THE INNOVATOR

ANATECH LTD
1020 Harts Lake Rd
Battle Creek, MI 49037
1.800.262.8324

Volume 2, Number 2 1998

FROM PATIENT TO EMBEDDING CENTER IN TWO HOURS OR LESS

The single biggest factor in health care today is cost, and all too often reducing costs means doing everything faster. Fixation time is usually the first to be sacrificed, with devastating results to specimen quality [for details, see The Innovator, 2 (1), 1998]. The remainder of the tissue processing program cannot be reduced significantly with conventional processors. Now, however, there is a way to produce specimens of astounding quality in an incredibly short time span. The secret is microwave fixation and processing, with a new twist.

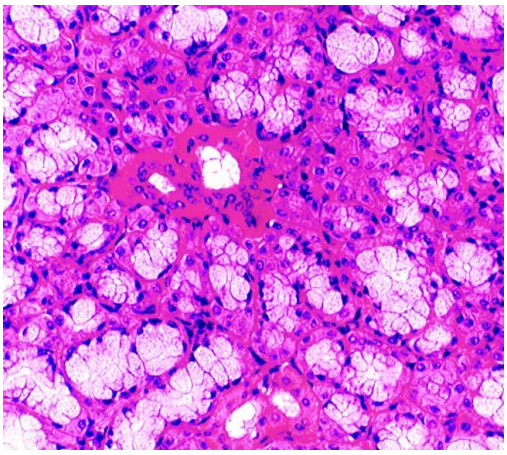

Figure 1. Initial fixation: Prefer, 2 hours; post fixed in Preserve and processed with 1 mm schedule. Mixed salivary gland (raccoon), Hematoxylin – Normal and Eosin. 20x

Microwave technology has been used in our field for nearly 20 years, but to date, few laboratories have developed fixation and processing protocols that yield high quality results on a consistent basis. Challenged by this problem, we began a research program to investigate the principles behind the technology, then developed methods to produce very high quality results in the shortest possible time, keeping costs to a minimum. The details are described in the following pages; the results just might blow you away.

We began by defining our goals. Foremost was quality. We had to produce specimens that were as good as the finest possible with standard fixatives. Second, it had to be very fast, significantly faster than conventional fixation and processing. Third, it had to be compatible with the wide variety of fixed, partially fixed and unfixed specimens commonly encountered in surgical and

Welcome...

This issue of the Innovator covers some of our recent research into the emerging field of microwave processing. Many labs have not even considered this technology, but there are growing, compelling reasons to do so now. Competition from other labs, pressures for faster turnaround time, and increased workload all cry out for some quantum leap away from conventional methods. Microwave fixation and processing is the answer, and it will cost you far less than a regular tissue processor. Our story also shows what can be done when dedicated people and

veterinary pathology. Fourth, it had to be practical. Fifth, it had to be safe; absolutely no compromises here. Finally, it had to be reasonable in cost. We believe that we achieved all of those goals.

their independent companies pool their collective resources for a project. Steven Slap and Alan Berger from Energy Beam Sciences sparked the interest then made everything possible with their support.

Fixation is the key to our success. Well-fixed tissues withstand the rigors of processing without developing artifacts. This is true for conventional as well as microwave processing. Because speed is of the essence and safety is a must, most fixatives are not suitable. In the past, microwaved specimens have been treated with various procedures, few of which produced histological images comparable to conventionally processed tissue.

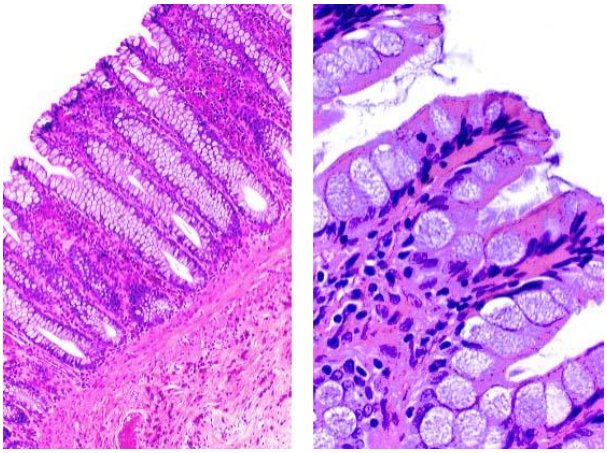

Figure 2. Initial fixation: NBF, 2 hours; post fixed in Preserve and processed with 1 mm schedule. Colon (raccoon), Hematoxylin – Normal and Eosin.10x (left) and 50x (right)

Preserve is compatible with a wide range of fixatives, including Prefer, NBF and zinc formalin, so nearly any specimen received in your lab can be treated with this program. Final appearance of the tissues will depend upon how long they have been in the primary fixative. Those fixed for hours in NBF, for instance, will look like high quality formalin fixed specimens. The same is true for the other fixatives. Tissues only lightly fixed, or unfixed, will resemble those exposed to Prefer for several hours. Red blood cells will be lysed, but nuclear chromatin patterns will be very sharp, cell membranes will be conspicuous and immunoreactivity will be retained very well, as seen in figures 2-4.

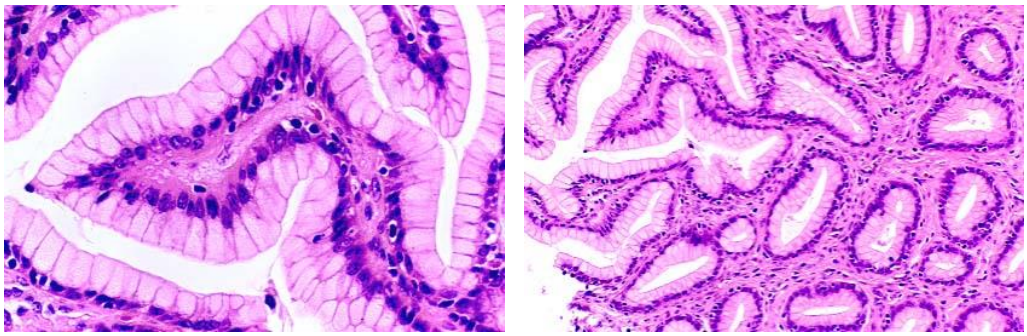

Figure 3. Initial fixation: NBF, 1 hour; post fixed in Preserve and processed with 3 mm schedule. Stomach (pig), Harris

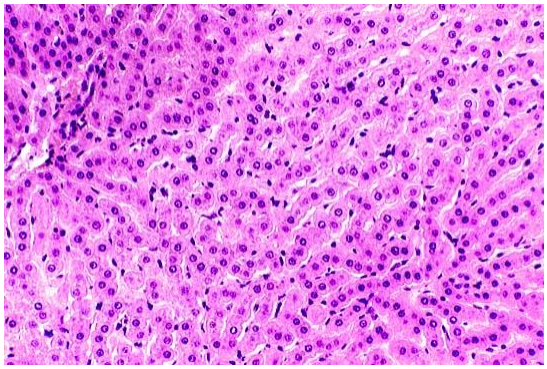

Figure 4. Initial fixation: Prefer, 8 days; post fixed in Preserve and processed with 1 mm schedule. Liver biopsy (cow), Harris Hematoxylin and Eosin. 20x

All of our work was done with an H2800 Microwave Processor from Energy Beam Sciences (Agawam, MA). The H2800 operates under entirely different principles from a household appliance, and is vented as well. Unlike a household oven, it will not cook specimens when used as directed.

We developed two schedules. If tissues are sliced no thicker than 1 mm, use the short program and be ready to embed ***in less than an hour after grossing***. For any sized specimens up to 3 mm in thickness, use the long program; it will not harm small tissues yet will adequately process thicker ones. Total time here from grossing to embedding is about an hour and a half. See table below for details.

Recommended fixation and processing schedules for 1 mm and 3 mm specimens

Step #	Activity	Reagent	Temp.	Time for 1 mm	Time for 3 mm
1	Initial fixation	your fixative	RT	variable	variable
2	Grossing	Preserve	RT	variable	variable
3a	Post fixation	Preserve	RT	20 minutes	30 minutes
3b		Preserve	55° C	4 minutes	16 minutes
4a	Dehydration	Ethanol	67° C	4 minutes	8 minutes
4b		Ethanol	67° C	4 minutes	8 minutes
4c		Ethanol	67° C	4 minutes	8 minutes
5	Infiltration	Wax	84° C	7 minutes	28 minutes
			Total scheduled time*	**43 minutes**	**98 minutes**
			Total time including manipulation & ramp up*	**58 minutes**	**113 minutes**

*** excluding initial fixation and grossing**

The procedure is simple. After receiving specimens in the fixative of your choice (or fresh), gross them into Preserve and set the cassettes in the special cassette holder. Keep them in Preserve at room temperature for 20-30 minutes. Remember to use a 20:1 ratio of fixative to specimens. The purpose of this hold time is to begin infiltration of Preserve while washing out the initial fixative.

Using fresh Preserve, finish fixing under microwave stimulation. We have found 55° C to be ideal for the times and tissue sizes indicated. During this step, tissue is chemically denatured in an aldehyde fashion. With Preserve, this occurs very quickly and uniformly throughout the specimen.

Dehydration is next, using ethanol (any variety will do as long as it is anhydrous). A second bath of fresh ethanol completes the function. Lipids from small fat deposits are liquefied and dissolved out. Many microwave protocols call for further processing with isopropanol, but we have found that this is not necessary with our protocol. As a result, we reduce total processing time by about 15%. Clearing also is not necessary.

The wax is heated to 84° C, which is sufficient to boil out the alcohol. Any good wax should perform satisfactorily. We use Surgipath's Infiltration Medium (Surgipath Medical Industries, Richmond, IL), followed by their Embedding Medium in the embedding center. Keeping the waterbath temperature low, we obtain hassle free sections with ease. You will find that sectioning these microwave-processed tissues is a pleasure.

Microwave processing is not automated, and some tech time is required to manipulate specimens through the steps manually. At first, we thought this would be cumbersome and out of synchrony with busy laboratory routine. After several runs, we worked out a system that goes smoothly with inconsequential interruption. Each lab will have to do this for their unique environment, but it really is no problem. By way of comparison, it is like hand staining with five steps. Removing specimens, changing the fluid, setting them back into the processor and resetting the controller takes less than 30 seconds
Although we at ANATECH LTD. developed the product and designed the protocols, we feel that Energy Beam Sciences is in a better position to serve the microwave market. Of course, you are welcome to talk with us about microwave fixation and processing. We certainly have had some experiences that could relate to your needs.

Ordering Preserve

Preserve is available only from:

Energy Beam Sciences
P.O. Box 468
Agawam, MA 01001
telephone 1-800-992-9037
fax 4143-789-2786
e-mail ebs@ebsciences.com.

For those unfamiliar with ramp up time, the H2800 Microwave Processor is controlled by setting the temperature and time. The magnetron (the microwave generator) cycles on and off very quickly until the set temperature is reached. At that point, the timer starts and temperature is maintained with bursts from the magnetron. With 600-650 ml of fluid and 24 cassettes, it takes 1.5-2 minutes for the fixative and alcohol to reach set temperature, and about twice that long for the wax.

Wax is rather unresponsive to microwave stimulation, and a variety of tricks have been used to heat it. We prefer using a Pyrex baking dish, rather than plastic containers with heating stones. It is quicker, more consistent and convenient.

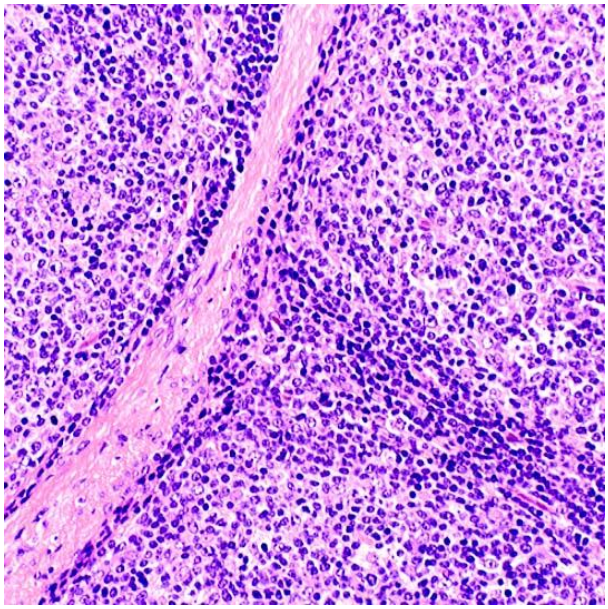

Figure 5. Initial fixation: NBF, 1 hour; post fixed in Preserve and processed with 3 mm schedule. Lymph node (pig), Harris Hematoxylin and Eosin. 20x

You can see from these photomicrographs that quality is extraordinary. These were not taken from the few best specimens in a run. All of the tissues processed together look like this, as long as none are grossed thicker than 3 mm and the program is followed. The only variation is due to initial fixation: type of fixative and exposure time. With the H2800, we have seen no variability due to number of cassettes (some users' process 96 at a time) or position in the processor. Properly fixed tissue does just fine in a microwave processor. Without Preserve, variability was rampant unless specimens were fixed initially for several (Prefer) to many (NBF, zinc formalin) hours.

Enjoy the pictures. Dream what your lab can now do in this highly competitive business.

1.800.262.8324 email@anatechltdusa.com