

Fig. 1. Kidney 4 mm, microwave processed in 48 min. Masson trichrome, 40X

## Microwave Technology in the Histology Laboratory

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### Introduction

Consumers first became familiar with microwave technology in the form of household microwave ovens that could cook or reheat foods in a fraction of the time required by conventional ovens. The use of microwave ovens in the histology laboratory started slowly, but today household microwave devices are commonly used to perform simple procedures such as specimen stabilization, staining, epitope retrieval, and some decalcification procedures. Laboratory-grade microwave devices are rapidly gaining popularity. They provide sophisticated systems for monitoring and controlling the energy, precise temperature control, agitation to prevent thermal layering, multiple safety features, and most importantly, appropriate ventilation. Laboratory microwave devices should be used for any technique that requires precise temperature control or involves the use of hazardous materials, especially toxic, flammable, or caustic reagents.

Microwave technology is routinely used to accelerate basic epitope retrieval techniques; enhance and accelerate staining procedures; stabilize and harden gross specimens for easier thin section dissection; facilitate the identification of lymph nodes in gross specimens; fix small and large specimens; decalcify bone; and rapidly, yet gently, process both small and large specimens. These procedures can be accomplished without compromising specimen morphology or antigenicity.

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### What happens inside a microwave?

Have you ever stopped to think about how it's possible to heat something in a chamber that remains at room temperature? It's a fascinating process.

Microwave devices use a power transformer to produce high voltage electricity (approximately 4000V). Through a complex process, the high voltage enables the magnetron in the instrument to produce microwaves, which are then channeled through a waveguide and delivered to the chamber.<sup>1,2</sup> A microwave (micro=small) is an electromagnetic wave with a frequency and wavelength that can be found about halfway between a radio wave and visible light in the electromagnetic spectrum. Most microwave devices operate at a frequency of 2.45 GHz (gigahertz) or 2,450,000,000 cycles per second. The microwaves generated have a wavelength of 12.2 cm in air but

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this wavelength is reduced when it enters a substance of greater density. The frequency of 2.45 GHz was selected for household microwave ovens because it is the frequency at which polar molecules, especially water molecules, respond strongly and the microwaves maintain good strength even at great depth. This capability is essential for cooking food and is also practical for histology laboratory work. Higher frequencies provide adequate penetration and lower frequencies cause the microwaves to penetrate well but be absorbed very weakly.<sup>3</sup>

### **How do microwaves affect tissue specimens?**

Routine histology procedures depend on relatively slow infiltration of solutions from the outer surfaces, and if heat is applied it must work its way into the interior of the specimen by thermal conduction. Exposing thin sections of specimens to microwave energy affects the entire specimen instantaneously and simultaneously, facilitating the exchange of solutions and accelerating reaction rates due to internal heat.

Microwaves can either pass through something with little or no effect, or they can be reflected or absorbed. Some substances, such as most plastics, glass, and paraffin pellets, are considered “microwave transparent” because they remain unaffected when exposed to microwave energy. Other substances, such as metal, will reflect microwaves. When substances absorb microwave energy they become excited and generate internal heat. It is widely accepted that as the microwave energy is absorbed in tissues, it is converted to *kinetic* and *chemical* energy.

When small dipolar molecules, such as water and the side chains of proteins, are exposed to microwave energy, they immediately attempt to align themselves within the electromagnetic field. They rotate rapidly through 180° at the rate of 2.45 billion cycles per second. The rotation alone does not produce heat. As the molecules collide, they absorb the microwaves

and convert the energy to thermal or *kinetic energy*. The rotation ceases immediately when the exposure stops. Large molecules are not as responsive because they cannot rotate as quickly in the electric field.

The *chemical energy* produced in tissue specimens during microwave exposure is less understood. The microwave energy packet (photon) can't ionize molecules and is too small to break even weak molecular bonds, but it is believed that the hydrogen bonds may be redistributed. It is presumed that microwaves affect the bound water that physically separates macromolecules and electrically insulates them from one another, and that as some of the bound water is removed, the macromolecules unwind, form new crosslinks, and then become locked into different configurations.<sup>4</sup>

In order to tie this information together, let me give you an example of what happens during microwave tissue processing. Microwaves enter the chamber; are reflected off the metal chamber walls; pass through the microwave transparent processing container and cassette; and excite the polar molecules in the solutions and the specimens. The resulting kinetic and chemical energy expedite the exchange of solutions within the specimens. This dramatically reduces the amount of time required for processing and eliminates the need for graded alcohols. Clearing solutions are not necessary because the alcohol is evaporated from the paraffin after infiltration. The solutions commonly used for processing are absolute ethyl (or reagent) alcohol, 99% isopropyl alcohol, and paraffin. The alcohol can be used several times and the paraffin can be reused many times, possibly for months. The specimens produced using this abbreviated process have excellent sectioning characteristics and the morphology and antigenicity are unaffected.

### **Incorporating the use of microwaves in the lab**

The pathology laboratory at Harris Methodist is a busy diagnostic

service that handles an annual caseload of 20,000 cases, which includes the preparation of 60,000 blocks, 24,000 special stains, and over 5,000 immunohistochemistry stains each year. In the 1980s, we explored the use of a domestic microwave device to decrease our special stains turnaround times. We purchased our first laboratory microwave device ten years later to reduce the time needed to process rush biopsy samples, selecting a device specifically designed for laboratory applications to enable us to avoid the ventilation problems associated with a household microwave. Our program was so successful that it became clear that additional devices would be beneficial, allowing us to process even larger samples. Today we have six microwave devices in use for various histology procedures throughout the department.

As our department continued to grow, the need for rapid tissue processing became apparent. Our early efforts included a shortened schedule on our conventional tissue processors which enabled same day reporting if the sample was received in the laboratory before noon. Despite this success, our clinicians complained about the restriction of submitting biopsies before noon in order to receive a quick turnaround. A further abbreviation of the schedule on the processor was not an option, and we also realized that a lot of time was wasted during solution exchange on the instrument.

Our exploration of microwave tissue processing was done with support from our instrument vendor. During the demonstration, we processed various tissue types in parallel using both microwave and routine processing. We found that the morphology we obtained was comparable for both techniques. We also performed immunohistochemical staining to assure that stain quality was not hindered by microwave processing. This yielded results comparable to conventionally processed tissues.

Initially we worried about damaging or cooking the specimens. We learned a lot from our experimentation. For example, we found that tissues were not damaged *if they were properly fixed*. We established that fixed biopsy samples could be microwave processed into paraffin in 20 minutes (see Tables 1A, 1B). Specimens must be completely submerged in solution and there must be sufficient space between the cassettes to allow fluid transfer. Just as in conventional processing, encapsulated specimens like skin tags or colon polyps must be bisected, and fatty samples require longer processing due to the amount of water in the sample. Microwave-processed samples section with ease on the microtome and the morphology and antigenicity are not affected.

The demonstration was a success for both the lab and the vendor, and we purchased our first laboratory device for processing in 1995. This enabled us to market our use of microwave technology to our clinicians by making it possible to turn around biopsy samples from specimen collection to report charting in 2 hours. In some instances results are available to the clinician before the patient leaves the outpatient recovery area. Cell blocks are also microwave processed after an initial fixation for 30 minutes. No longer do we have to wait until the next day to give the cell block slides to the cytotechnologist for reading. It has become a routine procedure in our laboratory when we receive a biopsy, cell block, or fine needle aspirate sample in the morning, to microwave process it, and even if special stains or immunohistochemical stains are required, the case can still be completed that same afternoon.

Our transition to microwave technology had an unexpected benefit. We found that by having our slides completed by afternoon and early evening for same-day surgeries, we could avoid the need to have a third shift perform this work.

**Table 1A\*†**

Solution	Wattage	Time	Temp	Comments
10% Formalin‡	750W	20 min	50°C	Loosely cover and agitate§

**Table 1B\*†**

Solution	Wattage	Time	Temp	Comments
10% Formalin‡	350W	30-45 min	40°C	Loosely cover and agitate§
10% Formalin‡	650W	10-15 min	40°C	Loosely cover and agitate§

\* **These are sample procedures only.** Procedures will need to be optimized in individual laboratories using nondiagnostic specimens.

† Fixation of 1- to 3-mm thick specimens in cassettes before processing. Tables 1A and 1B represent the two current theories on microwave fixation. Both techniques have produced good results.

‡ Buffered formalin solution is not necessary as long as the solution is freshly prepared.

§ Heated formalin solutions are very volatile and extremely hazardous. Do not allow fumes to enter the breathing zone. Cover containers before removing from the microwave and do not open until under a ventilation hood.

Our success with rapid biopsy processing led us to consider microwave processing for larger tissues. Extra devices were purchased and we began processing all of our tissues in three microwave instruments. Tissue samples had to be no more than 2 mm thick. Our initial attempt was successful and we continued this process for over 8 months. Slide quality declined when our pathologist's assistant left, and section thickness requirements were not followed. We placed the conventional tissue processors back into operation and began our research on how to improve the process. We eventually developed a microwave processing schedule for each sample thickness (see Tables 2A, 2B, 2C).

Our lab receives small samples in formalin from the operating room. Larger specimens are delivered to the lab fresh. These samples are dissected and processed that evening so that slides can be ready for the pathologist first thing the next morning. We struggle, as many labs do, to adhere to our turnaround time requirements while providing adequate fixation of our tissues. Our studies, using tissues of varying thickness processed in parallel by both conventional and microwave

procedures, have convinced us that microwave technology can provide results superior to conventional processing irrespective of section thickness.

Results from the laboratory microwave-processed specimens were remarkable. Not only did section morphology improve but also the morale of the microtomy staff. No longer did they have to struggle to obtain superior microscopic sections. All of this has led us to purchase another laboratory microwave device for our surgical pathology area. We are currently performing research on whole organ microwave fixation that will allow the pathologist to have a firm, well-fixed sample before dissection is performed. Sectioning 2-mm sections will not be as much of a challenge if the sample is firm prior to dissection. We believe that this additional investigation will permit us once again to abandon the use of conventional tissue processing.

Bone decalcification procedures can also be performed in a microwave device, decreasing turnaround time. Samples that can take days to decalcify with routine methods can be completed in a few hours in

**Table 2A. Processing FIXED Biopsy specimens (1 mm or less in thickness) that have been rinsed well in water\***

Solution	Time	Temp	Comments
100% Ethyl or Reagent Alcohol		RT	Rinse and discard
100% Ethyl or Reagent Alcohol	5 min	67°C	Loosely cover and agitate
Absolute Isopropanol	3 min	74°C	Loosely cover and agitate
Paraffin (60°C)	2 min	65°C	Uncovered <sup>†</sup> (Use same solution for 2nd step)
Paraffin	5 min	80° to 84°C	Uncovered <sup>†</sup>

**Table 2B. Processing FIXED 1-mm thick specimens (or bx specimens in sponges) that have been rinsed well in water\***

Solution	Time	Temp	Comments
100% Ethyl or Reagent Alcohol		RT	Rinse and discard
100% Ethyl or Reagent Alcohol	5 min	67°C	Loosely cover and agitate
Ethanol/ Isopropanol(50/50)	3 min	70°C	Loosely cover and agitate
Absolute Isopropanol	3 min	74°C	Loosely cover and agitate
Paraffin (60°C)	2 min	65°C	Uncovered <sup>†</sup> (Use same solution for 2nd step)
Paraffin	5 min	80° to 84°C	Uncovered <sup>†</sup>

**Table 2C. Processing FIXED 3-mm thick specimens that have been rinsed well in water\***

Solution	Time	Temp	Comments
100% Ethyl or Reagent Alcohol		RT	Rinse and discard
100% Ethyl or Reagent Alcohol	10 min	67°C	Loosely cover and agitate
100% Ethyl or Reagent Alcohol	10 min	67°C	Loosely cover and agitate
Absolute Isopropanol	10 min	74°C	Loosely cover and agitate
Absolute Isopropanol	10 min	74°C	Loosely cover and agitate
Paraffin (60°C)	10 min	65°C	Uncovered <sup>†</sup> (Use same solution for 2nd step)
Paraffin	10 min	80° to 84°C	Uncovered <sup>†</sup>

\* The sample processing protocols listed above have been run successfully on several 120V laboratory microwave models. Actual instrument wattage readings were between 650W and 1000W.

† During paraffin steps, stirring mechanisms may be used. Do not use air bubble agitation. Manually agitate specimen rack between paraffin steps.

such a unit. We are currently researching various decal solutions and using several different fixatives to obtain the best protocol for our lab. Our goal is to no longer delay reports while waiting for a decal sample.

Today, with the availability of true walk-away special staining instruments, we are performing fewer stains in the microwave, at least for those methods that have been successfully automated. This permits us to better utilize our staffing resources.

Epitope retrieval is a complex subject beyond the scope of this discussion. It should be stressed, however, that the retrieval method employed must be tailored to the antibody markers you are demonstrating and the detection system you are using. FDA-approved methods must be followed precisely or a disclaimer must be included in the pathology report indicating that the results reflect a departure from the approved method. Despite this, many have found microwave technology to be beneficial in achieving epitope recovery in formalin-fixed tissues for some markers. Some laboratory microwave instruments will print a completed run report that can provide useful information for your laboratory's quality control program. Reports may include a record of the temperatures achieved and their duration during antigen retrieval. It is not difficult to imagine that laboratory accrediting agencies may require such documentation in the future.

### Conclusion

Both domestic and laboratory devices can be used to perform many of the procedures in a routine histology laboratory but safety, reproducibility, and sample quality are important considerations when selecting the best device for your operation. In order to determine which device will best meet your needs, it is important to consider which procedures will be

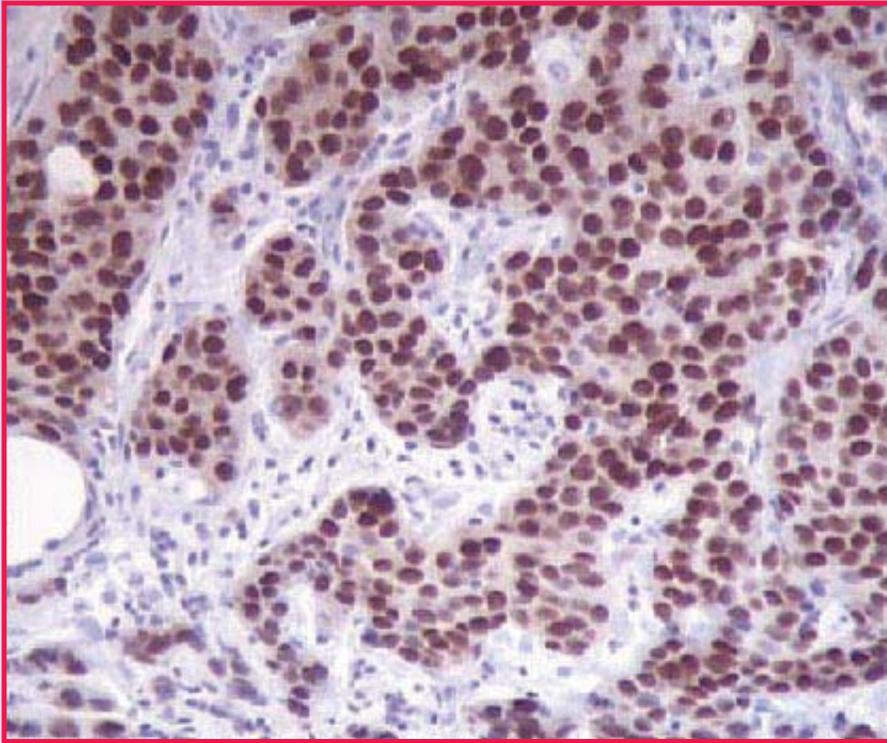


Fig. 2. Breast 1 mm, estrogen receptors stained with IHC.

performed; which chemicals/reagents will be required; how the temperature will be monitored and controlled; what wattage will be necessary for the desired procedures; and what the availability of power and ventilation will be in the desired installation area. Keep in mind that reagent costs will decrease because you use fewer reagents and smaller volumes than what is used in a routine processor. Paraffin costs also will decrease since the alcohol is evaporated off in the paraffin which may be cooled and reused.

It is encouraging to see the growth of this beneficial technology in our discipline. When used properly, it can decrease turnaround time and reagent costs. Most tangible of all, perhaps, is the diminished wait by patients for their diagnosis.

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Scientific papers and techniques for microwave tissue processing.

# Comparison of Seven Antibody Dilution Buffers for Immunohistochemistry

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## Abstract

Antibody dilution buffer provides a suitable environment for antigen-antibody interaction. The diluent can boost antibody affinity and enhance the quality of immunostaining.

The purpose of this experiment was to compare various antibody diluents for their ability to support antigen-antibody interaction, as well as to determine the shelf life of prediluted primary antibodies before they begin losing sensitivity against the epitope. One multitissue block was prepared using various normal and cancer tissues as a panel of cases to express various antigens for adequate comparison of antibodies. We compared seven buffers from various sources. Nine primary antibodies were diluted with each buffer and stored for further staining. The ability of these diluents to sustain antibody viability and to support antigen-antibody interaction was variable. Some dilution buffers showed selected advantage toward a specific type of antibody.

Overall, the DAKO antibody dilution buffer and the Ventana ChemMate antibody dilution buffer generated the strongest intensity of immunostaining and retained sensitivity of the antibodies relatively intact during the first 2-week period.

## Introduction

Immunohistochemistry is a complex method of testing histological tissue preparations with various antigen-specific antibodies. Antibody-antigen interaction is a delicate immunological process which

depends on numerous equally important factors for generating adequate immunostaining results.

Choosing the right primary antibody is perhaps the most important determinant for successful immunostaining. Meanwhile, choosing the right antibody dilution buffer is equally important, since antibodies require a suitable environment for adequate interaction with the tissue antigen. It is also important for antibodies to be in the proper environment for long-term storage.

### Materials and Methods

**Tissues:** The study included nine paraffin-embedded surgical biopsy specimens. Tissues were pre-selected to express various types of antigens for adequate comparison of antibodies. One multitissue block was prepared using various normal and cancer tissues: two normal tonsils, one normal lymph node, two mantle cell lymphomas, one normal pancreas, one chromogranin/synaptophysin positive tissue, and two melanomas.

**Primary Antibodies:** Nine primary antibodies were diluted in each buffer on day 1 and stored at 4°C for further staining and comparison.

**Antibody Dilution Buffers:** The study included one homemade and six commercially available antibody dilution buffers.

**Immunohistochemistry:** IHC staining was performed on day 1, day 2, and 1 week, 2 weeks, 4 weeks, and 8 weeks after diluting the antibodies. A standard immunoperoxidase method was applied according to the laboratory protocol with overnight incubation. The antigen retrieval procedure was performed in an autoclave at 105°C for 3 minutes.

### Evaluation and Analysis

The final analysis showed that antibodies behaved differently in each dilution buffer. A single diluent could not equally support

### Primary Antibodies Evaluated

Antibody	Clone	Titer	Source
Actin	HHF-35	1:500	DAKO
BCL-1	DCS-6	1:400	Neomarkers
CD3 (m)	PSI	1:400	Novocastra
CD4	1F6	1:200	Novocastra
CHR (m/p)	LK2H10(9)/poly	1:1K/1:32K	BM/DAKO
CK 5/6	D5/16B4	1:200	BM
Kappa (px)	Polyclonal	1:200	DAKO
Lambda (px)	Polyclonal	1:200	DAKO
S-100	Polyclonal	1:8K	DAKO

### Antibody Dilution Buffers Evaluated

Antibody Dilution Buffers	Cat #	Source
Antibody dilution buffer	CD200082	DAKO
ChemMate antibody dilution buffer Ventana (A)	ADB250	Ventana
Ventana (B)	Sample	Ventana
Ventana (C)	Sample	Ventana
PBS/BSA	SOP	Impath Inc.
Primary Antibody Diluent	750104	Research Genetics
Revival-Sky Blue	PD901L	Biocare Medical

all the antibodies for adequate immunostaining. For example, actin showed better immunostaining with the buffer supplied by Research Genetics, however, bcl-1, CD3, CK5/6 and S-100 did not stain as well with that same diluent.

Likewise, the performance of the CD3 antibody was unsurpassed with the Biocare Blue buffer, however, actin, bcl-1, kappa, and lambda antibodies stained inadequately with that buffer. This was probably because antibodies are highly sensitive and susceptible biological molecules, and each one requires a discrete chemical environment for optimum behavior. Slight environmental changes can influence the immunoreaction, creating fluctuations in the results.

Considering the practical inconveniences of using different diluents for different antibodies, we compared the overall achieve-

ment of all the antibodies obtained with each individual dilution buffer. In order to do that, we added the staining intensity scores of all antibodies that were obtained with the same antibody diluent and compared the total scores of different antibody dilution buffers.

The results were analyzed by comparing the sums of absolute and relative sensitivity values of all antibodies using various dilution buffers on the same day, as well as on different days of immunostaining (see Tables 1 and 2 on next page).

Ventana C dilution buffer was eliminated from the study due to high background and low reactivity observed with this buffer.

Immunostaining results were compared and evaluated semi-quantitatively by two independent investigators using light microscopy.

**Table 1. Comparison of Absolute Sensitivity of Antibodies\***

	DAKO	Ventana A	Ventana B	Biocare Blue	Research Genetics	PBS/BSA
Day 2	46	45	32	37	39	32
Week 1	47	46	31	35	43	36
Week 2	33	36	24	23	31	29
Week 4	37	37	26	26	34	32
Week 8	36	30	23	23	32	31
Total	199	194	136	144	179	160

\* Absolute sensitivity = intensity of specific staining.

**Table 2. Comparison of Relative Sensitivity of Antibodies†**

	DAKO	Ventana A	Ventana B	Biocare Blue	Research Genetics	PBS/BSA
Day 2	44	43	32	33	37	29
Week 1	46	44	31	30	42	33
Week 2	33	35	24	17	31	28
Week 4	36	34	24	22	33	29
Week 8	32	27	23	16	31	30
Total	191	183	134	118	174	149

† Relative sensitivity = absolute sensitivity – background staining results.

In order to minimize day-to-day subjective variations, results were compared from different runs of immunostaining on different days with the same antibodies to ensure that the same reference point was used consistently for comparison of antibody sensitivity. Each final score was recorded after two investigators came to a consensus regarding the score.

Three variables were scored: sensitivity, specificity, and background staining. The results were scored on a 0 to 6-plus scale. Each evaluation score had two components: A/B = sensitivity/background staining.

The results in Table 1 and Table 2 demonstrate that DAKO antibody dilution buffer had higher overall scores throughout the 8-week period when compared with the other dilution buffers. DAKO diluent retained antibody sensitivity relatively intact during the first 2-week period. In addition, antibodies that were diluted in DAKO dilution

buffer showed higher relative sensitivity (less background staining) than with the others.

#### Conclusions

1. DAKO antibody dilution buffer generated the strongest intensity of immunostaining.
2. Most antibodies showed overall decline in sensitivity on or after week 4 regardless of the antibody dilution buffer used.
3. All antibodies showed relatively consistent and stable results with the same dilution buffer during the 4-week study period.
4. All antibodies showed similar decline in sensitivity after the 4-week study period.
5. All antibodies showed similar variations during storage with the same dilution buffer.

## Microwave-Enhanced Fixation for Rapid Preparation of Tissue Sections for Microscopic Evaluation

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#### Background

Microwave use in the histology laboratory has increased dramatically over the last 10 years. Microwave technology has been applied to rapid tissue fixation, special staining procedures, and antigen retrieval for immunocytochemical techniques. Addition of heat to the fixation process increases fixative penetration into tissue resulting in rapid cross-linking of proteins and structural stabilization. Variations of temperature, tissue volume, and time can make it difficult to control for consistent tissue fixation. In addition, different tissues undergo the fixation process at different rates. Inadequate heat and length of fixation can also result in poor fixation of inner tissue. Excessive heat can result in shrinkage of smaller/thinner tissues. Different fixatives can be used including formalin, buffer solutions, commercial fixatives specified for microwave use, and others. Each can present different hazards from fumes and waste disposal. The generation of fixative fumes is accentuated with microwave use and requires adequate laboratory ventilation.

At Pfizer Global Research & Development, Ann Arbor, Drug Safety Evaluation, we have examined ways to utilize microwave technology to achieve high-quality, rapid fixation of

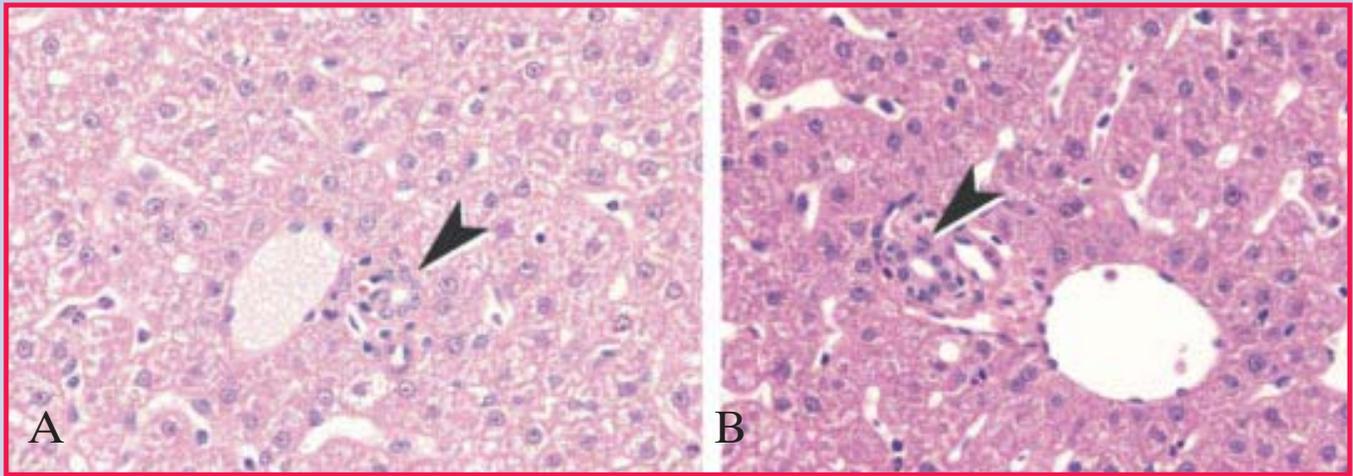


Fig. 1. Liver centrilobular hepatocytes: (A) immersion fixation; (B) microwave fixation. H&E, 20X

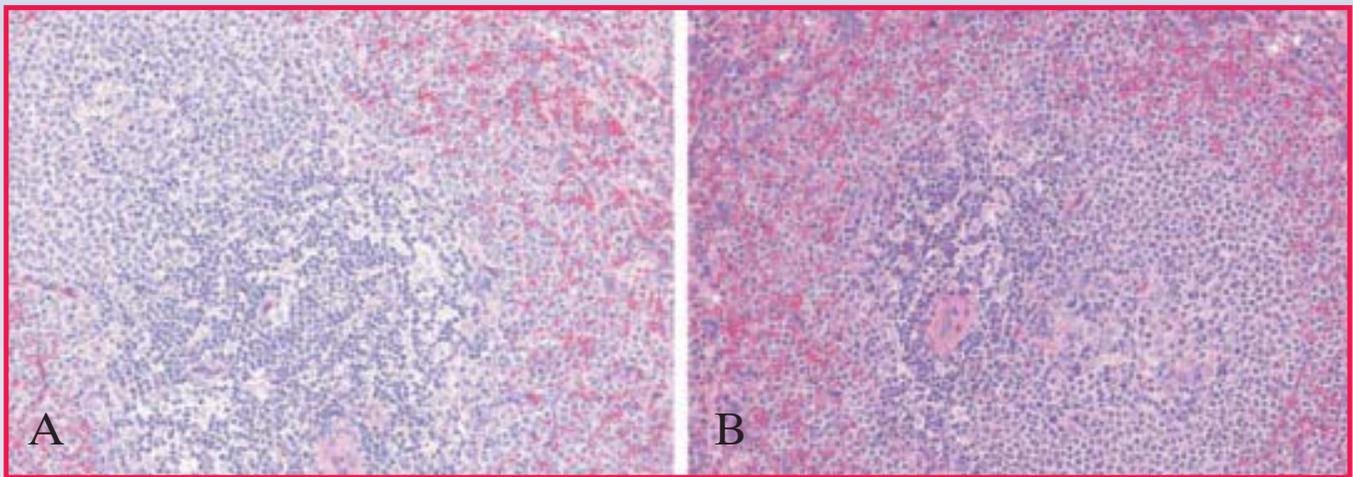


Fig. 2. Spleen white pulp with lymphoid follicle: (A) immersion fixation; (B) microwave fixation. H&E, 20X

research animal tissues. Our laboratory provides histology support for a variety of pharmaceutical preclinical safety studies in laboratory animals. It is sometimes necessary to evaluate tissues microscopically within a short time.

Other laboratories are able to produce histology slides rapidly, but often size and quality are compromised. Our goal was to accelerate the turnaround of our standard protocol tissue types and sizes while achieving optimal results. By using microwave fixation of our tissues, we have been able to achieve completion of tissues as large as 22 mm x 13 mm x 4 mm thick within 24 hours, as opposed to the four or five days that is typical of our work when conventional fixation and processing is used.

## Materials and Methods

### Equipment

The PELCO laboratory microwave (PELCO Model 3451, Ted Pella, Inc., Redding, CA) was chosen for our laboratory as it has many adjustable features that are useful in achieving optimal results. These include adjustable settings for wattage, temperature, and incorporated water load. Our unit was calibrated for specific wattage (personal communication, Richard Giberson, Ted Pella, Inc.). It also has a temperature probe and bubbler for use within the solutions placed inside the chamber.

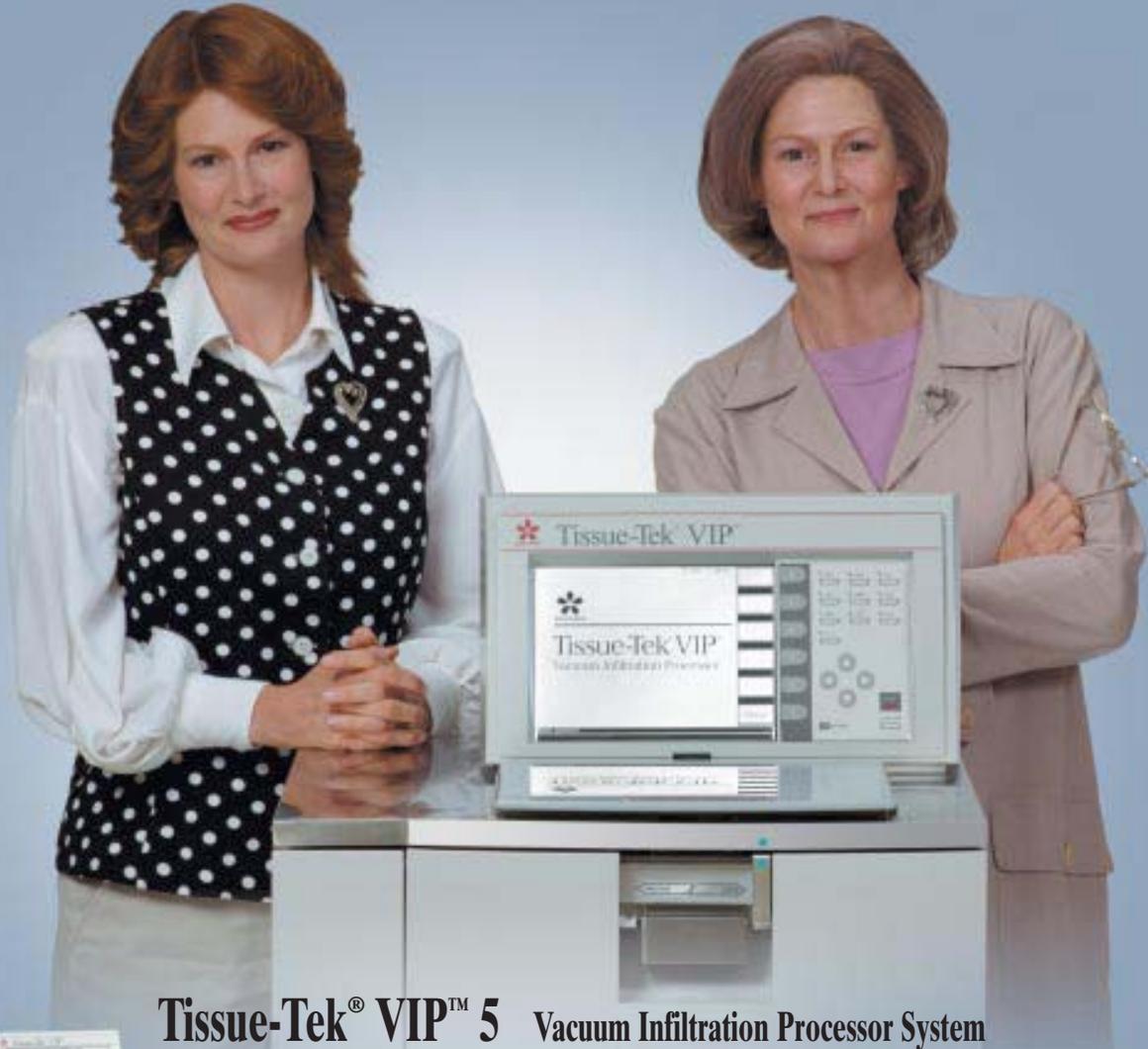
### Tissue Collection, Fixation, and Processing

All animal testing within the Pfizer facility is conducted in accordance with current guidelines for animal welfare (Guide for the Care and

Use of Laboratory Animals, 1996) and reviewed by internal committees. Tissues were selected based on exploratory study protocols including spleen, colon, kidney, liver, pancreas, and mesenteric lymph nodes. Each was collected and fixed the same way.

Cynomolgus monkey tissues were collected during a necropsy procedure. The tissues were placed in room temperature 10% Neutral Buffered Formalin (NBF) for 30 minutes. All were trimmed and cassetted according to internal standards, no larger than 22 mm x 13 mm x 4 mm. Cassettes were placed in fresh 10% NBF in a plastic PELCO cassette rack system specifically designed for microwaving, and placed into the microwave chamber.

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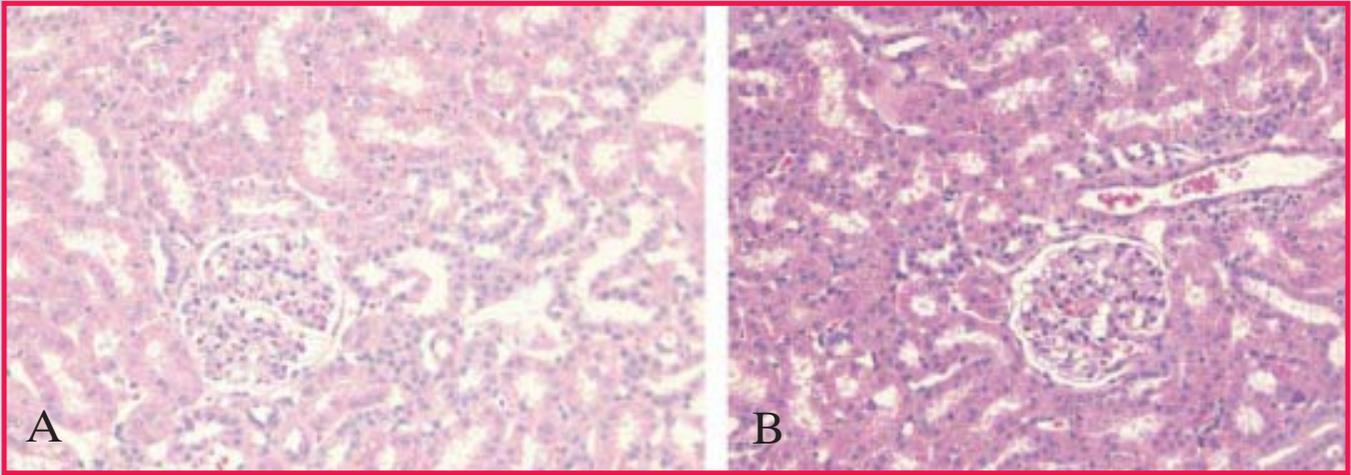


Fig. 3. Kidney glomerulus and tubules: (A) immersion fixation; (B) microwave fixation. H&E, 20X

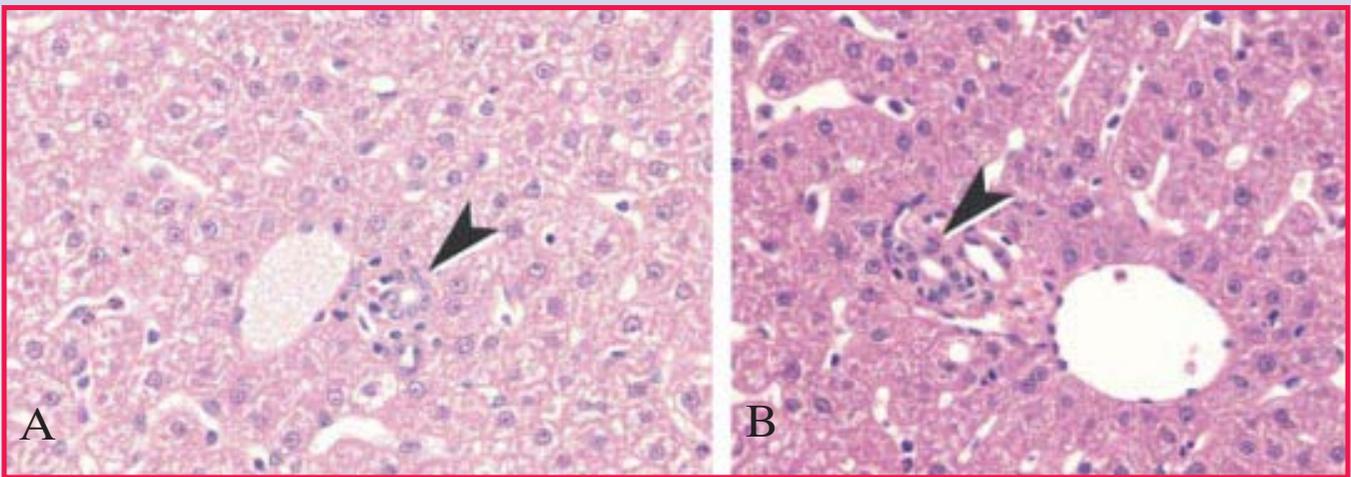


Fig. 4. Liver portal tract, bile duct (arrowhead): (A) immersion fixation; (B) microwave fixation. H&E, 20X

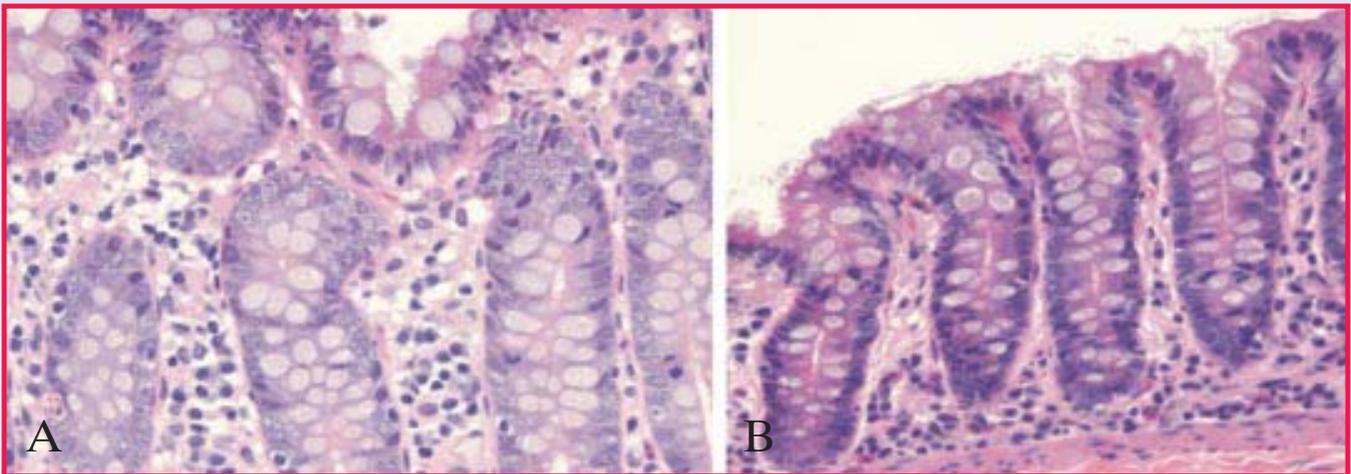


Fig. 5. Colon mucosa: (A) immersion fixation; (B) microwave fixation. Some mucosal shrinkage was evident with microwave-fixed specimen. H&E, 20X

The microwave was set at 45°C, wattage setting 3 (315 watts), medium-high power, for 70 minutes. Following the formalin, tissues were placed in Preserve™ fixative (Energy Beam Sciences, Agawam, MA)

at 45°C, wattage setting 2 (210 watts), medium-high power, for 40 minutes. Cassettes were then rinsed in tap water briefly and processed overnight in a conventional tissue processor

(Tissue-Tek® VIP™, Sakura Finetek, Torrance, CA) beginning with 70% specially denatured ethyl alcohol, through 100%, xylene, and paraffin (Paraplast X-tra, Oxford Labware, St. Louis, MO).

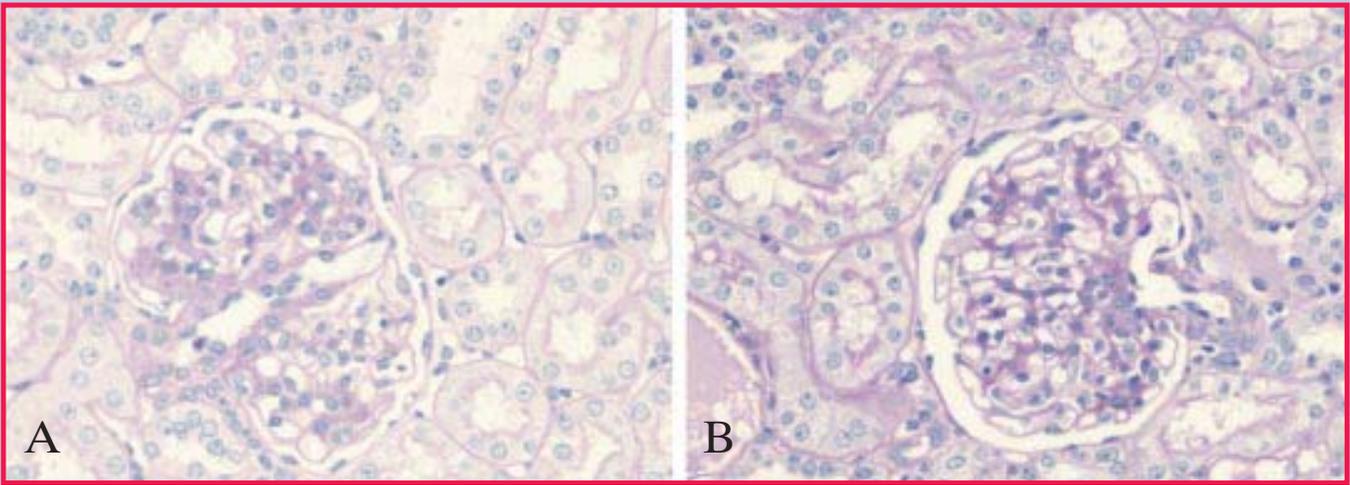


Fig. 6. Kidney glomerulus and tubules: (A) immersion fixation; (B) microwave fixation. Enhanced detail of basement membranes in microwave-fixed specimen. PAS, 40X

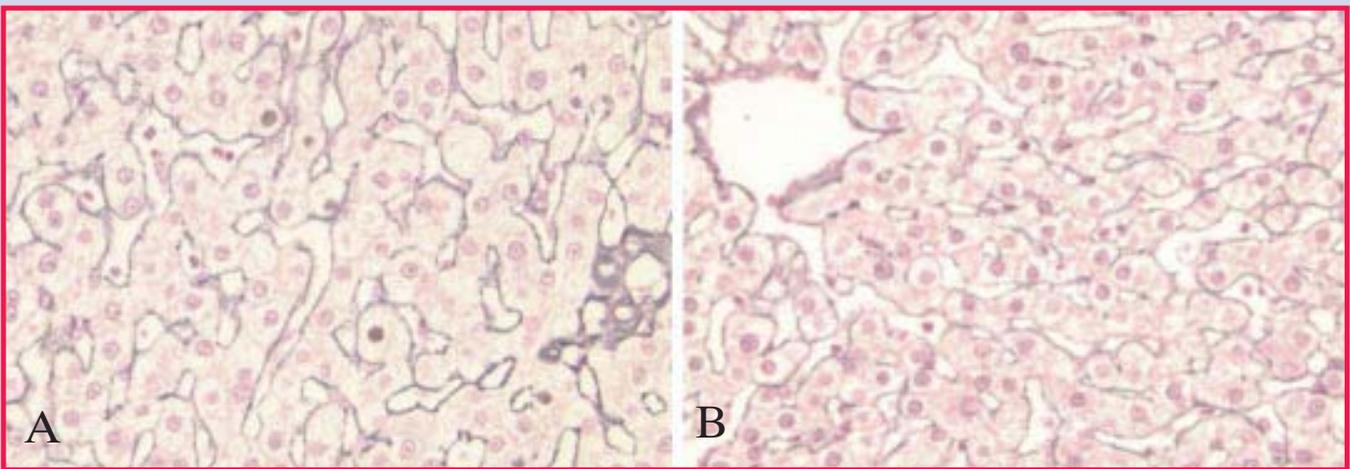


Fig. 7. Liver reticulin: A) immersion fixation; (B) microwave fixation. Reticulin staining comparable in both specimens with enhanced nuclear fast red counterstain in microwave specimen. Reticulin stain/nuclear fast red, 20X

### **Embedding, Sectioning, and Staining**

The next morning the tissues were embedded in Paraplast® embedding medium, (Oxford Labware, St. Louis, MO). Blocks were sectioned at 3 $\mu$  and stained with Hematoxylin & Eosin (H&E), Periodic Acid Schiff (PAS), and/or Reticulin stain using standard protocols.<sup>1</sup>

For comparison, tissue sections from the same tissue samples were prepared using standard immersion fixation in 10% NBF for 2-4 days and then processed using the overnight processor as described above. Embedding, sectioning, and staining were also done as described above, as well as per internal standard operating procedures.

### **Results**

Tissue sections prepared from microwave-fixed samples were of high quality with excellent fixation and uniform staining characteristics. In general, staining was enhanced in tissues processed by microwave fixation. With H&E staining, both nuclear detail and cytoplasmic staining were excellent (Fig. 1 *liver*, Fig. 2 *spleen*). Epithelial preservation was also excellent (Fig. 3 *kidney*, Fig. 4 *liver detail portal area*), however, when compared to immersion-fixed tissue, limited mucosal shrinkage was evident in sections of colon (Fig. 5 *colon*). In sections of kidney stained by the PAS procedure, there was excellent preservation/staining of basement membranes (Fig. 6 *kidney PAS*). Similarly, reticulin staining in the liver was comparable

for microwave- and immersion-fixed tissue (Fig. 7 *liver reticulin*). In general, microwave tissue fixation resulted in tissue sections of excellent quality, which was comparable for sections prepared from tissues using standard immersion-fixation procedures.

### **Conclusion**

Microwave-enhanced tissue fixation is a valuable technique for rapid production of quality tissue sections for microscopic evaluation. Microwave-fixation procedures can be applied to a variety of tissue types with production of tissue sections within 24 hours. In internal exploratory study protocols where time is an important factor, this new methodology could prove to be very useful in determining dose levels for further study in continuing

drug discovery. The use of microwave technology in clinical and industrial settings is now more commonplace as laboratories become aware of its value in achieving quality histologic preparations in less time.

#### Reference

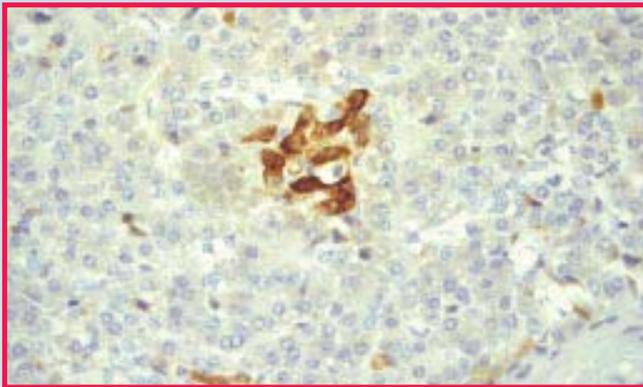
1. Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis, Mo: C.V. Mosby Co;1980.

## Prolonged Storage of Antibody Stock Solutions for Immunohistochemistry: A Three-Year Study

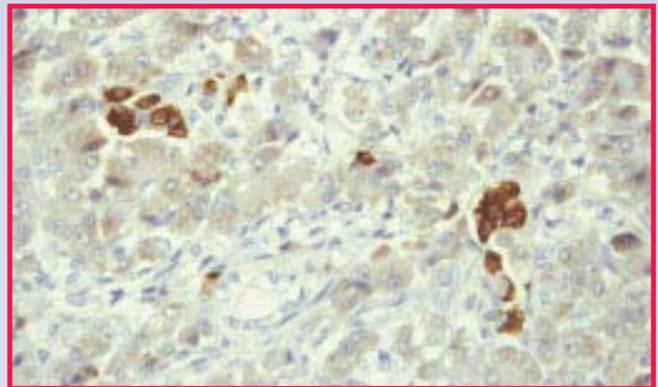
**Marilyn E. Beissenherz, HT(ASCP)**  
**J. A. Ramos-Vara, DVM, PhD, ECVP**  
Veterinary Medical  
Diagnostic Laboratory  
University of Missouri-Columbia

#### Introduction

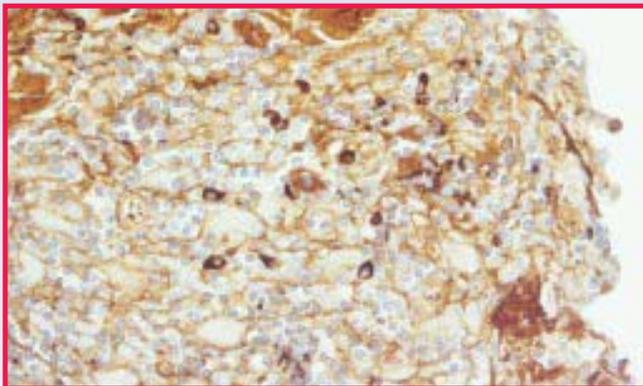
The effect of storage of antibody stock solutions on the immunoreactivity of these antibodies is not well documented. We tested the immunohistochemical reactivity of stock solutions of antibodies stored for several years at the University of Missouri-Veterinary Medical Diagnostic Laboratory (UM-VMDL) immunohistochemistry laboratory, using formalin-fixed, paraffin-embedded tissues. Determination of immunoreactivity was qualitative (staining or no staining).



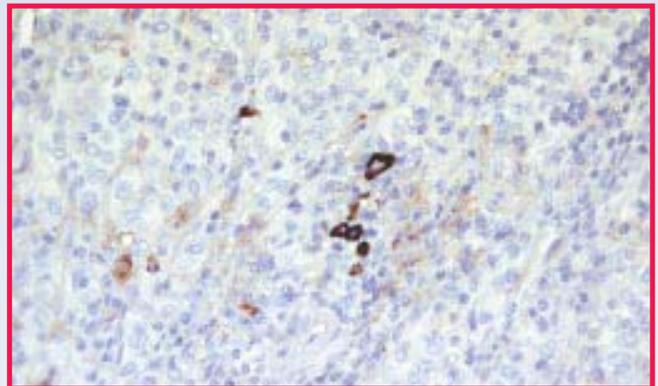
Glucagon 1998



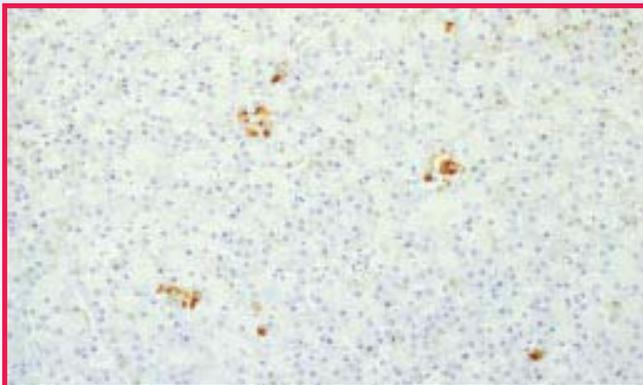
Glucagon 2001



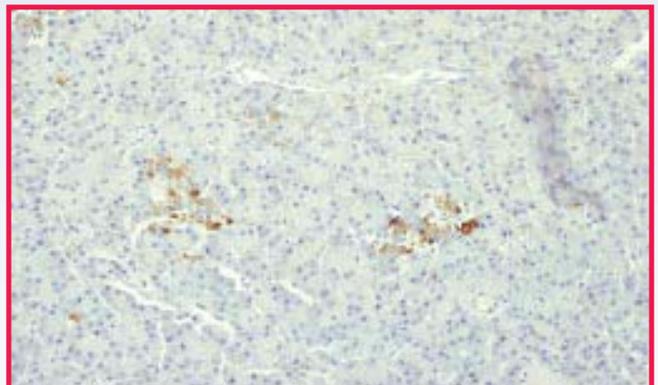
IgG 1998



IgG 2001



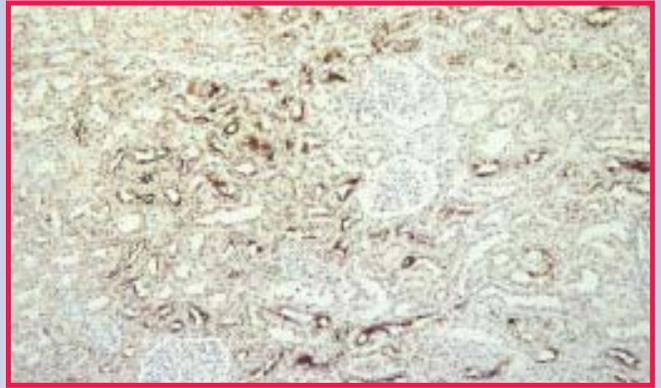
Insulin 1998



Insulin 2001



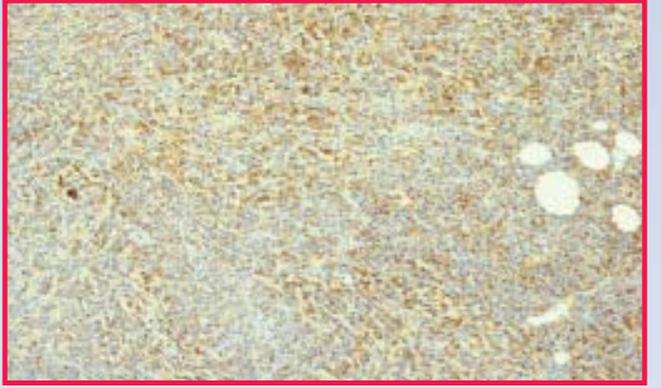
*Leptospira* 1998



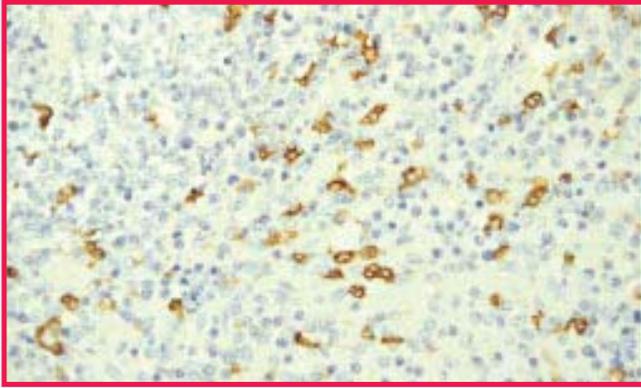
*Leptospira* 2001



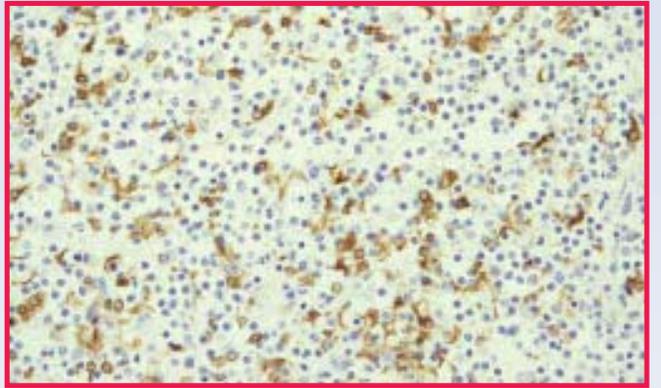
Lambda light chains 1998



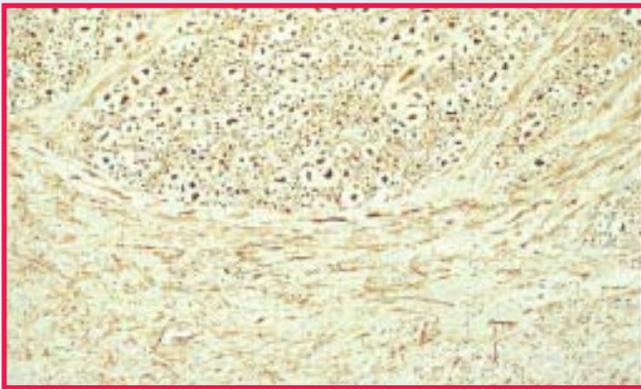
Lambda light chains 2001



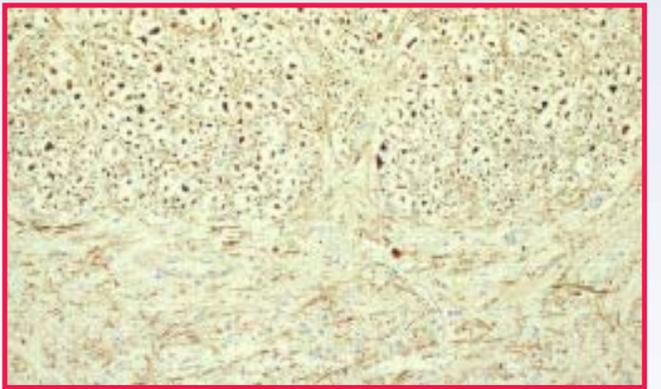
Myeloid/histiocytic antigen 1998



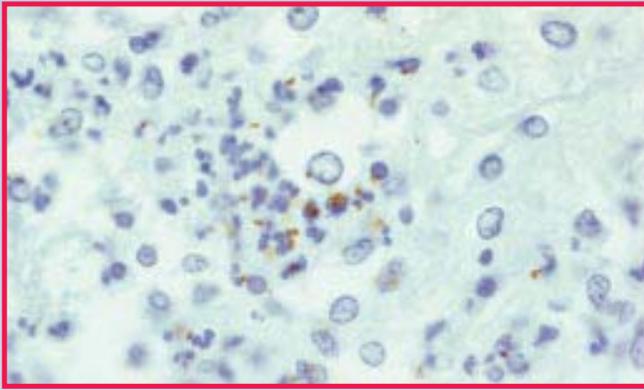
Myeloid/histiocytic antigen 2001



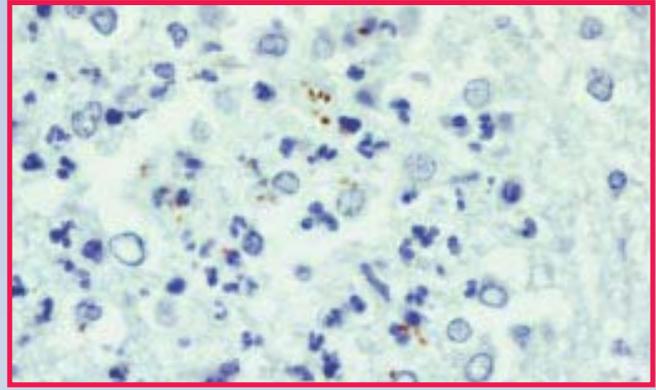
Neurofilament 1998



Neurofilament 2001



Listeria 1998



Listeria 2001

### Materials and Methods

The final dilution of each antibody was made from a stock solution (1:100). Stock dilutions were prepared from concentrated (undiluted) antiserum using DAKO diluent, which was kept in plastic capped vials and stored at 4°C. The final volume for these stock dilutions was 5-10 mL. Immunohistochemical methods included labeled streptavidin-peroxidase, ABC, and EnVision Plus-peroxidase.

### Results

Of 100 antibodies used at the UM-VMDL, stock solutions of 15 antibodies have been in use with no apparent loss of staining since October 1998. These antibodies are:

- Calcitonin (DAKO; polyclonal)
- *Campylobacter* sp. (KPL; polyclonal)
- GFAP (DAKO; polyclonal)
- Glucagon (DAKO; polyclonal)
- Canine IgG (Sigma; monoclonal)
- Insulin (Zymed; monoclonal)
- Laminin (DAKO; polyclonal)
- *Leptospira* sp. (NVSL; polyclonal)
- *Listeria O* antigen (Difco; polyclonal)
- Lambda light chain (DAKO; polyclonal)
- Myeloid/histiocytic antigen (DAKO; monoclonal)
- *Mycobacterium bovis* (DAKO; polyclonal)
- Myoglobin (DAKO; polyclonal)

- Neurofilament (Sternberger Monoclonals; monoclonal)
- Somatostatin (DAKO; polyclonal)

### Conclusion

The reason for the variability in the immunoreactivity of antibodies after prolonged storage is unknown. Although most of the antibodies that remained immunoreactive after three years of storage were polyclonal, several were monoclonal. Variations in the immunoreactivity of antibodies stored as stock solutions may be due to diluent used, protein concentration, class and subclass of immunoglobulins, among others.

## Staining Artifact Associated With Bouin's Substitute for Masson Trichrome Staining on Formalin-Fixed Liver Biopsies

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Cleveland Clinic Foundation  
Cleveland, OH

### Introduction

Masson trichrome staining is commonly requested on formalin-fixed, paraffin-embedded liver biopsies. Formalin-fixed tissue

sections stained with Masson trichrome require Bouin's fixative as a mordant. However, when using a non-picric-acid-based Bouin's substitute, microscopic examination reveals an artifact demonstrating an overall pale red appearance and intense blue areas often around the perimeter of the section.

### Materials and Methods

The use of Bouin's fixative (picric acid, formaldehyde, acetic acid, and water) as a mordant in preparation for Masson trichrome staining was compared to the use of a Bouin's substitute (chloroplatinic acid, formaldehyde, acetic acid). Duplicate paraffin-embedded, formalin-fixed needle biopsies of liver were sectioned at 4 microns on twelve different liver biopsy cases. One slide was stained with a Masson trichrome procedure using Bouin's fixative (saturated picric acid) and the duplicate slide was stained with the same Masson trichrome procedure, but with a Bouin's substitute (saturated chloroplatinic acid). Afterward, the slides were compared for quality.

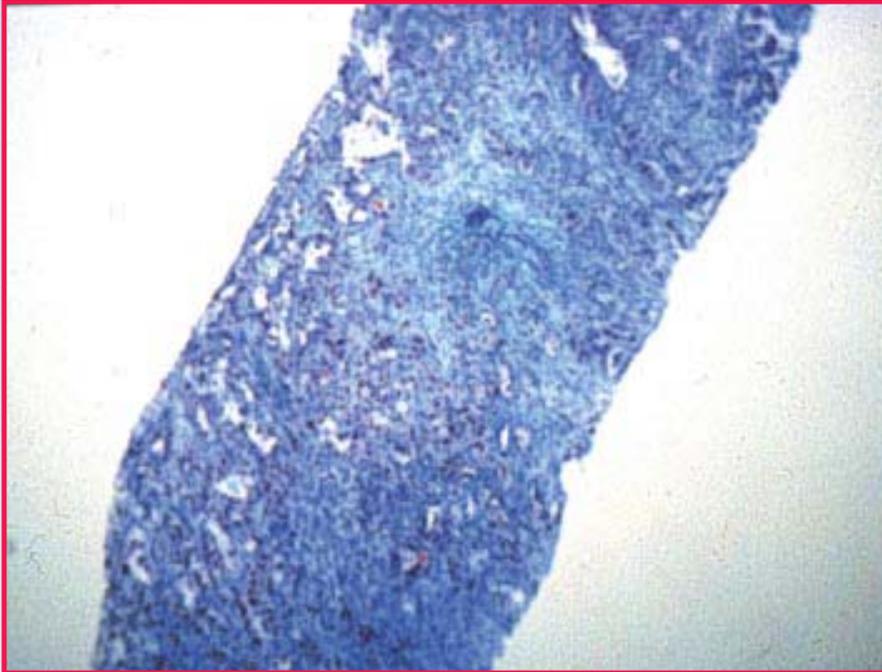
### Results

All Masson trichrome-stained slides using Bouin's substitute demonstrated diminished red staining of the Biebrich scarlet acid fuchsin solution with a dark blue appearance as a result of intensified aniline blue staining. All duplicate slides stained using Bouin's fixative demonstrated homogeneous Biebrich scarlet acid fuchsin and aniline blue staining.

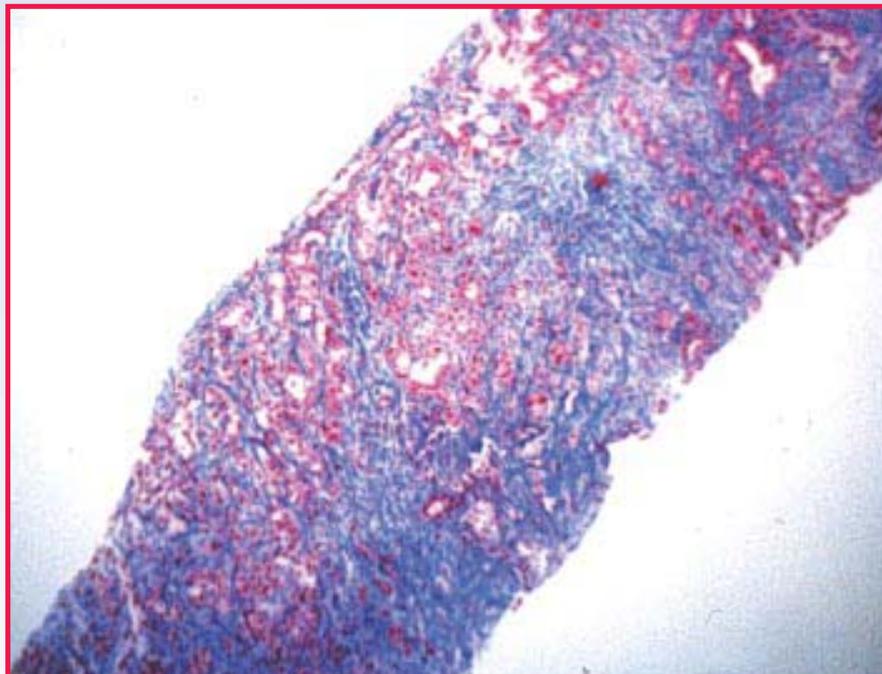
## Conclusion

Adequate staining of Biebrich scarlet acid fuchsin solution is essential for diagnosis of formalin-fixed, paraffin-embedded liver biopsies. An artifact demonstrating pale Biebrich scarlet acid fuchsin staining and intense aniline blue staining may interfere

with the microscopic interpretation of formalin-fixed liver biopsies. To reduce this artifact, we recommend the use of Bouin's post-fixation mordanting using a saturated picric acid solution for Masson trichrome staining of formalin-fixed liver biopsies.



Masson trichrome stain with Bouin's substitute mordanting. 150X



Masson trichrome stain with Bouin's mordanting. 150X

## Living With Formaldehyde in Today's Healthcare Workplace

**Ted R. Bryan, BS**  
**Consultant**  
**Environmental Technologies**

As we all know, aqueous formaldehyde, more commonly referred to as formalin, has been used extensively in most fields of histology for many years. However, the issues of safety and disposal are topics that until recently were mostly relegated to the back burner. Due to the inherent caveats of formalin use, there has been much discussion about the implementation of chemicals that can be used in place of formalin. What most histologists don't realize is that many of these substitutes present their own problems with respect to exposure and disposal, without offering the superb morphological patterns found only in formalin fixation. For those of you who favor a fixative that offers better immunoreactivity, zinc formalin has been around for many years and would be the fixative of choice. In either case, the "perfect fixative" has yet to be discovered and even when it is, it will take years before its use is widely adopted.

In light of this, it seems only logical that more attention be dedicated to the safety of the 1.4 million health care professionals who presently come in contact with the hazards of formalin on a daily basis. Much dialogue has been devoted to the evils of formalin but too little on discussing recent developments regarding exposure control and safe disposal. While examining each of these issues, it is important to remember that exposure control is largely regulated by OSHA, whereas disposal is regulated by the EPA and local Publicly Owned Treatment Works (POTWs).



Personal protective equipment required when working with formaldehyde in a poorly ventilated area.

### Exposure Control

Over the last few years, advances in technology have dramatically decreased the risk of exposure to histotechnologists. Regulated by OSHA laws (29CFR 1910.134), formalin exposure can be substantially mitigated by employing general ventilation and simple, low-cost work practice controls. By definition, work practice controls “reduce the likelihood of exposure by altering the manner in which a task is performed.” For instance, in grossing areas where ventilation may not be adequate, employment of appropriate respirators and neutralizing pads can dramatically decrease vapor exposure. With respect to the pads, it is important to note that when implementing the use of these types of controls, you are advised to thoroughly investigate the product to insure that it does actually have the ability to neutralize the formalin. Reading the MSDS and posing questions to the manufacturer are both essential in order to guarantee that the product functions as represented. Neutralization capabilities vary dramatically and companies often make performance claims with respect to neutralization that cannot be substantiated.

Formalin spills are another source of exposure which can be quickly remedied if an appropriate work practice control is in place. For small spills, neutralizing cloths are fast and effective; large spills will require a spill control agent that is easy to apply and not only absorbs the spill rapidly, but completely neutralizes it as well. Once again, buyer beware! Be sure to do your homework and investigate the products you use. Although some spill control agents may neutralize the formalin, the resulting mixture is at a pH that is below 1 and is therefore still considered hazardous waste.

### Formalin Disposal

In 1976, the US Environmental Protection Agency (EPA) passed the Resource Conservation and Recovery Act (RCRA) in order to protect the environment from improper hazardous waste management practices. This law mandates “cradle to grave” responsibility for users of hazardous waste material. As the awareness of our environmental issues increases, the methods we employ for disposal of hazardous chemicals becomes an issue each of us must face. Since pollution does not recognize the political boundaries separating states and nations, the responsibility for safe disposal belongs to all individuals united in this cause. The decisions we make regarding these issues will ultimately affect the global ecosystem. For this reason it is important to set stringent standards that are both economically feasible and relatively simple to follow.

With respect to formalin, we should all be thankful that the days of disposing untreated waste directly into a sink are fast coming to an end. Those institutions that still practice this will have to consider the repercussions (see 40CFR 403) and chronic effects on aquatic degradation, bioremediation, and human health. In the not too distant future, all municipal POTWs will be required to adhere

to regulatory laws and avoid the effects of careless disposal practices. This is easier to accomplish than it sounds due to the availability of disposal options that are not only in compliance with hazardous waste disposal guidelines, but are economical and easy to use as well.

One such option for waste disposal is hazardous waste hauling (off-site disposal). This remains a viable option as long as it is provided by EPA-licensed, reputable firms. However, this method can be expensive, labor intensive, and it carries a potentially high liability due to exposure and spill risks.

A viable alternative to off-site disposal is the use of a treatment technology known as neutralization. In most cases, treatment can be trouble-free and convenient because it takes place at the site of waste generation and does not require transportation out of the department. However, in recent years there has been considerable controversy about the neutralization claims made by the various manufacturers of these treatment technologies. In response to this, and long considered a model for environmental responsibility, the California EPA has developed its unique Environmental Technology Certification Program. The purpose of this program is to “provide an independent technical evaluation of technologies to identify those meeting applicable quality standards, so as to facilitate regulatory and end-user acceptance.” In a nutshell, this program certifies the scientific and engineering claims made by a manufacturer regarding their technology. This is your guarantee that the product claims have been substantiated by an independent source other than the manufacturer. The program is significant not just for those who reside in California, but for everyone in every state and country who values the importance of safe waste disposal.

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At one time there were three certified formaldehyde treatment technologies. Two of these were originally certified under a pilot program that involved a very rudimentary level of testing not involving 10% NBF wastes. The other technology, Tissue-Tek®\* Neutralex®†, was certified under a more stringent program that included the treatment of actual formalin waste. When the manufacturers of the technologies certified under the pilot program decided to apply for recertification, the California EPA decided that they had to be held to the same testing standards as were performed on Neutralex®. As a result of this testing, both of the other two technologies were denied recertification. The data determined that after treatment in both technologies “about one-half of the samples in each batch were more toxic after treatment than before treatment, and more toxic than the hazardous waste threshold.” As a result of this denial, health care facilities in California are no longer authorized to treat their 10% NBF hazardous waste with either of these technologies. Neutralex® is presently the only technology certified by the California EPA. This is the type of regulatory responsibility that should also be a model for every institution that has a need to dispose of formalin waste.

### Conclusions

Regulatory requirements affecting the use of hazardous chemicals both domestically and globally are changing as our awareness of the effects of these substances becomes better understood. Contrary to the belief of some, we can safely co-exist with formalin in the workplace as well as protect the integrity of our fragile environment. Through the use of reliable engineering and work practice controls, vapor exposure can easily be maintained within the boundaries of OSHA laws. The California EPA Environmental Certification Program will set a standard for safe disposal that can

be duplicated worldwide. We now have the technology to effectively control the integrity of our workplace and environment. Let's face it — formalin will be a part of most histology departments for years to come. With a concerted effort by all affected, we can navigate a course that is safe, economical, and effective.

\* Tissue-Tek® is a registered trademark of Sakura Finetek U.S.A., Inc.

† Neutralex® is a registered trademark of SCIGEN Inc.

## *Helicobacter pylori* Stain Using Diff-Quik Solution as a Counterstain

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### Abstract

*Helicobacter pylori* is a spiral-shaped bacterium found in the gastrointestinal tract. It is now known to cause more than 90% of duodenal ulcers and up to 80% of gastric ulcers, hence its diagnostic significance. Our lab uses the Diff-Quik procedure<sup>1</sup> to identify *H pylori*. We recently began to use a polyclonal *H pylori* concentrated antibody (DAKO), which aids in rapid visualization of the bacteria, especially when present in small numbers. Another advantage is the specificity of staining which permits *H pylori* to be distinguished from non-*Helicobacter* organisms. We have found that using Diff-Quik Solution II instead of hematoxylin as a counterstain results in a more aesthetically pleasing stain which is preferred by many pathologists. Histological tissue detail is improved with no significant loss of contrast, which aids in finding the organisms in tissue sections. Non-*Helicobacter* organisms are also easier to identify with the

Diff-Quik counterstain. This is a fail-safe method to provide chromogenic back-up staining of *H pylori* in the rare event of immunoperoxidase technical failure. An added benefit may be the visualization of *Helicobacter* strains other than *H pylori* with the Diff-Quik counterstain, such as *Gastrospirillum hominis* (*Helicobacter heilmannii*)<sup>2</sup> which has unknown antibody reactivity.<sup>3</sup>

### Materials and Reagents

**Fixation:** Hollande's Fixative or 10% buffered formalin

**Technique:** Paraffin sections cut at 3-4 microns

### Reagents:

1. Tris Buffer with Tween 20 — DAKO Corporation
2. Proteinase K — DAKO Corporation
3. *H pylori* concentrated polyclonal rabbit antibody — DAKO Corporation
4. EnVision Plus rabbit detection system — DAKO Corporation
5. Diff-Quik Solution II — American Scientific Products

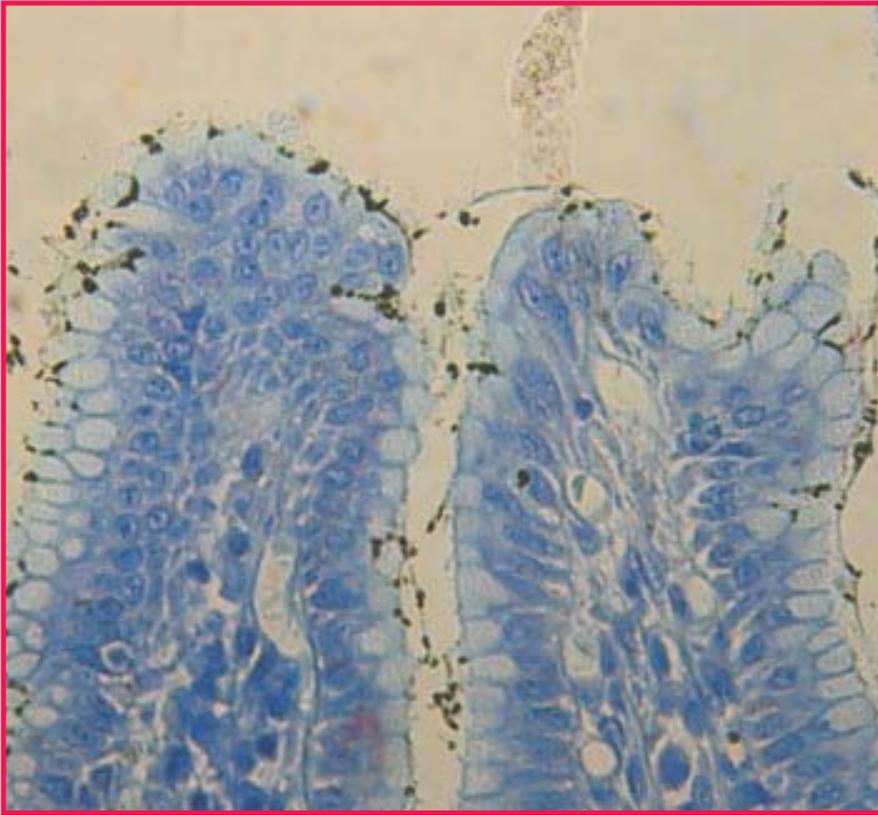
### Results

The *H pylori* antibody stains the organisms dark brown to black. The Diff-Quik counterstain gives a bright blue background demonstrating improved tissue detection over hematoxylin. Non-*Helicobacter* organisms are stained blue by the Diff-Quik solution.

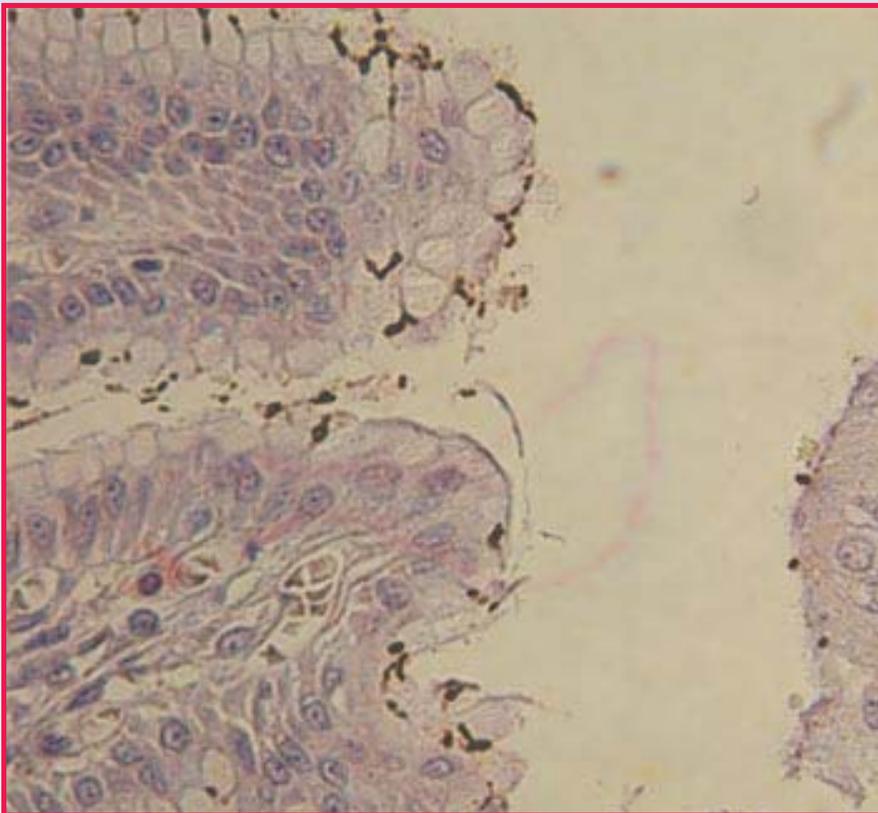
### Conclusion

Immunoperoxidase stain for *Helicobacter pylori* using DAKO polyclonal antibody and Diff-Quik counterstain offers the following advantages:

1. More rapid, lower magnification identification of *H pylori*.
2. Smaller numbers of *H pylori* are more easily detected.
3. Specific antibody staining of organisms is possible.
4. Fail-safe staining of bacteria



IHC-stained *H pylori* with Diff-Quick counterstain, oil immersion.



IHC-stained *H pylori* with hematoxylin counterstain, oil immersion.

in the rare event of immunoperoxidase technical failure.

5. Detection of bacteria other than *H pylori*, which may be clinically significant e.g., *Gastrospirillum hominis* (*Helicobacter heilmannii*) by the Diff-Quik counterstain.

#### References

1. Skipper WR, Destephano DB. A rapid stain for *Campylobacter pylori* in gastrointestinal tissue sections using Diff-Quik. *J Histotechnol.* 1989; 12:303-304.
2. Andersen LP, Boye K, Blom J, Holck S, Norgaard A, Elsborg L. Characterization of a culturable *Gastrospirillum hominis* (*Helicobacter heilmannii*) strain isolated from human gastric mucosa. *J Clin Microbiol.* 1999;37(4):1069-1076.
3. Personal communication, Karen Atwood. DAKO Corporation.

## Use of Commercially Clarified Methyl Green in the Methyl Green-Pyronin Stain

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 Department of Pathology  
 & Laboratory Medicine  
 Mayo Medical Center  
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#### Introduction

Methyl green-pyronin is used in histology labs to stain for DNA and RNA, which is especially beneficial for demonstrating plasma cells, mast cells, and immunoblasts. The purpose of this study was to observe the staining results of methyl green-pyronin solution [when made with methyl green 0.5% aqueous purified with chloroform from Poly Scientific (Bay Shore, NY)] on formalin-fixed paraffin tissue sections. Observations were made by comparing these results to the classical method touted by many texts which requires that the stain be purified using a 24-hour chloroform separation procedure to clarify the methyl green. Slides were also compared to the staining results of the same tissue stained in a ready-made methyl green-pyronin solution from a commercial source (Sigma Diagnostics).

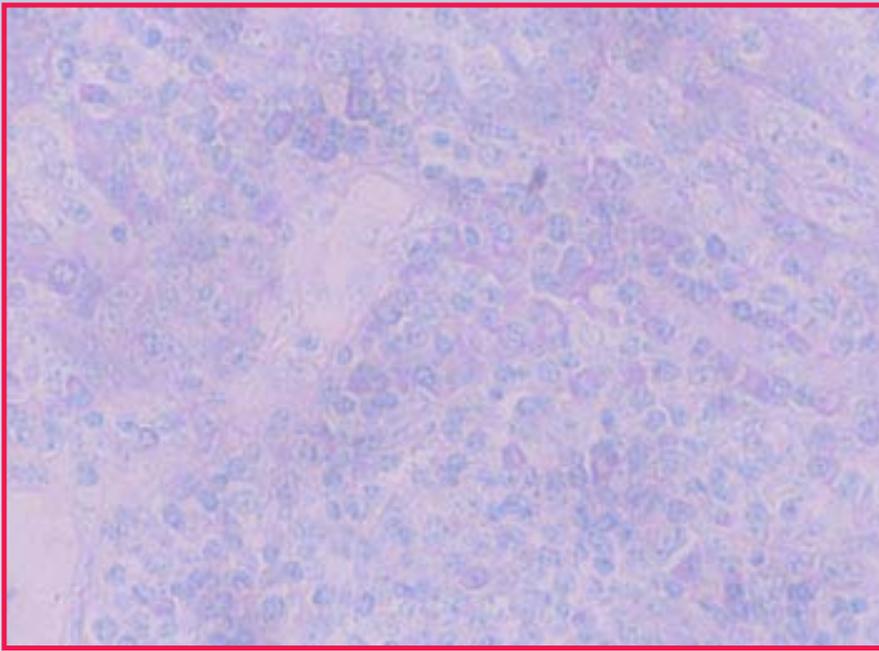


Fig. 1. Tonsil #1 stained with 0.10% methyl green-pyronin Y solution for 10 minutes.

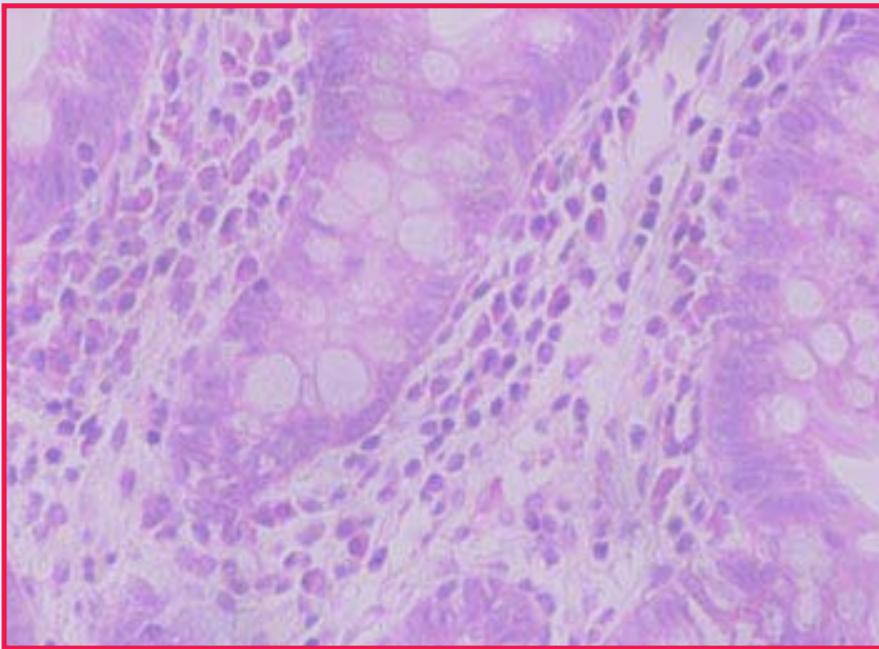


Fig. 2. Small intestine #3 stained with 0.10% methyl green-pyronin Y solution for 15 minutes.

### Procedure

#### Tissue:

- 3 paraffin blocks of small intestine cut at 5  $\mu\text{m}$ , 1 section per slide, 51 slides per block
- 2 paraffin blocks of tonsil cut at 4  $\mu\text{m}$ , 1 section per slide, 51 slides per block

#### Trials: 5 separate trials were conducted

- Each trial consisted of 10 sets of 5 slides, one slide of each of the tissues
- 5 sets were stained in 0.10% methyl green-pyronin Y solution; each set was stained for either 5, 10, 15, 20, or 25 minutes

- 5 sets were stained in 0.15% methyl green-pyronin Y solution; each set was stained for either 5, 10, 15, 20, or 25 minutes
- 1 set of slides was stained with ready-made methyl green-pyronin Y solution for comparison

### Reagents

#### 0.10% Methyl Green-Pyronin Y staining solution

140 ml 0.5% chloroform-clarified methyl green\*

0.14 g pyronin Y (CI 45005) (mixed thoroughly, filtered, and adjusted to pH 4.2 with 3% acetic acid or 3% sodium hydroxide)

#### 0.15% Methyl Green-Pyronin Y staining solution

140 ml 0.5% chloroform-clarified methyl green\*

0.21 g pyronin Y (CI 45005) (mixed thoroughly, filtered, and adjusted to pH 4.2 with 3% acetic acid or 3% sodium hydroxide)

*Ready-made Methyl Green-Pyronin staining solution†* (adjusted to pH 4.2 with 3% acetic acid or 3% sodium hydroxide)

### Method

1. Deparaffinize slides and hydrate to water.
2. Stain slides in filtered 0.10% and 0.15% methyl green-pyronin Y solutions at either 5, 10, 15, 20, or 25 minutes.
3. Briefly rinse the slides with distilled water – 3 dips.
4. Blot sections dry with filter paper.
5. Dip the slides in two changes of acetone – 15 dips.
6. Dip slides in acetone/xylene – 15 dips.
7. Dip in two changes of xylene – 15 dips.
8. Place in xylene for 5 minutes.
9. Mount with synthetic resin.

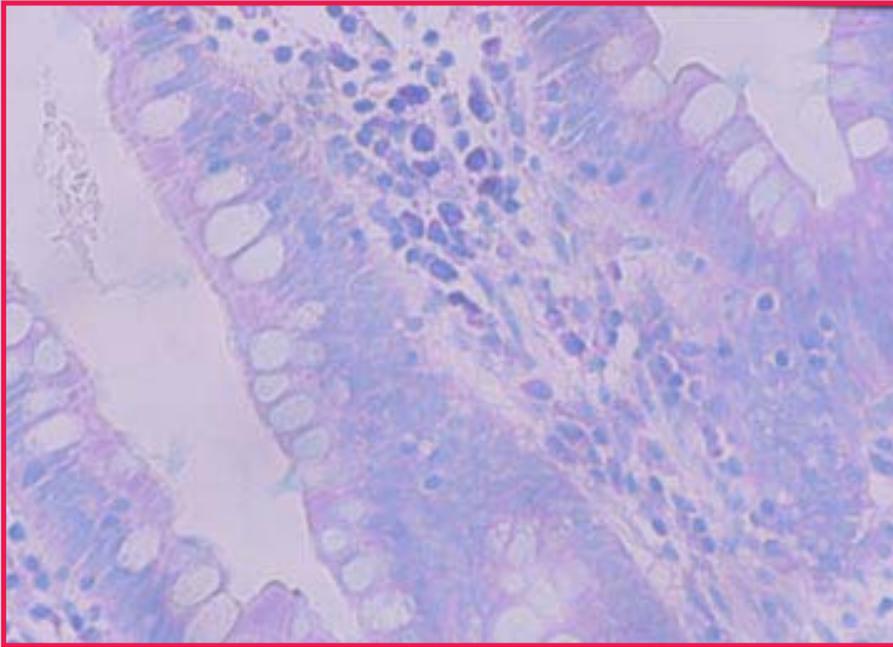


Fig. 3. Small intestine #3 stained with ready-made methyl green-pyronin solution.

**Results**

DNA .....Green to blue green  
 RNA .....Red  
 Goblet cells .....Mint green  
 Background .....Pale pink to colorless  
 Immunoblast and plasma cell cytoplasm ...Intense red  
 Nuclei .....Green to blue-green

**Discussion**

Staining solution preparation time was shortened from 24 hours to 20 minutes. Tonsil demonstrated the best results with the 0.10% pyronin Y solution at 10 and 15 minutes. Small intestine stained best with 0.10% at 15 minutes. Small intestine and tonsil had pale results at 5 minutes with both 0.10% and 0.15% pyronin Y concentrations. At 20 and 25 minutes, tissues demonstrated background staining, uneven staining, and poor contrast between nuclei and cytoplasm. Test slides demonstrated more nuclear and cytoplasmic contrast and clarity than slides stained with the ready-made methyl green-pyronin Y solution. Pyroninophilic tissue components were harder to distinguish from the nuclei and background in the ready-made

methyl green-pyronin stained slides. Slide results were consistent throughout the 5 trials.

**Conclusion**

Based on the results of this study, staining solutions made from the manufacturer-clarified methyl green provide an excellent alternative to both the chloroform separation procedure and the ready-made methyl green-pyronin Y solution.

\*Methyl green 0.5% aqueous purified with chloroform, Poly Scientific.  
 †Methyl green-pyronin, Sigma Diagnostics.

**Making a List...Checking it Twice**

**Wanda G. Smith, BHS, HT(ASCP)  
 Medical University  
 of South Carolina**

No, I'm not talking about Santa's Christmas list to see if we've been naughty or nice. I'm talking about all the checklists we make in our histology laboratories to ensure we have done ALL the jobs, large and

small, so that no job duty falls through the cracks. However, inevitably, there will be something we have forgotten, and then... WE WILL MAKE ANOTHER CHECKLIST!!!!

We start with our Histopathology Worksheet that the residents fill out at the grossing table. The resident lists the worksheet number, the date of grossing, and their last name, so we will know who to yell at (Oh...I mean talk to), when a block is missing. The accession numbers, patient names, specimen and block numbers, along with the number of pieces in each block and the CPT code for that specimen are included on the histopathology worksheet. Now, given that these are trained professionals you would think this would be something they could get right. Right??? Yeah right!!! We use this worksheet to document any discrepancies between the resident and the laboratory. We also document who embedded the blocks, as well as the number of blocks embedded, trying to catch any missing blocks at this time. We document which technologist entered data into our Cerner computer system, who cut the blocks, stained the slides, and who was responsible for labeling the slides corresponding to this worksheet.

Next, we have our Work Task list, which includes all the technologists with their corresponding duties. Duties include everything from cutting, staining, running the Ultrarush specimens to putting the extra cut slides in order for future staining. These duties rotate on a weekly basis. Each month a tech is also assigned a tissue processor to be responsible for the cleaning, changing, and rotating of solutions. We have a checklist for that too. We have a Decal Log Sheet, so we will know which resident to give decals to when they are finished. We have a Stainer Checklist to document changes and rotations on our automatic slide stainer as well as our H&E hand staining set-up.

We have an On Call list for the year, and an Orientation and Training checklist. We have a Competency Assessment checklist, which is used at 6 months for new employees and yearly for the long-timers. We have our EPMS checklist, which stands for Employee Performance Management System, also known as our evaluation forms. We have a checklist for when each Competency Assessment and EPMS is due.

When all of the slides are out for the day, we tally the total number of cases, blocks, and slides on the Block Count Worksheet. We also document mislabeled cases, blocks, and slides, and processor malfunctions (which we now seem to avoid since we got rid of one particularly "moody" processor named Herman and we now have all VIP tissue processors!). We document any other notes pertaining to what tissues should have been decaded. (I'm sure no one else has THIS problem!!!), and any other sectioning difficulties we have encountered.

You would think after all these checklists we would not forget ANYTHING... well, we have an Evening Checklist for the last person leaving for the day. This person does a walk-through to check off everything else including that the tissue processors are set correctly and running, no specimens are left out on the grossing tables, and the embedding centers and waterbaths are turned off. We even check to make sure the radio and cordless telephone are locked up in their cabinets.

Whew!!! I hope I have not forgotten anything. Oh yeah...

Dear Santa,  
I would really like one of those really fast motorized microtomes for Christmas. I've been really good this year....

## **NSH to Offer New Programs in the Coming Year**

**Vinnie Della Speranza  
Scientific Editor**

The National Society for Histotechnology has completed negotiations that will make two new educational programs available to practitioners of the discipline. These new initiatives will further enhance the society's efforts to reach practicing histotechs who have been unable to attend educational conferences due to cost.

In February, the College of American Pathologists Board of Governors voted to accept a partnership proposal proffered by the NSH to market a proficiency testing program to the histotechnology community in the United States. The first of its kind, this program will make it possible for pathology laboratories to benchmark the quality of their stains and tissue sections against those from other participating facilities by completing periodic exercises.

The NSH program was developed as a result of the efforts of Sue Lewis of the University of Iowa, and Bert Dotson of Duke University who conducted an NSH-funded pilot study in 1997. Twenty volunteer laboratories participated in the pilot which helped to refine the program to offer the greatest benefit to participants. In its final form, the program is expected to offer guidance for improvement as well as educational information; this will enable a laboratory's staff to enhance their understanding of a method's theoretical basis, a component often lacking in existing proficiency testing programs that currently target clinical testing laboratories. A steering committee composed of key representatives from each organization began meeting in late April to hammer out the final details for an anticipated 2003 rollout.

In another exciting venture, the NSH Board of Directors met on March 22 to select a vendor to host its new virtual library expected to be made available later this year. This program will allow subscribers to experience a variety of educational topics that will be accessible on the worldwide web from a link on the NSH homepage. This will make it possible for histotechs to obtain their continuing education without ever leaving their homes. In contrast to teleconferences which can only be experienced at the scheduled time, the virtual library can be experienced whenever it is convenient for the participant. Participants may complete a workshop in installments, coming back as many times as necessary to complete the full experience. The program will bookmark where you left off, provide an online exam as evidence of completion, and track a participant's continuing education units which may be printed out along with any handout material accompanying the workshop.

The NSH anticipates high demand for this product, especially from those unable to secure travel funds from their employer. Employers may find the program a useful adjunct to their in-house continuing education program for staff. Further details are expected to become available by mid-summer.

Be sure to frequent the NSH website at [www.nsh.org](http://www.nsh.org) for more information on these exciting programs.

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Vinnie Della Speranza, *HistoLogic*® Editor, 165 Ashley Avenue, Suite 309, Charleston, SC 29425. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.



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