microwave techniques.

TABLE 1 TRADITIONAL DECALCIFICATION TIMES
(Number of Days)*

BONE TYPE	MOUSE	RAT	RABBIT / GUINEA PIG*	FERRET	DOG / MINI-PIG	MONKEY
STERNUM	(Overnight) 1	2-3	3-4	4-5	4-5	4-5
RIB	(Overnight) 1	2-3	3-4	4-5	4-5	4-5
FEMUR	(Overnight) 1	(Trim after 2 days) 3-4	(Pre-trimmed) 4-6*	(Trim after 2-3 days) 3-5	(Pre-trimmed) 6-7	(Pre-trimmed) 7-10
FEMUR/JOINT	1-2	(Trim after 2 days) 3-5	(Pre-trimmed) 4-6*	(Trim after 2-3 days) 3-7	(Pre-trimmed) 7-10	(Pre-trimmed) 10-12
TURBINATES	(Trim after 1 day) 1-2	(Trim after 2 days) 3-6	(Pre-trimmed) 4-6	(Pre-trimmed) 4-7	(Pre-trimmed) 7-10	(Pre-trimmed) 10-12

* Guinea pigs, trim after 2-3 days

TABLE 2 MICROWAVE DECALCIFICATION TIMES
(Number of Hours) *

BONE TYPE	MOUSE	RAT	RABBIT / GUINEA PIG	FERRET	DOG / MINI-PIG	MONKEY
STERNUM	1 - 4	4 - 8	7 - 12	8 - 10	10 - 12	12 - 14
RIB	1 - 4	4 - 8	7 - 12	8 - 10	10 - 12	12 - 14
FEMUR	1 - 5	(Trim after 8-10 hrs) 4 - 12	(Pre-trimmed) ** 9 - 16	(Trim after 9-14 hrs) 14 - 20	(Pre-trimmed) 18 - 22	(Pre-trimmed) 20 - 24
FEMUR/JOINT	2 - 5	(Trim after 8-10 hrs) 4 - 12	(Pre-trimmed) ** 10 - 16	(Trim after 9-14 hrs) 14 - 22	(Pre-trimmed) 20 - 26	(Pre-trimmed) 20 - 26
TURBINATES	(Trim after 2-5 hrs) 2 - 7	(Trim after 8 hrs) 7-14	12 - 16	(Pre-trimmed) 12 - 18	(Pre-trimmed) 16 - 22	(Pre-trimmed) 18 - 24

*Bones may remain immersed in decalcification solution for several hours following microwave completion.
**Guinea Pigs trim after 9 - 14 hrs.

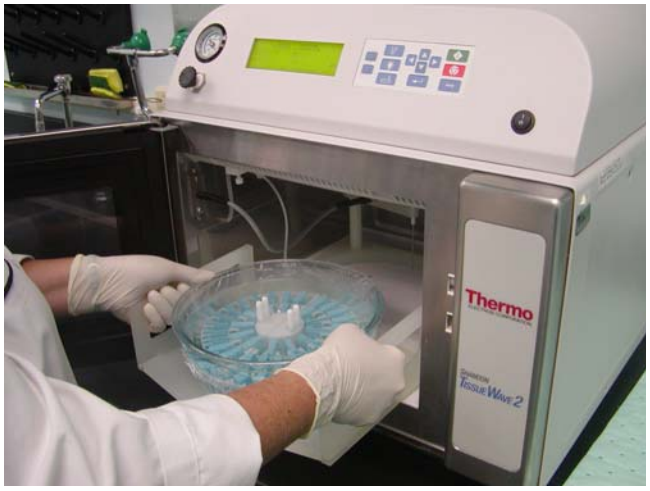

Fig. 1 TissueWave2 Microwave Processor

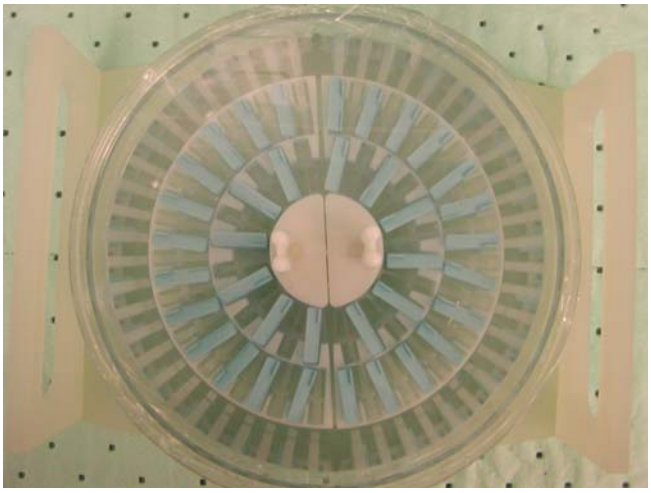

Fig. 2 Microwave Processing Chamber

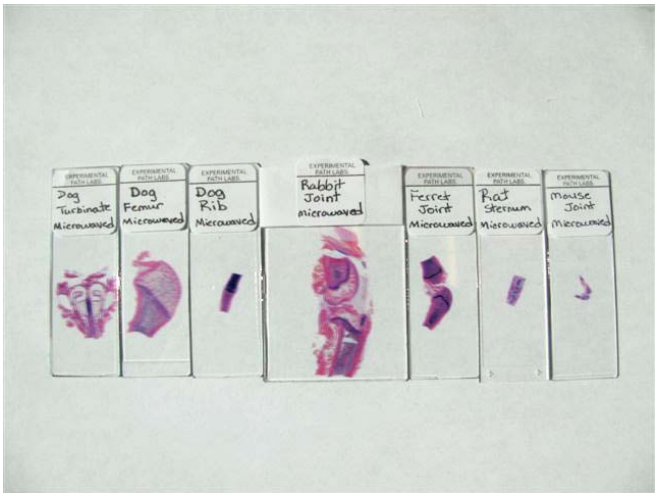

Fig. 3 Various Species of Microwave Decalcified Bones

Fig. 4 Comparison of Routine & Super-Sized Slides/Cassettes

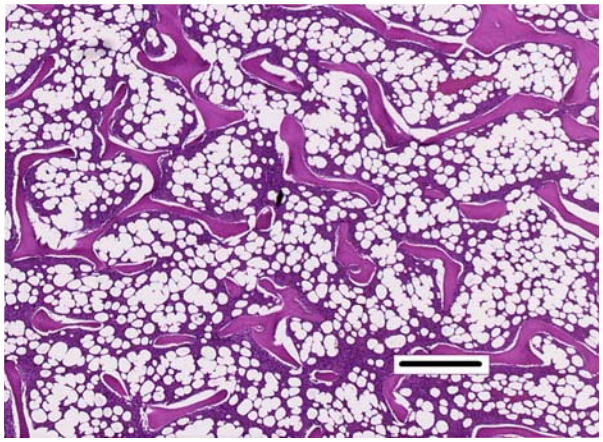

Fig. 5 Traditional Decalcified Dog Femur (Bar - 500 microns)

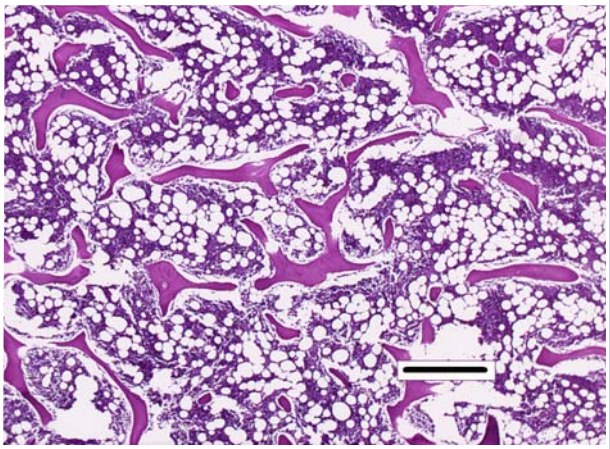

Fig. 6 Microwave Decalcified Dog Femur (Bar - 500 microns)

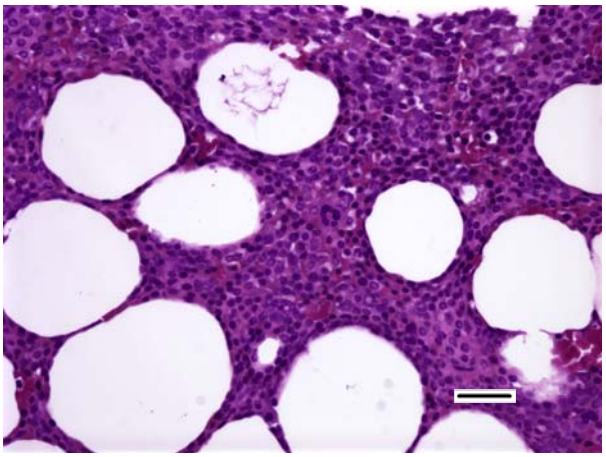

Fig. 7 Traditional Decalcified Dog Femur (Bar - 25 microns)

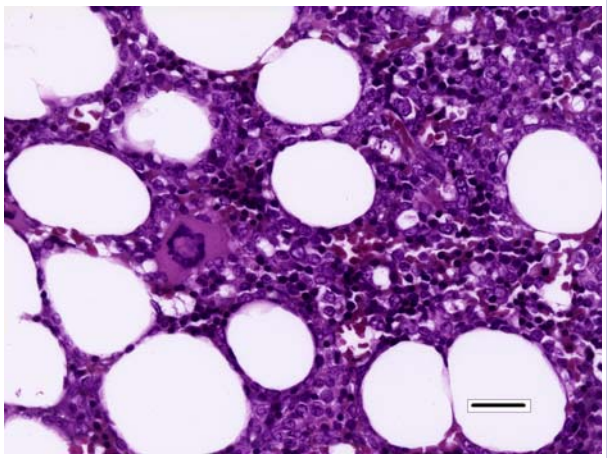

Fig. 8 Microwave Decalcified Dog Femur (Bar - 25 microns)

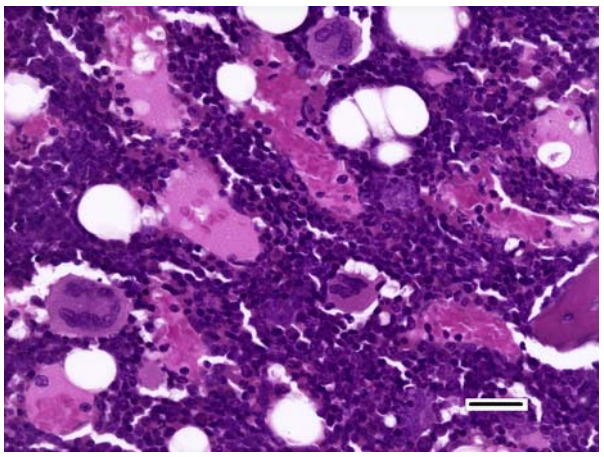

Fig. 9 Traditional Decalcified Rat Sternum (Bar - 25 microns)

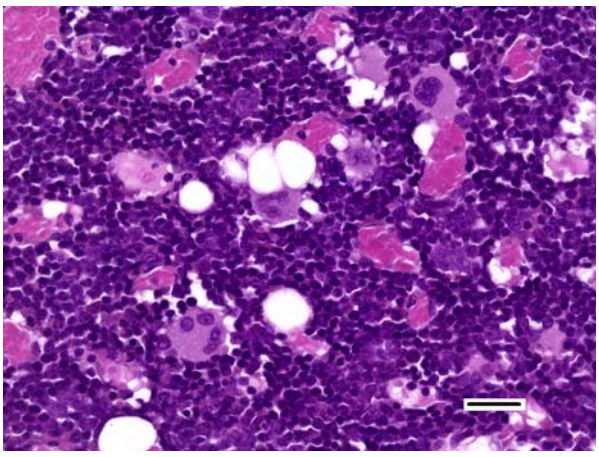

Fig. 10 Microwave Decalcified Rat Sternum (Bar - 25 microns)

Materials and Methods

Various types of bone, including sternum, rib, femur, femuro-tibial joint and nasal turbinate were collected from the following eight animal species: mouse, rat, rabbit, guinea pig, ferret, dog, mini-pig and monkey. Larger animal bones were pre-trimmed to an approximate thickness of 3-4 mm, and small animal bones (femurs, joints and nasal turbinates) were left whole initially and then trimmed after partial decalcification as needed. Any bone that was too large initially to fit into a standard tissue cassette (Premiere®, CNA Scientific, Manassas, VA) was wrapped, along with its cassette, in gauze during decalcification.

The TissueWave™ 2 microwave processor (Thermo Scientific®, Kalamazoo, MI) was used for microwave processing *(Figure 1)*. Bones were loaded into the processing basket and placed in the processor chamber. The chamber was filled to the required level with Formical 2000™ decal solution (Decal Chemical Corp., Tallman, NY), the primary active ingredient of which is formic acid. Although the capacity of the processing basket was listed as 74 cassettes, it was determined that the placement of cassettes in every other slot (for a maximum of 37 cassettes) produced superior results *(Figure 2)*. The processing temperature of the decalcification solution was set at 40 degrees Celsius, the air agitator was set to "on", and the power control was set at 100%. The temperature of the decal solution during microwave processing was 38-40 degrees Celsius.

Small animal bones were examined at 1 hr intervals and large animal bones were examined at 2 hr intervals until decalcification was complete. Bones were removed from the processor, rinsed in tap water and loaded on a Tissue Tek® VIP®5 (Sakura Finetek, Torrance, CA) tissue processor for overnight processing. The bones were embedded in Paraplast® (Leica Micro Systems, St. Louis LLC) paraffin embedding medium and microtomed at 4µm thickness. After the slides were stained with Hematoxylin 2 and Eosin-Y (Thermo Scientific® Richard Allan Scientific®, Kalamazoo, MI) and coverslipped, they were submitted to three board-certified toxicologic pathologists (EPL Sterling, VA) for evaluation.

Results

Compared with a traditional bone decalcification method, microwave decalcification significantly reduced bone decalcification time *(Tables 1 and 2)*. Time was reduced for all species *(Figure 3)* and each type of bone evaluated, but was most significant for large animal bones. During subsequent studies in which super-sized slides and cassettes were required for very large bone samples, microwave processing was also found to reduce decalcification times significantly *(Figure 4*).

The quality of bone sections following microwave decalcification was considered to be very good to excellent by the reviewing pathologists. No adverse effects on structural preservation were noted, and nuclear detail was demonstrated to be sharp *(Figures 5 thru 10)*.

Conclusions

Significantly reduced processing times were achieved when bone samples, especially from large animals, were decalcified in a microwave processor. Tissue quality following microwave decalcification was determined to be comparable or superior to tradition decalcification methodology. Microwave processing proved to be an efficient and reliable procedure for the decalcification of bones from laboratory animal species.

Acknowledgements

The authors would like to thank Bernie Wolfe (Technical support), Dr. Jeffrey C. Wolf (photographs and slide evaluation), Dr. Kathleen Funk (slide evaluation), Nelson Wilson (manuscript review) and Cyndi Bono (poster design) from Experimental Pathology Laboratories Inc., Sarah Hale (slide review) and Theresa Sharp (supplied some of the bones for method development) from Covance Laboratories, Inc.