

# Effects of Microwave Fixation and Decalcification on Rodent Tissue

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## Abstract

The histological use of laboratory microwaves was proposed by Mayers in 1970. Since then, microwaves have become widely used in histology laboratories for fixation, decalcification, and processing; however, protocols must be carefully optimized to ensure the maintenance of optimal cellular morphology and antigenicity. In the current study, we sought to decrease laboratory turnaround time and optimize specimen preservation conditions by using microwave fixation. Mouse and rat tissues were harvested and divided into two groups (A and B). Tissues in group A were fixed for 24 h in 10% neutral buffered formalin (NBF), whereas tissues in group B were fixed for 2 h in 10% NBF before being microwaved at 50°C for varying times. Tissues from the two groups were compared by a pathologist. We found that the optimal microwave formalin-fixation times necessary to preserve morphology were 29 min for mouse tissues and 30 min for rat tissues. These times allowed specimens to be placed on a processor 3 h after tissue collection and processed for embedding the following day. We also determined optimal times for microwave decalcification of mouse and rat sternums and femurs. Decalcification of rat femurs with the combined use of microwave fixation and decalcification saved nearly 2 days of specimen turnaround times. To assess the effect of microwave fixation on antigenicity, rat intestine samples from groups A and B were stained with Ki-67 and caspase 3 antibodies. When compared with tissues that had been bench-fixed for 24 h, microwave-fixed tissues showed no loss of antigenicity. We conclude that rodent fixa-

have been found to have many uses such as for RADAR, food preparation and, more recently, in the histology laboratory. The histological use of laboratory microwaves was proposed by Mayers in 1970 (1) for the process of tissue fixation. The standard home and laboratory microwave are most often used at a frequency of 2.45 GHz. According to Giberson and Galvez (2), the household microwave differs from the laboratory microwave in the duty cycle time of the magnetron (the time the magnetron is on divided by the time base). Laboratory microwaves generally have a cycle time of 1 s, whereas a conventional microwave has a much longer cycle of approximately 10 s or more. If a laboratory microwave operates at a 50% duty cycle, the magnetron is on for half a second and then off for half a second. A household microwave at the same percentage duty cycle is on for 5 s and off for 5 s. The magnetron of the household microwave is off for a much longer period of time, which leads to a greater fluctuation in temperature. For this reason, the laboratory microwave is much more suitable for reproducible results during scientific research than the household microwave. The main focus of our study was to determine optimal laboratory microwave decalcification and formalin-fixation times for rodent tissues by using routinely used reagents and to investigate whether those tissues were adversely affected by the microwave exposure by comparing the results to routine processes. We also sought to determine whether tissues fixed in the microwave provided comparable results with those of routine processes when performing immunohistochemistry (IHC).