Expedited Bone Throughput Using Microwave Decalcification

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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. 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, 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 compromising 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 (eg, mice and rats), and every 2 hours for larger animals (eg, 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 from a period of 1-6 days required with standard immersion decalcification to 1-16 hours when using microwave decalcification. For larger animal bones, the amount of time for decalcification was reduced from 3-12 days to 8-26 hours. Microwave processing significantly reduced the time for bone decalcification and, as a result, we suggest using this method as the standard for larger animal bones, and on a case-by-case basis for small animal species. By using this methodology, slide submission by the histology laboratory to the pathologist can be expedited significantly.
**Introduction**

In our laboratory, there was an increased need to expedite preparation of slides for pathology review, so 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 compromising 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 microwave technique was compared to traditional decalcification by evaluating both the time required to achieve adequate decalcification as well as tissue quality.

**Materials and Methods**

Various types of bone, including sternum, rib, femur, femorotibial 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 pretrimmed to an approximate thickness of 3-4 mm; small animal bones (femur, joints, and nasal turbinate) 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 (Fig. 2). 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), which includes formic acid as its main ingredient. 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 (Fig. 3). The processing temperature of the decalcification solution was set at 40ºC, 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 38ºC-40ºC. Small animal bones were examined at 1-hour intervals and large animal bones were examined at 2-hour intervals during working hours until decalcification was complete. Bones were removed from the processor, rinsed in tap water, and loaded on a Tissue-Tek® VIP® 5 tissue processor (Sakura Finetek USA, Inc., Torrance, CA) for overnight processing. The bones were embedded in Paraplast® (Leica Micro Systems, St. Louis, MO) para”n-embedding medium, and
sectioned at 4 μm 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 (Table 1). Time was reduced in all species 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. The quality of bone sections following microwave decalcification was considered to be very good to excellent by reviewing pathologists. No adverse effects on structural preservation were noted, and nuclear detail was demonstrated to be sharp (Figs. 4-9).
Conclusions

Significantly reduced processing times were achieved when bone samples (Fig. 10), especially from large animals, were decalcified in a microwave processor. Tissue quality following microwave decalcification was determined to be comparable or superior to the results obtained using traditional decalcification methodology. Microwave processing proved to be an efficient and reliable procedure for the decalcification of bones from laboratory animal species.

Acknowledgments

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Table 1. Traditional vs Microwave® Decalcification Times

<table>
<thead>
<tr>
<th>BONE TYPE</th>
<th>TRADITIONAL DECALCIFICATION TIME (DAYS)</th>
<th>MICROWAVE DECALCIFICATION TIME (HOURS)</th>
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<th>MICROWAVE DECALCIFICATION TIME (HOURS)</th>
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<tbody>
<tr>
<td>MOUSE</td>
<td>1 d (overnight)</td>
<td>1-4 hr</td>
<td>2-3 d (short after 2 d)</td>
<td>4-8 hr</td>
<td>3-4 d (short after 2 d)</td>
<td>7-12 hr (short after 6-10 hr)</td>
<td>4-5 d (short after 3 d)</td>
<td>8-10 hr (short after 5-6 hr)</td>
<td>4-5 d (short after 3 d)</td>
<td>10-12 hr (short after 7-14 hr)</td>
<td>4-5 d (short after 3 d)</td>
<td>12-14 hr (short after 8-10 hr)</td>
</tr>
<tr>
<td>RAT</td>
<td>1 d (overnight)</td>
<td>1-4 hr</td>
<td>2-3 d (short after 2 d)</td>
<td>4-8 hr</td>
<td>3-4 d (short after 2 d)</td>
<td>7-12 hr (short after 6-10 hr)</td>
<td>4-5 d (short after 3 d)</td>
<td>8-10 hr (short after 5-6 hr)</td>
<td>4-5 d (short after 3 d)</td>
<td>10-12 hr (short after 7-14 hr)</td>
<td>4-5 d (short after 3 d)</td>
<td>12-14 hr (short after 8-10 hr)</td>
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<tr>
<td>RABBIT/GUINEA PIG</td>
<td>1 d (overnight)</td>
<td>1-4 hr</td>
<td>2-3 d (short after 2 d)</td>
<td>4-8 hr</td>
<td>3-4 d (short after 2 d)</td>
<td>7-12 hr (short after 6-10 hr)</td>
<td>4-5 d (short after 3 d)</td>
<td>8-10 hr (short after 5-6 hr)</td>
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<td>FERRET</td>
<td>1 d (overnight)</td>
<td>1-4 hr</td>
<td>2-3 d (short after 2 d)</td>
<td>4-8 hr</td>
<td>3-4 d (short after 2 d)</td>
<td>7-12 hr (short after 6-10 hr)</td>
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<td>4-5 d (short after 3 d)</td>
<td>12-14 hr (short after 8-10 hr)</td>
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<tr>
<td>DOG/MINI-PIG</td>
<td>1 d (overnight)</td>
<td>1-4 hr</td>
<td>2-3 d (short after 2 d)</td>
<td>4-8 hr</td>
<td>3-4 d (short after 2 d)</td>
<td>7-12 hr (short after 6-10 hr)</td>
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<td>12-14 hr (short after 8-10 hr)</td>
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<tr>
<td>MONKEY</td>
<td>1 d (overnight)</td>
<td>1-4 hr</td>
<td>2-3 d (short after 2 d)</td>
<td>4-8 hr</td>
<td>3-4 d (short after 2 d)</td>
<td>7-12 hr (short after 6-10 hr)</td>
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<td>12-14 hr (short after 8-10 hr)</td>
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* Bones may remain immersed in decalcification solution for several hours following completion of microwave decalcification.
* Guinea pig, trim after 2-3 days with traditional decalcification.
* Guinea pig, trim after 9-14 hours with microwave decalcification.

Fig. 10. (Top) Slides of bones from various species following microwave decalcification. (Bottom) Larger bones in super-size cassettes, which can also be decalcified in the microwave chamber.