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Histo-Logic

Technical Bulletin for Histotechnology • Managing Editor, Gilles Lefebvre • Scientific Editor, Terri Staples, HT (ASCP), BS, HTL
Vol. XXVI, No. 2, September 1996

Microwave Periodic Acid-Methenamine (Jones) Silver Staining of Renal Glomerular Basement Membranes in Paraffin- and Glycol Methacrylate-Embedded Tissues

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Introduction

In our laboratory it was found that Jones' periodic acid-methenamine silver method (PAM) was inconsistent in demonstrating lesions in renal glomerular basement membranes (BM). Snook's reticulum method appeared to be more reliable than the latter procedure. Jones' method proved to have several drawbacks which rendered it difficult to control, including prolonged mordanting with noxious agents, the use of a buffer to regulate pH, lengthy incubation in hot silver with concomitant breakdown of staining solution, and the necessity of microscopic evaluation of staining intensity throughout silver incubation. A successful attempt was made to investigate requirements for the above parameters and establish reliable, simple, rapid, routine histopathologic evaluation of glomerular lesions in renal biopsies from tissues embedded in paraffin and in glycol methacrylate.

Staining Procedure

Fixation: 10% neutral buffered formalin

Tissue Sections: 4.0-µm paraffin section or 3.5-µm GMA sections

Reagents:

5% Zinc Chloride

Zinc chloride ..... 2.5 g
Distilled water ..... 50.0 mL

0.5% Periodic Acid (freshly prepared)

Periodic acid ..... 0.25 g
Distilled water ..... 50.0 mL

Silver Solution Stock

A: 5% Silver Nitrate
Silver nitrate ..... 5.0 g
Distilled water ..... 100.0 mL

B: 3% Methenamine (Hexamethylenetetramine)
Methenamine ..... 3.0 g
Distilled water ..... 100.0 mL

C: 5% Sodium Borate (Borax)
Sodium borate ..... 5.0 g
Distilled water ..... 100.0 mL

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Impregnating Solution
Mix 5.0 mL of 5% silver nitrate into 100 mL of 3% methenamine. Refrigerate in an amber bottle for storage up to 1 month. Immediately before use, mix 25 mL of the above with 25 mL distilled water containing 2.0 mL of 5% sodium borate. Use acid-cleaned glassware throughout the procedure.

0.2% Gold Chloride
Gold chloride.....0.2 g
Distilled water.....100.0 mL

2% Sodium Thiosulfate
Sodium thiosulfate.....2.0 g
Distilled water.....100.0 mL

0.2% Light Green Solution
Light green SF yellowish.....0.2 g
Distilled water.....100.0 mL

All microwave steps are carried out in loosely covered plastic coplin jars.

Procedure
1. Decerate and hydrate paraffin sections. Immerse GMS slides in distilled water.
2. Mordant slides in 5% zinc chloride for 50 seconds at power level 8.

   This laboratory uses an Amana Radar Range RS50 microwave rated at 750 watts full power.
3. Wash in running water for 5 minutes. Rinse in distilled water.
4. Oxidize in freshly prepared 0.5% periodic acid for 20 minutes.
5. Wash in running water for 2 minutes. Rinse in distilled water.
6. Prepare impregnating silver solutions and microwave at power level 6 (450 watts) for 40 seconds.
7. Immerse slides — agitate each slide up and down in solution with wax-coated forceps.
8. Reheat at power level 6 for 40 seconds and agitate slides for an additional minute.
9. Wash in four changes of distilled water.
10. Tone in 0.2% gold chloride for 30 seconds.
11. Wash in four changes of distilled water.
12. Place in 2% sodium thiosulfate for 1 minute.
13. Wash in running water for 2 minutes.
14. Counterstain in 0.2% light green for 20 seconds.
15. Briefly wash in distilled water.
16. Dehydrate, clear, and mount.

Results
Basement membranes of renal glomeruli and reticular fibers.....black
Background.....light green

Discussion
Jones[1] introduced PAM in 1951 as a method for demonstrating BM in renal glomeruli. The original method is tedious, labor intensive, and requires considerable skill on the part of the technologist in determining the stain end point. The use of microwave bombardment has helped overcome many of the shortcomings of silver stains. Brinn[2] first utilized microwave acceleration of several silver stains. He found that not only did the microwave hasten the process, but it also helped yield cleaner, sharper silver deposition. Others have modified similar procedures for use with various plastics such as Epon or LR White embedding matrix.[3-5]

In this laboratory, renal biopsies are routinely embedded in paraffin. However, in the event more rapid analysis or more criterial cytologic detail is required, we have found that rapid GMA processing and microwave PAM staining may be considered as a viable alternative.

Since lengthy mordanting in Zenker's or Bouin's fluid had been recommended earlier to improve PAM staining of BM,[6] a successful attempt was made to introduce a rapid microwave mordant of 5% zinc chloride.[7] Our impression is that this mordant effectively assists in enhancing the argyrophilia of BM without recourse to primary fixation in a picric acid- or mercuric chloride-based fixative and eliminates the potential need for dezenkerization.

The periodic acid solution should be freshly prepared. Silver impregnation is achieved rapidly in two steps in the microwave oven and generally is optimal under the conditions outlined without recourse to constant microscopic monitoring. This method has proven successful with both formalin-fixed paraffin sections and GMA-processed renal tissue (Fig 1) with generally lessened background staining than conventionally stained counterparts.

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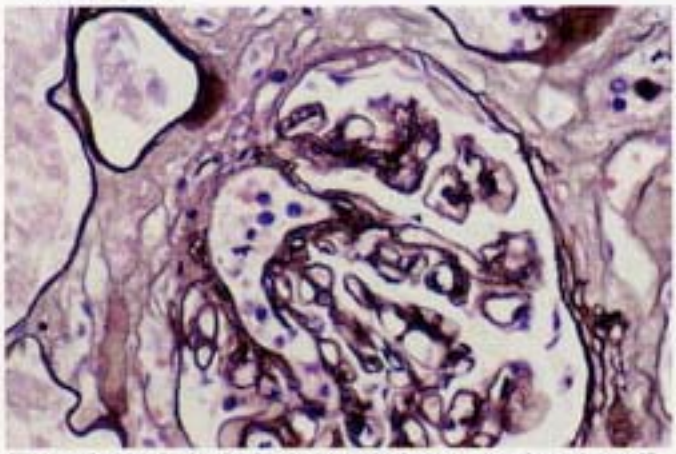

Fig 1. — PAM stain showing black glomerular basement membrane in paraffin-processed normal kidney tissue, 400×.

Announcing the Winner of the Oldest Tissue-Tek® VIP™ Tissue Processor Still in Use Contest

1981. It was a great year. The 52 Americans being held hostage in Iran were released; the first reusable spacecraft — the Space Shuttle Columbia — was sent into space and returned 2 days later; and Sandra Day O'Connor became the first woman appointed to the Supreme Court of the United States.

It was also the year in which a Tissue-Tek® VIP™ Tissue Processor, Model 4660, was installed in the Straub Clinic & Hospital, Inc. in Honolulu, HI. The winning Tissue-Tek VIP, Serial Number 4660-81001, was installed by Gary Toponce, according to the laboratory coordinator, Ms. Lynn M. Wong, who sent in the winning entry. Other entries included Tissue-Tek VIPs manufactured in 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989, 1990, and 1991.

The world's most reliable tissue processor, Tissue-Tek VIP, proves its world-record claim once again. Lynn, your limited edition VIP polo shirts are in the mail. Thanks to you — and to all of you — who took the time and made the effort to enter the contest.

Zinc Chloride: A New Additive fo Improved H&E Stains

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Pine Bluff, AR 71603

Introduction

Fixation of tissue has been, and still is, controversial issue. We have numerous fixatives ar combinations of fixatives on the market. It interesting that today we are still concerned wi fixing the cells as soon as possible and protecting t tissue from the harms of the processing cycle. selecting the correct fixative for our lab, we mu work with many fixatives and then select t procedure that works best for us. The majority of t laboratories in our country use 10% neutral buffer formalin. Why? We have used it forever, it is chea and it is easy to use. It does provide consistent ar adequate preservation. In addition, the pathologis can readily recognize the artifacts caused by formal fixation.

Zinc chloride, used in the new zinc formali fixatives, is an excellent mordant. The addition the zinc (a heavy metal) reduces denaturation ar attaches to the proteins. My pathologist suggest we try the zinc formalin for fixation. We used t same time frame for fixation that we were using f 10% formalin, which is 9 hours. This proved to b an unacceptable time for good fixation in the zi formalin. We did not have the well-fixed tissue w were accustomed to working with, and the zi formalin did not fix fat in the length of time w allowed. However, the pathologists did like t staining effect that we received by using the zi formalin. Our H&E stains, special stains, and i munoperoxidase stains showed bright colors, goo contrast, and good nuclear detail.

Taking into consideration the fact that the patho ogists liked the improvements in staining, a ne procedure was adopted for staining our slides usi zinc chloride as a mordant. We have a line automatic stainer. We added 0.2 g of zinc chloride the two absolute alcohol hydration steps on t stainer. The alcohol baths are changed daily ea morning as the stainer is prepared for use. W discovered that this gave us the excellent staini that was seen with the zinc fixative procedure (Figs and 2). The zinc chloride acts as a mordant to li the dye to the tissue by attaching itself to the protei allowing the hematoxylin or other stain to atta better.

Procedure

The following procedure is used on our linear stainer, which incorporates 28 stations at 1 minute for each station.

Station	Solution
1-3	Xylene
4-5	Absolute alcohol with 0.2-g zinc chloride
6	95% Alcohol
7-8	Running tap water
9-13	Hematoxylin
14	Running tap water
15	Acid alcohol
16	Running tap water
17	Bluing solution
18	Running tap water
19	95% Alcohol
20-22	Eosin
23	95% Alcohol
24-26	Absolute alcohol
27-28	Xylene
Results:	Nuclei — blue to blue-black Cytoplasm, muscle, and collagen — various shades of pink to red-orange

Discussion

Formaldehyde alters the ability of certain positively charged tissue elements to bind eosin. Zinc chloride increases tissue basophilia of cationic or positively charged dyes. Because binding of hematoxylin by negatively charged groups is unaffected, the tissue reaction is basophilic.

Zinc chloride used in the two absolute alcohol hydration steps in our hydrating procedure gives us the bright distinct nuclear detail in our H&E stain, good staining of immunoperoxidase stains, special stains, and DNA hybridization. Using the zinc chloride on our automatic stainer is less expensive than using it in the fixative, there is no damage done on our processor by the zinc chloride, and we meet our objective of improved staining.

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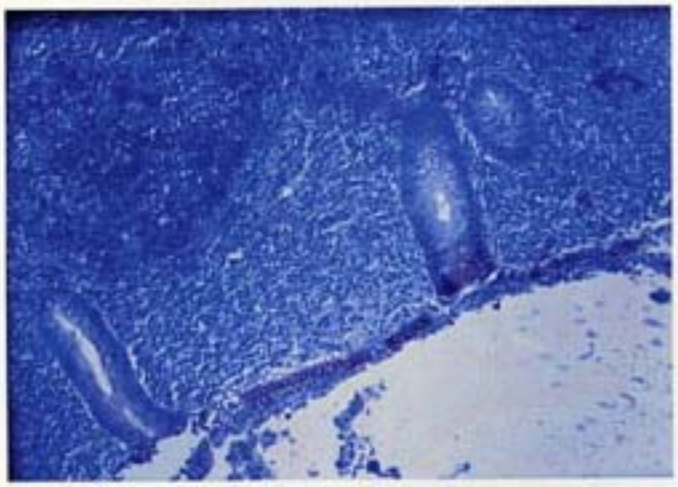

Fig 1. — H&E stain of intestine, 10×.

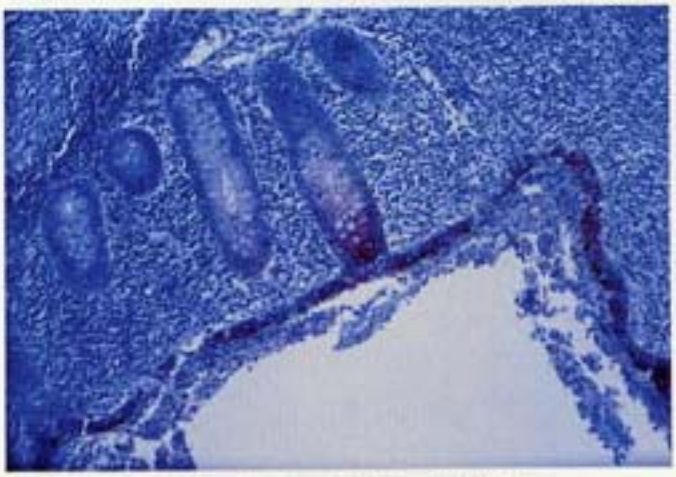

Fig 2. — H&E stain of intestine using zinc chloride-alcohol, 10×.

Rehydration of Biopsy Specimens to Facilitate Sectioning

Terri C. Staples, HT (ASCP), HTL
Baptist Health System
Birmingham, AL

A common problem in the Histology laboratory is the overprocessing of small biopsy specimens. The overprocessing occurs when these small tissue fragments are placed on the "routine" overnight schedule with other surgical and/or autopsy specimens. The extended exposure to alcohol, clearing agents, and hot paraffin causes the removal of bound water molecules within the biopsy tissue, resulting in tissue sections that are less than adequate for diagnosis.

The Histology Laboratory at Montclair Baptist Medical Center has adopted a modified processing schedule to improve the quality of the tissue sections obtained from small biopsy specimens.[1] However, there are occasions when a biopsy cassette does get processed overnight. When this occurs, they have been able to salvage the specimen by rehydration of the tissue in the paraffin block before sectioning.

Microscopic examination of biopsy specimens that are overprocessed demonstrates folds and wrinkles that may not be obvious on the waterbath (Figs 1 and 2). This may be due, in part, to the excess dehydration of the tissue, which causes the section to absorb water on the flotation bath, thus creating microscopic folds and wrinkles. Rehydration of the tissue during microtomy helps eliminate these folds and wrinkles by allowing the tissue to absorb water before it is placed on the bath. The resulting sections are of greater diagnostic quality (Figs 3 and 4). Although a modified processing schedule for biopsy specimens is preferred, it is possible to salvage overprocessed specimens with this technique.

Procedure

1. Carefully trim the block until the surface of the biopsy specimen is exposed.
2. Wet the exposed surface of the tissue with water from the tissue flotation bath using a small gauze sponge (alternatively, you may wet your thumb and place it on the surface of the block) for 10 to 15 seconds.
3. Obtain the next tissue ribbon and place it on the flotation bath.
4. Continue the process until the required number of sections has been obtained.

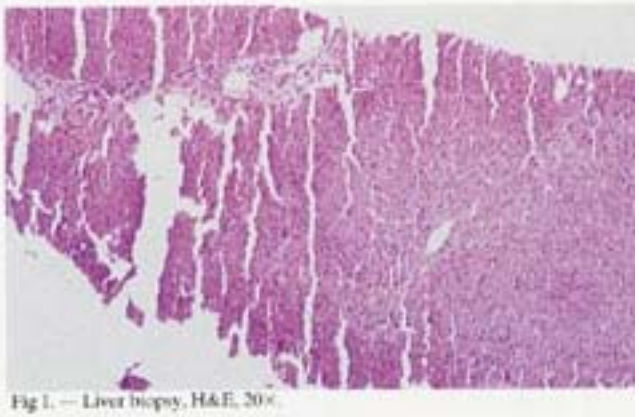

Fig 1. — Liver biopsy, H&E, 20×.

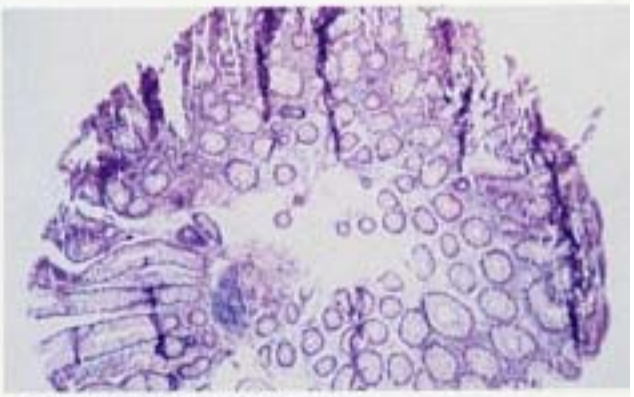

Fig 2. — Gastric biopsy, H&E, 20×.

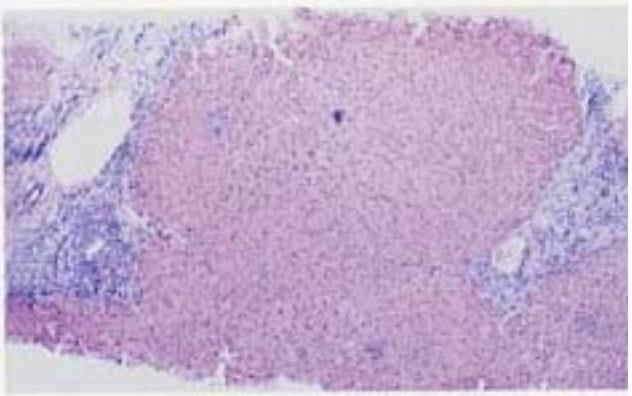

Fig 3. — Liver biopsy after rehydration, H&E, 20×.

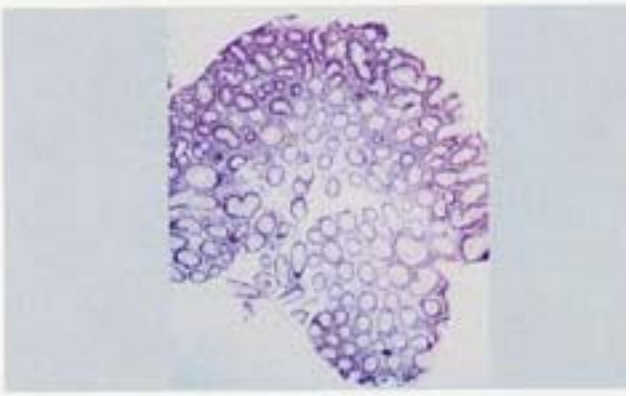

Fig 4. — Gastric biopsy after rehydration, H&E, 20×.

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Questions in Search of an Answer

Question 1. We have been doing Warthin Starry stains on *Helicobacter pylori* for approximately 3 to 4 years. We usually have had excellent results. About 1½ years ago, we noticed the edges of the tissue biopsies that we were staining were colorless. It was not consistent among the total number of slides in the coplin jar nor was it happening every day. We do *Helicobacter* stains depending on volume three times a week, usually Monday, Wednesday, and Friday. We QC the slides daily, and for weeks the stain will be fine and then will fail again.

In a coplin jar of five slides in the developer, two would develop normally and three would be colorless. Our control slide was always good. This rules out the staining solutions.

We processed the tissues on two different processors with two different processing times from regular overnight schedules to 10-minute schedules in each solution. I can't see where it is a processing problem.

We have explored every avenue, including the physician performing the procedure, medication taken by the patient prior to the procedure, fixation time, etc. I have asked Freida Carson, Lamar Jones, and many other reputable histologists. No one seems to have an answer, but there must be one. Our pathologists have been very patient up until recently. Could it be a sectioning problem? We deparaffinize and hydrate a rack of campy's together before we go into the 1% silver nitrate.

Also, silver precipitation sometimes poses a problem. We use plastic forceps when transferring the slides. I am open to suggestions on how to prevent this as well. Any and all suggestions will be greatly appreciated.

Terri DeCarli
Department of Pathology
North Arundel Hospital
Glen Burnie, MD

Question 2. Our laboratory has discovered that staining of gastrointestinal and endometrial biopsies can be greatly improved by controlling the temperature at which the tissue sections are dried prior to staining. At temperatures above 80°C, there is a tendency for the nuclei to take on a "ghost" appearance (Fig 1). Sections from the same tissue section dried at 60°C exhibit better nuclear detail (Fig 2). Can anyone explain the reason for this variation in staining?

Baptist Health System Laboratory
Birmingham, AL

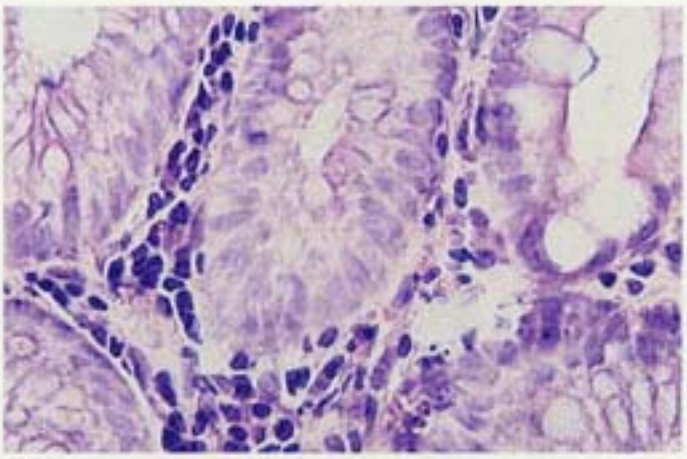

Fig 1.

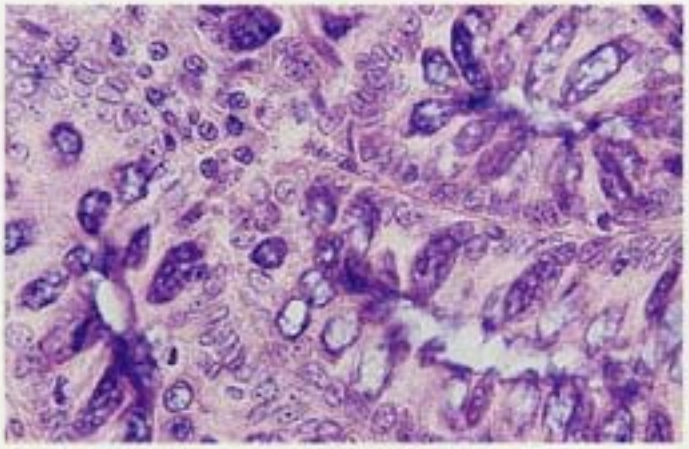

Fig 2.

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Letter to the Editor

This letter is in response to a recently publish
letter in this journal regarding uranyl nitrate. As t
Radiation Safety Officer at Markham Stouffvil
Hospital, I would like to clarify the information
that letter. Uranyl nitrate does not "give off a go
deal of radiation"!

Uranyl nitrate is a dry chemical that is derived fr
uranium, a natural *mining* product. It can be stor
in a regular storage container on your storage she
There is a detectable amount of radiation (25 cou
per second or 0.03 mR/h) at the surface of t
container. This amount of radiation is 2 to 3 tim
our background radiation (11 counts per second
0.01 mR/h), which surrounds us all the time. A
distance of 30 cm from the container, I was unable
detect radiation (11 counts per second or 0.01 mR
equal to background).

In addition, the 1% solution (diluted chemica
measured 21 counts per second or <0.025 mR/h
the surface, and at 30 cm I was unable to dete
radiation. On all other surfaces where uranyl nitra
is used, there was no radiation detected.

I hope this information helps you come to the sa
conclusion that we did. The storage and handling
uranyl nitrate is not a "real radiation hazard."

Sheryl Shears, NMT(N), RSO
Maureen Rose, WT, ART
Markham Stouffville Hospital
Markham, Ontario, Canada

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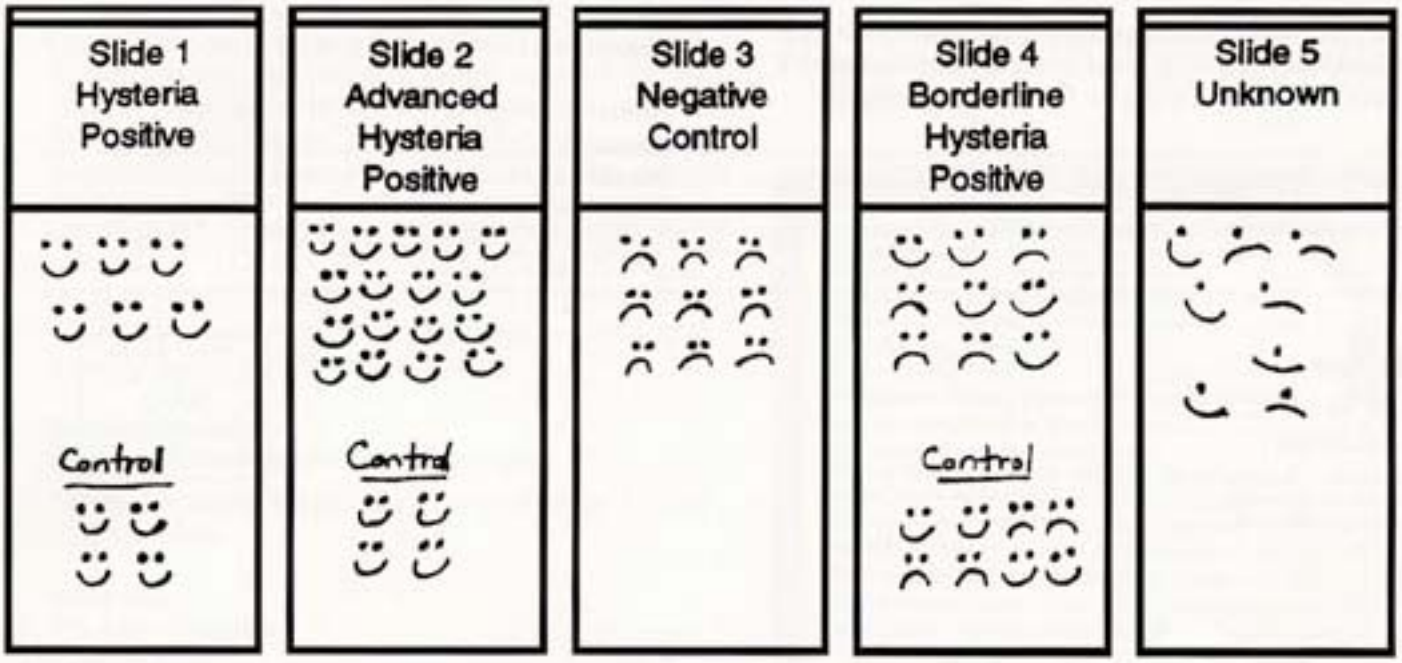
Histo-Humor



submitted by
Caroline Hass, HT (ASCP)
St. Jerome Hospital
Batavia, NY

Meet You at Twilight at the Twilight Cafe

NSH in Albuquerque means meeting old and new friends under the starlight at the Twilight Cafe.

Imagine seeing them now. Imagine the bright, clear evening skies — the brilliant moonlight and countless stars. Now you're beginning to hear the music and feel the rhythm of a song you'd almost forgotten.

And, yes, it's time again for you to plan your visit to the one and only **Twilight Cafe,** at the Hyatt Grand Ballroom, where only the most select of NSH society venture to meet.

That's right. This year's NSH Party will be in Albuquerque, NM, at the Hyatt Regency, on Sunday evening, October 20, 1996. Sakura is proud this year to have **Allegiance** (Baxter) as a cosponsor of this now-traditional party to end all parties.

You're welcome to get fancy this year. Tuxedos and black ties and party dresses are preferred but, of course, optional. *Ooh la la!* Festivities, complete with the great food and wonderful music you remember, begin at 8:30 PM and go till 11:30 PM.

Mark your social calendar now. We'd love to see you and look forward to a great evening together under the stars at the **Twilight Cafe** at the Hyatt in Albuquerque.

Don't Forget!

Location: Hyatt Regency Hotel, Grand Ballroom

Date: Sunday, October 20, 1996

Time: 8:30 PM to 11:30 PM

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