Days Can Be Converted To Hours, Minutes or Even Seconds When Using Microwave Technology in the Lab

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What happens when a cup of water and two ice cubes, contained in a bowl, are placed side by side in an 800 watt microwave oven and microwaved for two minutes at 100% power? The answer may not be obvious, even to the most ardent microwave user. The water will boil and the ice will remain essentially unmelted. This dichotomy of the results may appear puzzling to the casual observer, or may explain why your food does not defrost quite evenly. To the microwave, however, it all makes sense. Water is a dielectric and absorbs microwave energy. The net result is that it heats up. The ice under the above conditions is essentially transparent to the microwave and does not heat. It would take an ice wall greater than 30 meters thick (3.4 cm for 25° C water) to protect you from the microwave energy emitted by the standard household microwave, whereas a >170 nm thick piece of aluminum foil is all that is required to reflect that same energy.

Understanding how microwaves interact with different materials and chemicals is important in making the instrument a useful tool in the laboratory. Information on how microwaves interact with different materials can be found in a number of references[1,2]. Anyone who has used a microwave to heat food knows that the initial heating can be uneven and that hot and cold spots can result. A frozen burrito will demonstrate this point well. Identifying hot and cold spots quickly in the microwave oven is important when it is being used as a piece of laboratory equipment. A simple tool, neon indicator lights[3], can be used to identify these areas of high and low energy. Areas where the lights come on are called hot spots and areas where they remain off are cold spots. The identification of these areas is important when it comes to sample placement in the microwave oven.

Another variable is the actual wattage of the microwave oven being used. This will influence the time parameters chosen as well as sample location. One watt equals 14.33 calories per minute. Therefore, the amount of energy being transmitted to the sample will vary greatly depending on the wattage of the oven, the time period chosen and the sample location.

The use of the microwave oven makes it possible to shorten standard protocols for transmission electron microscopy[4,5] (TEM) and DNA extraction[6,7] from days to hours, minutes or seconds, depending on the step being performed. The problems come from not understanding how the microwave irradiation interacts with the sample, sample container or the solution the sample is in. In our TEM research with the microwave oven we used a sample holder called the Prep-Eze™ from Ted Pella, Inc. to process tissue (mouse liver, kidney and Pacific yew needle). We inserted a Teflon® plunger with a detachable tissue basket into a 4 dram shell vial. Fluid volumes for fixation, dehydration, etc., were 3.75 to 4 ml. The tissue handling system was subject to fairly rapid heating (>70° C in 20 sec.) in the 800 watt Model 3440 Microwave Oven (Ted Pella, Inc.) used in our research[4,5]. In our studies we froze the 4 dram vial in a 100 ml polyhethylene beaker containing ~70 ml of tap water and broke up the 20 sec. of 100% power into two 10 sec. intervals separated by 20 sec. of 0% power. Incorporating this technique solved the problem of sample overheating during fixation.

A series of tests were run on 1.5 ml microcentrifuge tubes containing 400 µl of stain extraction buffer used for DNA extraction for PCR[7]. Sixteen microcentrifuge tubes in a plastic rack, not encased in ice, were microwaved for two 8 second periods of 100% power separated by a 20 sec. interval of 0% power. Little heating (~4-6° C) was detected in any of the tubes. Yet these conditions were adequate to promote efficient DNA extraction from four different forensic samples (unpublished data). Results of the DNA extraction are shown in fig. 1. Continuous microwaving of the microcentrifuge tubes at 100% power for a minute produced temperature increases in the range of 10-14° C.

We have recently published a three hour protocol for rapid microwave processing of tissue for electron microscopy[5]. The results of this study are seen in Figures 2 and 3. Routine processing of tissue for TEM is at the minimum one day endeavor, but usually requires two or more days. The same is true for DNA extraction techniques which normally take two days. This latter process can be shortened to two hours with the extraction step taking only seconds when the microwave is used. All types of tested forensic samples to date have yielded amplifiable DNA[7]. It also appears that the use of the microwave will inactivate some of the inhibitors to PCR such as heparin[8].

Use of microwave technology in the laboratory can greatly shorten the processing times associated with routine protocols while yielding results of equal or better quality. The caveat, however, is that any change in conditions will likely influence the outcome. The change in shape, size and volume of sample containers (4 drain vial versus a 1.5 ml microcentrifuge tube) can dramatically affect sample heating. The wattage of the microwave will directly influence the amount of power reaching the sample over a given time and sample placement in a hot or cold spot is an important consideration as well. A temperature probe, which comes with the Model 3440 Microwave Oven, was the tool that made it possible to shorten many of the steps and create appropriate experimental conditions when

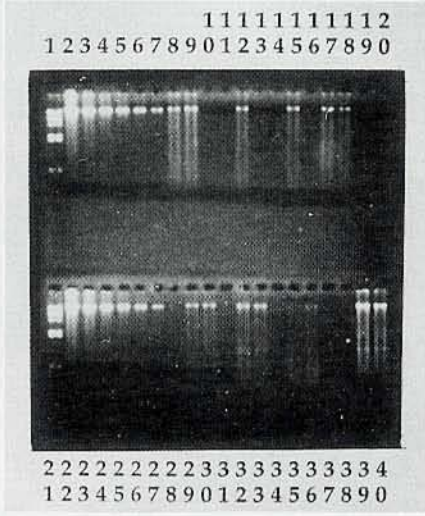

Figure 1. Total genomic DNA extracted from blood stains, hair, fingernails and semen with a computer driven microwave.

Samples were placed into a 1.5 ml microcentrifuge tube containing 400 µl of stain extraction buffer (SEB - 10 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 2.0 % Sodium Dodecyl Sulfate. The samples were tightly capped, briefly finger vortexed and then incubated at 85° C for 10 minutes in a heat block. The samples were then placed in the computer driven microwave (Pelco) and irradiated on full power for a total of 16 seconds. The total time was split by microwaving once for 8 seconds, waiting 20 seconds and then again microwaving for 8 seconds. After microwaving, 10 µl of Proteinase K (20 mg/ml) was added and the samples were then incubated at 56° C for 15 minutes. 400 µl of phenol : chloroform : isoamyl alcohol mixture (24:24:1 -TE 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA equilibrated) was then added to each of the samples. Samples were mixed by vortexing for 3-5 seconds and then centrifuged at 13,000 g for 2 minutes. The aqueous phase (top layer) was removed and placed into TE in a Centricon 100 (Amicon) to a total of 2 ml. Samples were centrifuged 20 minutes at 1000 g (2500 RPM in a Hermle centrifuge). TE wash and centrifugation were repeated as described at least 2X with 2 ml TE. The nucleic acids remaining in the retentate were collected by inverting the Centricon into the collection tube and spinning 2 minutes at 500 g (1800 REM in Hermle). The retentate was transferred to a 1.5 ml microfuge tube.

Agarose gekls are prepared using 1 % agarose (FMC BioProducts) in 1X TBE buffer (TBE). A total of 3 µl (~10 %) of the extracted DNA and 1 µl of loading buffer (0.25 % Bromophenol Blue and 30 % Glycerol in TE) are loaded for each sample. Lane 1 and 21 contain Lambda Hind III digested DNA (BRL). Lanes 2-7 and lanes 22-27 contain standards of 250, 100, 50, 25,12.5 and 6 ng of the cell line K562 DNA (BRL). Lanes 8, 9, 12, 15,17,18, 29 and 30 contain DNA extracted from bloodstains. Lanes 32 and 33 contain DNA extracted from 0.031 g of manual hair shavings. Lanes 35 and 36 contain DNA extracted from 0.012 and 0.015 g of fingernails. Lanes 37 and 38 contain the negative reagent buffer control and lanes 39 and 40 contain DNA extracted from liquid semen. 5 volts/cm was applied for 30-45 minutes. The gels were then post stained in EtBr (0.5 mg/ml) for 45 minutes and then destained in dH_20 for 15 minutes and photographed under UV onto Polaroid #57 film. The lanes 10, 11, 13, and 14 contain a sample of the retentate from a second Centricon 100 wash using 20 µl TE volume. The results demonstrate no significant amount of remaining DNA is left on the Centricon membrane after the first collection.

DNA was extracted from every tested sample type including bloodstains, hair shavings, fingernails and semen.

doing these studies. We have demonstrated in two totally different protocols that controlled conditions in the microwave can produce excellent results quickly and reproducibly. The secret is trying to understand how the microwave interacts with the sample in its environment. ■

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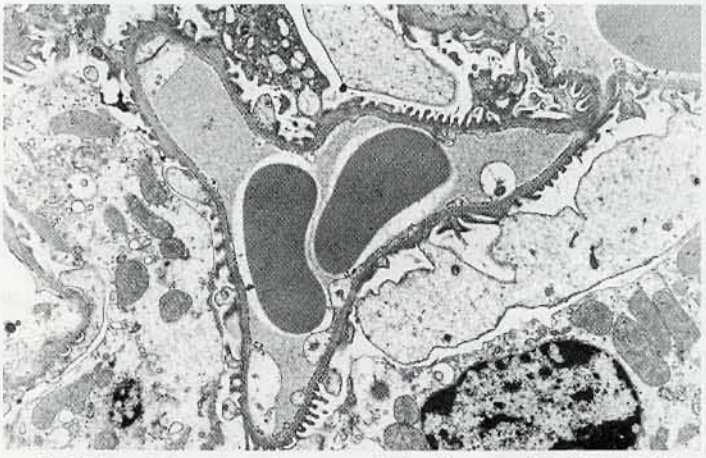

Fig. 2 Mouse kidney glomerulus processed by the 3 hr. protocol for TEM. 14,000X

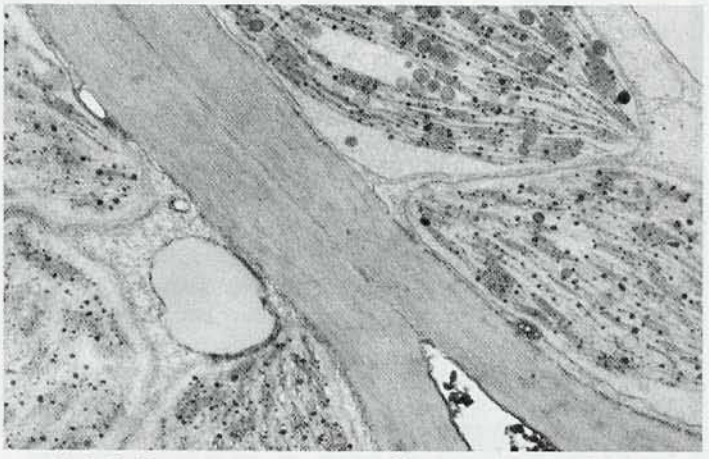

Fig. 3 Pacific yew needle processed by the 3 hr. protocol for TEM. 35,000X