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# Phenol Myth Unveiled! Were Ziehl-Neelsen and Kinyoun Mistaken?

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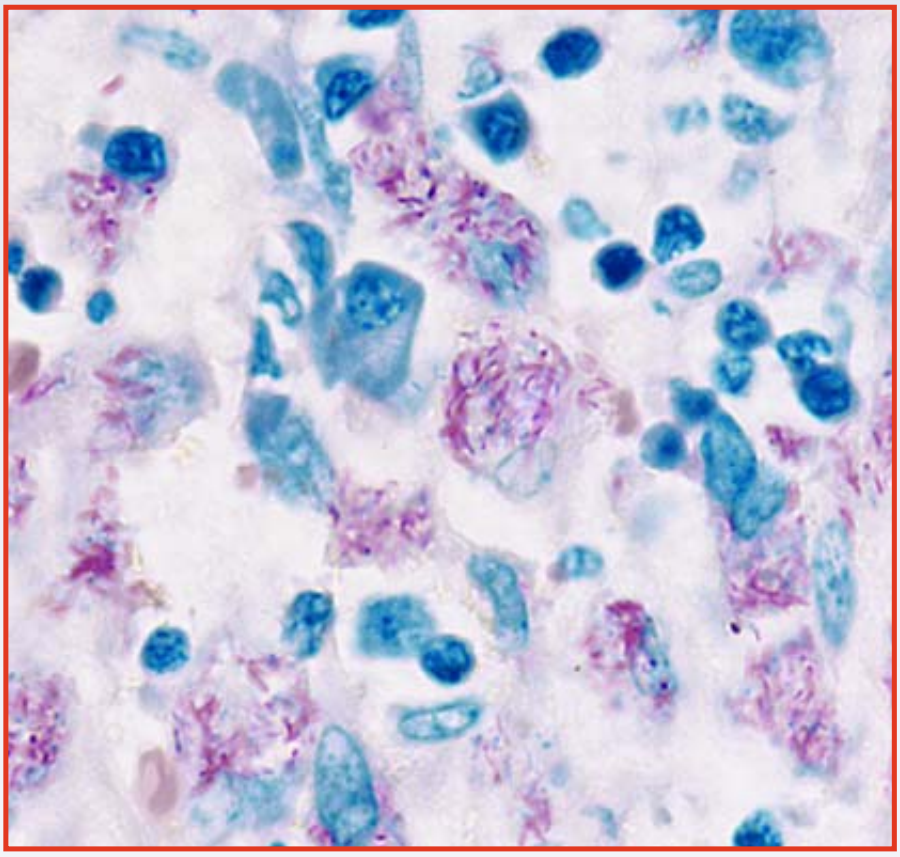

Fig. 1. *Mycobacterium avium*, aqueous basic fuchsin, oil immersion.

*The dust of exploded beliefs may make a fine sunset*

*Geoffrey Madan*

**Abstract**
Acid-fast staining of biopsy tissues continues to be an important adjunct to the diagnosis of tubercle disease. Classic light microscopic techniques utilize carbol fuchsin solutions which incorporate the hazardous solvent phenol. On a quest to identify a safer, yet reliable method for demonstrating this important human pathogen, we discovered that a dilute aqueous solution of basic fuchsin readily penetrates the mycobacterium cell wall of at least two species, dispelling the commonly held belief that a lipophilic agent (ie, phenol or detergent) is necessary for staining.

## Introduction

Tuberculosis remains a major global health problem. The World Health Organization estimates that 8 million new cases and 3 million deaths are directly attributable to the disease each year.[1] It has been suggested that between one-quarter and one-half of the world's population is infected.[2] In recent years, there has been a new sense of urgency to report positive acid-fast findings, prompted by the resurgence of tuberculosis outbreaks involving multidrug-resistant strains.

Laboratory methods to visualize mycobacterium in tissues remain in demand and are utilized almost daily in the clinical histology laboratory. Ehrlich's work with methylene blue in 1878 was of great importance, for he discovered that the addition of aniline oil to this dye allowed it to penetrate the tubercle organism,[3] the first apparent acid-fast method. Ziehl later used phenol in place of aniline oil to achieve staining. The still popular and perhaps best known Ziehl-Neelsen stain was introduced the following year (1879) using a dilute carbol fuchsin solution.[3] In 1915, Kinyoun

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recommended the use of a supersaturated carbol fuchsin solution at room temperature to stain mycobacteria,[4] a popular method that allows one to avoid heating the highly volatile phenol. These methods remain largely unchanged and in use today in laboratories around the world.

Anyone who has worked with phenol quickly becomes familiar with its strong, distinctive odor. Even at room temperature, its low vapor pressure, yet high vapor density allows phenol vapors to escape into and linger in room air. The high toxicity of this solvent makes its use in small, poorly vented laboratories problematic. Chronic poisoning following prolonged exposures to low concentrations of the vapor or mist results in digestive disturbances (vomiting, difficulty in swallowing, excessive salivation, diarrhea, loss of appetite), nervous disorders (headache, fainting, dizziness, mental disturbances), and skin eruptions. Chronic poisoning may terminate fatally in cases where there has been extensive damage to the kidneys or liver.[5]

Surprisingly, carbol fuchsin methods remain popular despite their known hazards and the availability of other methods utilizing alternative lipophilic agents. Perhaps this is due to the fact that the classic carbol fuchsin methods are touted in virtually every respected histology text, or that methods utilizing alternative lipophilic agents are simply not well known. Ellis and Zabrowarny[6] recommended the use of Liquid Organic Cleaner (LOC), a liquid detergent, to be used in place of phenol. Cserni[7] utilized this same agent in the Auramine fluorescent method.

Our investigation of alternative lipophilic agents began with an examination of two readily available surfactants, Tween 20 and Triton X, which we substituted for phenol in the Kinyoun's formulation. Both detergents allowed us to achieve acceptable staining results. Quite unexpectedly however, we discovered that excellent staining of mycobacterium species may be achieved with a simple, dilute (0.5%) aqueous solution of basic fuchsin, eliminating the need for any additional agents to penetrate the organism wall.

**Methods**

Initial investigations included the use of Tween 20 and Triton X, surfactants readily available from scientific supply houses, in place of phenol in the same proportions utilized in the Kinyoun's AFB method (4 g basic fuchsin, 20 mL of ethanol, 8 mL of surfactant, and 100 mL of water).[8]

Staining was carried out at room temperature, as well as in microwave and convection ovens, although we preferred to avoid heating alcohol for obvious safety reasons. In order to verify that the surfactant was indeed responsible for the positive staining results we observed, we utilized a dilute, (0.5%) solution of basic fuchsin in water. We expected to get no appreciable staining with aqueous basic fuchsin, due to the widely held belief that this organism's high cell wall content of hydrophobic lipids render it impervious to dye solutions that do not utilize a lipophilic agent. Staining with this dilute, aqueous basic fuchsin was carried out at room temperature and at 60° C using a convection oven, microwave oven, and water bath.

**Results**

Replacement of phenol with surfactant (Tween 20 or Triton X) yielded satisfactory staining of acid-fast organisms, both heated (15 minutes) and at room temperature (60 minutes), supporting the notion that a lipophilic agent facilitates entry of the dye into the organism's waxy cell wall. While these results succeeded in eliminating the hazards of phenol from the method, we had reservations about promoting a technique that requires heating ethanol, which poses its own hazards. Surprisingly, hot (60° C) solutions of 0.5% aqueous basic fuchsin yielded results comparable to that of the surfactant method, allowing one to achieve excellent staining in 15 minutes, yet this same dye solution at room temperature was ineffective at incubation times up to 1 hour. We could find no known hazards associated with the heating of an aqueous solution of basic fuchsin. It is important to note however, that this dye is considerably less soluble in water (3 mg/mL) than it is in alcohol (70 mg/mL).[9] As a result, the positively stained organisms appeared an intense fuchia not unlike that achieved with a PAS stain, as opposed to the dark purple-rose commonly seen with the classic Kinyoun method. As you can see from Figures 1 and 2, less dye in the cell wall does not hamper organism identification.

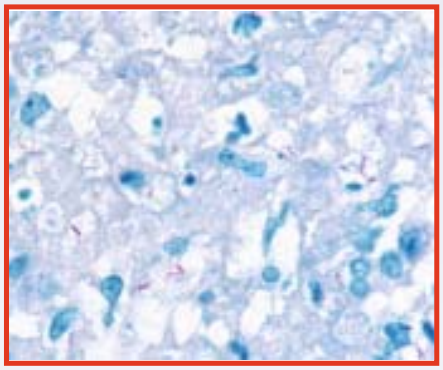

Fig. 2. *Mycobacterium tuberculosis*, aqueous basic fuchsin, oil immersion.

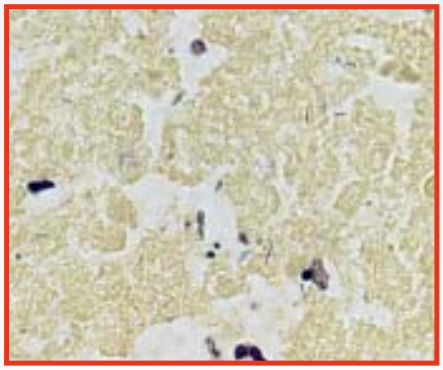

Fig. 3. *Mycobacterium tuberculosis*, Brown-Brenn stain, oil immersion.

**Method:** Aqueous Method for AFB

**Purpose:** To identify acid-fast bacteria in tissue sections

**Fixation:** 10% neutral buffered formalin

**Technique:** Paraffin sections cut at 3-5 microns

**Quality Control:** Tissue known to be positive for acid-fast bacteria

**Reagents:**

*0.5% Aqueous Basic Fuchsin*
Basic Fuchsin (CI#42510) ... 0.5 g
Deionized water ... 100.0 mL

*1% Acid Alcohol*
Ethanol, 70% ... 99.0 mL
Hydrochloric acid, concentrated ... 1.0 mL

*Methylene Blue Solution (Stock)*
Methylene blue ... 1.4 g
Ethanol, 95% ... 100.0 mL

*Methylene Blue Solution (Working)*
Methylene blue solution (stock) ... 5.0 mL
Deionized water ... 45.0 mL

**Staining Procedure:**

1. Filter 0.5% aqueous basic fuchsin into glass coplin jar and place in 65° C water bath (see remarks).
2. Deparaffinize and hydrate to distilled water.
3. Place slides in 60ºC basic fuchsin for 15 minutes.
4. Rinse thoroughly in running water.
5. Differentiate in 1% acid alcohol until no more color runs off and tissue is pale pink.
6. Rinse in 3 changes of water.
7. Counterstain in working methylene blue for 20 seconds.
8. Dehydrate through graded alcohols, clear in xylene and mount in permount.

**Results:**

Acid-fast bacteria ... bright pink to red
Background ... light blue

**Remarks:**

We recommend filtering the solution before use to avoid the possibility of precipitate on sections. Keep the coplin jar covered during staining to maintain solution temperature. In our hands, we found that a water bath temperature of 65° C reliably maintained the solution temperature at 60° C.

**Discussion**

The resurgence of mycobacterium infections in recent years has caused considerable morbidity and mortality worldwide. Especially alarming are the multidrug-resistant (MDR) strains that have arisen in patient populations who fail to complete their course of therapy. *Mycobacterium tuberculosis* (TB) infections in the U.S. have a higher prevalence in medically underserved ethnic minorities, homeless persons, prison inmates, alcoholics, and injecting drug users, as well as foreign-born persons from areas of high prevalence.[1] The organism is carried in particles known as droplet nuclei that are generated when patients with pulmonary tuberculosis cough. These particles, 1-5 microns in size, remain suspended in air by normal air currents, increasing the risk of transmission.

In addition to TB, *Mycobacterium avium* complex (MAC) poses particular difficulties for those who are immunosuppressed. MAC organisms are ubiquitous in nature and have been isolated from water, soil, plants, house dust, and a myriad of environmental surfaces. While the organisms are normally of low pathogenicity and may colonize without causing disease, they are an important cause of pulmonary and gastrointestinal disorders in patients with HIV infection or those who are therapeutically immune suppressed, and may lead to disseminated infection.[1]

Traditionally, mycobacteria have been considered to be surrounded by a thick, waxy coat. In fact, lipids account for as much as 60% of the dry weight of the cell wall, including the characteristic mycolic acids- long chain fatty acids containing up to 90 carbon atoms in length. The hydrocarbon chains of the mycolic acids are intercalated with those of numerous wall-associated lipids and glycolipids.[10] While the high lipid content is believed to render the mycobacterial cell wall hydrophobic, the mycolic acid residues are thought to impart the acid-fast characteristic by retaining the dye even in the presence of acid.[1]

Although a number of basic dyes may be used to stain acid-fast organisms, basic fuchsin is most frequently used in acid-fast stains since it is an extremely brilliant dye.[4] Berg used crystal violet, and Lillie suggested the use of night blue or victoria blue. The intense pink-violet offered by basic fuchsin remains the most popular method, however.

Our staining results using dilute, aqueous basic fuchsin call into question the belief that a lipophilic agent is essential to demonstrate acid-fast organisms. These results are not inconsistent with the observation that TB is gram-positive when stained with aqueous crystal violet in the Brown-Brenn stain.[8] Although this dye is chemically very similar to basic fuchsin, it requires no heat to bind, as did the basic fuchsin in this investigation.

Recent work by Daffee and Etienne[2] indicates that a bacterial capsule (as opposed to the cell wall) surrounds the organism *in vivo.* This capsule actually contains very little lipid (2%-5%) and is composed rather of polysaccharide and protein. The ratio of protein to polysaccharide varies, however, according to species. Perhaps the basic fuchsin in our method is in fact binding to the capsule instead of the cell wall, but it is doubtful that this reaction would require heat, nor is it likely to impart acid-fast qualities, since proteins are amphoteric. That is, at a pH below their isoelectric point, proteins exhibit a positive charge and would not bind the basic, positively charged basic fuchsin dye.

It is worth considering that Ziehl and his contemporaries did not have the precise methods of temperature control now available. They were forced to rely upon direct flame heating or steaming from boiling water in order to heat their solutions. This may help to explain their interest in aniline oil and phenol to stain acid-fast organisms. We may also find ourselves working with purer lots of dye. Is it possible that they had not investigated the use of a simple, dilute aqueous dye solution?

Further work is needed to determine if the heat simply makes it possible for the aqueous dye to penetrate the hydrophobic structures only to become trapped upon cooling (ie, physical staining), or if the heat allows the dye to gain access to chemical moieties where true chemical binding occurs.

The value of this simplified staining procedure is apparent. It is simple, rapid, and reproducible without need of hazardous reagents. It also provides a cost savings to the laboratory through the elimination of alcohol, phenol, or surfactant required with other methods.

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# It's a Jungle Out There! How to Avoid Hiring the Wrong Person


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With a national vacancy rate approaching twenty percent, histology supervisors are finding it more difficult than ever to find qualified individuals to staff the laboratory. Insufficient numbers of training programs, low professional esteem, and low pay, ever-increasing pressure to produce more work with less staff, and forced overtime, all contribute to this high attrition rate and overall gloomy picture. If your situation is anything like mine, we simply cannot afford to make bad hiring choices. Unfortunately, current market circumstances make this ever more likely because we find ourselves with fewer applicants to select from.

*Past behavior predicts future performance!*

If you've ever had to discipline, or worse yet, terminate a bad hiring choice, you understand how difficult and painful it can be to correct a problem situation. If your institution drags its feet on termination, you risk losing good employees who are frustrated because of the appearance that a situation with a problem employee will continue indefinitely. Somehow a bad situation manages to take its toll on everyone and seems to get worse before it gets better.

Okay, I know what you are thinking. You don't need anyone to tell you how and who to hire. After all, you trust your gut instinct and you are a good judge of character. Well, I thought so too until about six years ago when I hired an individual who left me wondering how I could have exercised such poor judgement.

Yes, I had to blame myself, but in retrospect, do you think it would be that difficult for someone to be on their best behavior and mislead you during an interview? I recently did a search at Amazon.com for books on interviewing. Those advising the applicant outnumber those aiding managers by three to one! I came across one title that really made my blood boil: " How to Get the Job You've Always Wanted Using Acting Skills You Never Knew You Had!" ACTING SKILLS?! Well now, doesn't that just leave you with a warm, fuzzy feeling? Whatever happened to plain, old honesty and being yourself? Believe me, we managers need all of the help we can get because it IS a jungle out there! We simply cannot afford to rely upon gut instinct to make our hiring selections.

I've made it a point to do quite a bit of research on the topic of hiring and interviewing and I am happy to share with you what I have learned. I've had individuals I've hired tell me that my interview with them was the most challenging they had ever had. GOOD! This means I'm on the right track. I'll show them ACTING SKILLS!

Let's get back to those wonderful instincts of yours. Think you are a good judge of character? Is that really what you are judging? Or were you influenced by the applicant's appearance, dress, smile, or ability to speak in full sentences? Studies have shown that we make an impression on others within the first sixty seconds of meeting them. You've probably heard the expression " we don't get a second chance to make a good first impression," which means that our own biases may sway us in favor of the wrong candidate or away from the right one, in the first SIXTY seconds.

So let's be honest. The job interview is not a fashion show or a beauty contest. Do you need a smooth talker with a nice wardrobe and a nice smile on your staff? Not likely unless you are hiring for a public

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relations position or a television anchor spot. The fact is, our biases can lead us to make incorrect choices. So if you can open your mind and put aside stereotypes and prejudices, you just may find yourself a real gem.

*Our biases lead us to make judgements about others within 60 seconds of first meeting them.*

Obviously you are interviewing because you've had turnover. Maybe your former employee left under his own steam or maybe you had to provide the incentive for his departure. The work is piling up, the staff is looking tired, and they are getting on each other's last nerve. You are under great pressure to get the vacancy filled.

Have you thought about what got you into this situation? Is it possible that when you hired that last individual, you failed to get enough information during the interview? Did you successfully match the candidate with the job? Maybe you didn't really take time to properly evaluate that individual's resume, or you settled for rehearsed, superficial answers to your questions. Do you use questions that the applicant anticipated and prepared for, like those which have been used since the beginning of time . . . *"what are your greatest strengths and your greatest weaknesses?"* Do you really think that I would tell you my greatest weaknesses?

The most important point that I can leave you with is that ***Past Behavior Predicts Future Performance!*** Don't we all want to know how the applicant really will behave and perform? The key is to be able to learn how the applicant behaved in the past. This is your single best predictor of what you might expect from this individual. In fact, isn't this really what we are hoping to learn with a reference check? Unfortunately in today's litigious society, many facilities are reluctant to answer probing questions during a reference check. This leaves us wondering what the applicant is really like in the workplace.

Now, can you get the information you really need from the applicant, or will you simply be stuck with what he or she wants you to know?

In an anonymous survey of 1000 job applicants, 34% admitted that they lied, embellished, or exaggerated on their resumes. Surprised? Well you shouldn't be! Pick up any number of references on how to write a resume and you'll be encouraged to do just that. If 34% admitted that they did this, how many did so but didn't admit it? You can quickly see that the deck is stacked against us. Don't believe everything you read! Several years ago I had an individual put down that she developed a new system for icing paraffin blocks. I don't have to tell you that this piqued my interest. When I explored this with her further, I learned that she used a rubber band to hold an ice cube against the block face on the microtome chuck. Creative perhaps, but hardly the "development of a system."

If you are an individual who is too busy to look thoroughly at a resume until five minutes before the applicant arrives, you are setting yourself up for failure. Every resume under your consideration should be put under a microscope. Have the candidate's previous jobs been of short duration? Has the candidate changed jobs frequently? Has the applicant offered previous direct line supervisors as references, or only coworkers and pathologists (major red flag!)? Frankly I want you to disbelieve everything you read on a resume until you satisfy yourself during the interview that there is merit in how the applicant represented himself/herself. Make yourself a working copy of the resume or employment application and using a red pen, note everyplace there is information that you will want to explore in further depth with the applicant at interview.

I believe that the style of questioning can be key to getting the information you need about the applicant. Authors offer widely divergent opinions of the types of questions one should use, but I can boil this down for you to two very practical recommendations. First, avoid if at all possible, hypothetical questions. Interviewers like to use situational problem solving questions to try to better understand how an applicant might behave in a given situation. An example might be *"What would you do if you discovered a coworker using the department telephone to make personal calls?"* In my opinion, the use of hypothetical questions is a flawed approach. This gives the applicant the license to sit back and theorize about what answer will impress you. This gets no closer to anticipating what their true behavior in the described situation might be. I remember reading somewhere that *imagination* is centered in a different locus in the brain than *memory*. When you ask an applicant to remember something, they begin searching their memory, making it much less likely that they will come up with an imagined answer to your question. Therefore, in the above scenario, you might ask instead *"Tell me about a time when you discovered an employee violating hospital policy and how did you handle it?"* If the candidate tells you that they never experienced an employee who violated policy, I think that you may have an integrity issue on your hands.

*In an anonymous survey of 1000 job applicants, 34% admitted that they lied, embellished, or exaggerated on their resumes.*

A second type of questioning that I urge you to use as much as possible is what is called "domino" questioning. It is no secret that many applicants rehearse for an interview by trying to anticipate the subject matter that you are likely to ask them about. Every available reference out there offers them the best answers to otherwise difficult questions. For example, if I was your applicant and you asked me to tell you about my shortcomings, I would tell you that *"I tend to be a perfectionist which can frustrate those I work with. This is an aspect of myself that I am working to improve."*

Now I ask you, how many of you would disqualify someone from consideration because they are a perfectionist?! I think you get my point.

The purpose of domino questioning is to have two or three follow-up questions on a subject in order to force the candidate to go further in depth in their answer. In essence, you go well beyond the rehearsed answer and may be better equipped to discern if the applicant was sincere and honest or simply attempting to tell you what you wanted to hear. Let's look at an example:

*"Tell me about a time when you became very angry in the workplace."* This question allows you to explore the emotional control of the candidate. The applicant may be tempted to share with you an incident where they felt that they had been mistreated or wronged in some way, allowing you to decide if there was any merit to their reaction to the circumstances. Follow-up (domino) questions might then be: *"Why did you get this angry?"; "What did you do to resolve your anger?"; "Tell me of a time when another's anger was misdirected at you";* and *"How did you resolve it?"*

Many interviewers fail to have a strategy for the interview. Surely you need to know more than if the applicant can cut sections. Is the applicant a team player? Will this individual be loyal to you or bypass you on the chain of command in order to get noticed by those above you? Will the individual mesh with your existing team or cause your team to become unhappy and hence, less productive? Will this individual bring stability or will he or she stay for only a short time? Is the applicant high strung or cool under fire? Will this person be dependable in a crisis? Is the candidate rigid and inflexible or adaptable to change? Our needs in a busy laboratory go well beyond whether the individual has the skills and experience to do the job!

Okay, maybe you've decided that you've found the person you want. Do you know that this individual will be happy with you, your work environment, and your expectations? What do you know about the applicant's value system and needs? After all, turnover was how you got yourself here, wasn't it? I find that many interviewers fail to explore the applicant's needs and whether the applicant will be able to give you his or her best based upon your management style. If you haven't made this a focus of past interviews you would do well to reconsider your strategy. Success in previous positions is not necessarily a guarantee that this individual will succeed with you.

Occasionally I break my own rule and use the following hypothetical question. *"How would you spend $2500 if the hospital gave it to you to spend as you wish?"* You are thinking, "How ridiculous!" It occurs to me though, that if the applicant responds by saying that he'd take his family on vacation, you may be speaking to an individual who values his personal time a great deal and therefore might not be very happy with the mandatory overtime that you know is a reality in your lab. If the candidate replies *"I'd pay off my credit card balance"* or *"I'd put it in the bank,"* you are probably talking to someone who is not into immediate gratification, but rather someone who may willingly work hard to achieve their goals. They may in turn work very hard for you in order to achieve those personal goals. Of course, there is no "right" answer to this question and it is up to you to decide if the reply is beneficial to you.

Have you ever worked with or known someone who believed that if they could achieve something, it must not have been very difficult or valuable? There is a personality type out there who thinks in just this way. Such a person continually strives for something else or more, and is unlikely to be with you long. So if you are looking for a stable person to replace the one who retired after twenty years of loyal service, this candidate is not likely to be the one. If you have a new graduate sitting opposite you whose dream is to be a physician, do you really want to invest a year to train this person to work in your lab? I don't. I find that some candidates think they will impress me by telling me that medical school is a professional goal. Instead I hear in my mind, "more turnover!"

Shortly after accepting my current position but prior to my arrival here, it became necessary for me to interview an individual by telephone. She had applied for a vacancy in the immunohistochemistry lab and appeared to have strong experience. When I first reviewed her resume, my gut told me that she changed jobs too often (spanning several states), so I probed this during my conversation with her on the phone. She became defensive and offered the explanation that two of the facilities were bought by a major hospital corporation and that she only wants to work for a quality lab. An impressive answer but a REHEARSED one! While she tried to impress me, what I really heard from her was, *"When things don't go the way I like, I leave."* I also noticed that she kept emphasizing how she pleased the pathologists at each of the facilities where she worked. I told her that I would not accept references from pathologists and would require them from her immediate line supervisors. I learned later that this apparently upset her and she was never heard from again. I have nothing against my pathologists being happy, but have you ever worked with someone who kissed up to the doctors, yet made their supervisor and coworkers miserable because they weren't a team player?

I'm going to close this discussion with examples of some useful questions I have used. You don't have to like or use them. But perhaps they will serve as food for thought.

*"What does your supervisor do to get your best work from you?"* Sometimes the candidate will trash their boss. Do you want them doing that to you? Besides, you shouldn't have to do anything to get their best work.

*"Give me an example of a crisis situation you were involved in."* Does the applicant describe circumstances that are an everyday occurrence in your lab?

*"Tell me about a time when you had to go above and beyond the call of duty at work."* I once had an applicant for a supervisory role who answered, "I called in a service technician for an instrument that was down." Isn't this task just a routine part of a supervisor's job?

*"Tell me about an accomplishment that you are truly proud of"* or *"What makes an individual successful?"* Such questions speak to an individual's attitude and may provide a glimpse of how hard they are willing to work in order to achieve their goals.

*"Tell me about the most difficult job-related task you have faced."* The applicant's definition of difficult may very well describe what you view as a routine, everyday occurrence.

Let's talk briefly about silence. Yes, silence can be golden. Less experienced supervisors find themselves doing most of the talking during the interview and as a result, learning very little about the applicant. We tend to be very uncomfortable with silence. Place two people in a room with absolutely nothing in common and I guarantee you one of them will feel the need to break the silence. So why not use this as a tool during the interview? It has been my experience that the less I speak during the interview, the more the applicant will. For example, if you've asked a question and the applicant provides an answer, be slow to speak in acknowledgment. You can nod or smile or shake your head to acknowledge that you've heard the applicant's reply, but don't speak. You will find that the applicant will continue to speak in order to fill the void. The more the candidate speaks, the more information you receive and the more you will learn. I use this technique quite frequently and you would be amazed at how much additional information I have learned. If you don't believe me, try it.

Perhaps you will consider sharing your interviewing or hiring experiences (or nightmares!) with our readers. We managers can learn through each other's experiences and mistakes. After all, it is a jungle out there! Feel free to forward your correspondence to me at ***Histo-Logic***.

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## Histology In the Movies

Just before press time, an undercover source notified ***Histo-Logic*** that a Sakura VIP and Coverslipper appeared to be blown up in the new Columbia Pictures release, "Hollow Man." It seems that Sakura-Finetek was asked to provide laboratory instrumentation for the film.

Not to worry though. It turns out that Hollywood used the original instruments provided by Sakura to create replicas that became props in the explosion. It would be such a waste to blow up a perfectly good VIP!

# An Anatomic Perspective of Selected Colonic Stains


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**Cobleskill, NY**


The normal adult large intestine, or colon, has multiple functions including water and electrolyte absorption, mucous synthesis and secretion, and the propulsion of the luminal contents for excretion. As in all parts of the gastrointestinal tract, the colon is organized into four functional layers: the mucosa, the submucosa, the external muscle layers (muscularis externa), and the serosa. Of these layers, it is the mucosa that is the target of most special stains performed in the histology laboratory, and this mucosa is the focus of this paper.

The colonic mucosa consists of three components: the epithelium, the lamina propria, and the muscularis mucosa. A simple columnar epithelium lines the entire length of the colon. Typically the surface is flat with straight tubular glands, called crypts, interspersed at regular intervals (Fig. 1). The surface can, however, be highly irregular even in normal tissues. Immediately beneath the epithelium is the lamina propria, a loose connective tissue which is variably cellular depending on the degree of infiltration. Solitary lymphoid nodules, or lymphatic follicles, are common. Each lymphoid nodule tends to be organized in a circular pattern with a darker-staining peripheral or cortical region, and a less basophilic central region called the germinal center. The decreased staining intensity of the germinal center reflects the presence of proliferating lymphoblasts (derived from activated B cells), which are larger cells with increased cytoplasm and more space between adjacent cells.

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Multiple groups of lymphoid follicles may coalesce to form a larger collection of lymphoid tissue called a Peyer's patch. In some areas the Peyer's patches can displace the glands and distort the overlying epithelia and/or the deeper submucosa and external muscle layers. Since most of the cells within the Peyer's patches are small lymphocytes which have a large nucleus and minimal cytoplasm, the Peyer's patches appear dark blue in a routine hematoxylin and eosin stain.
The third component of the mucosa, the muscularis mucosa, is a layer of smooth muscle which separates the mucosa from the deeper submucosa. The latter has abundant collagen and is the pathway for large blood vessels and nerves. A good trichrome will clearly define the bright red smooth muscle of the muscularis mucosa from the blue (Mallory's trichrome), or green (Gomori's One Step) collagen of the submucosa.
It may be noted in a trichrome stain that there is also a distinct layer of collagen immediately beneath the basement membrane of the surface epithelium. In certain disease states such as collagenous colitis, this layer may be considerably thickened.

The surface epithelial cells are tall columnar absorptive cells that are involved in fluid and electrolyte uptake and transport, in a mucosal to serosal direction. One of the primary electrolytes is sodium, which is actively transported; water follows passively. The sodium pump, Na/K ATPase, is located on the basolateral membrane of these cells and the latter is highly convoluted to maximize surface area. This helps to explain the eosinophilic property of these surface cells. Within the crypts, the goblet cells predominate.
As their name implies, goblet cells are shaped like a wine goblet. A large columnar nucleus and abundant cisternae of rough endoplasmic reticulum fill the narrow base of the goblet. These structures are intensely basophilic. In contrast, the expanded apex of the cell is filled with large secretory granules filled with mucins. These goblet cells continually synthesize and secrete mucins at low levels, so-called baseline secretion. When this occurs, individual secretory granules fuse with the apical membrane and release their product into the lumen.[1] Upon stimulation, however, there is massive secretion and loss of the apical granule mass as the individual granules fuse with one another and with the apical plasma membrane.[2,3] The stimulus for this compound exocytosis may be either physiologic, (eg, acetylcholine release from enteric nerves), or artifactual, (eg, manual manipulation or forceps). Assuming there has not been excessive loss of the mucins, it is this product that is stained by many special stains such as Alcian blue, PAS (Fig. 2), mucicarmine (Fig. 3), and High Iron Diamine (HID) (Fig. 4). The specific staining of the mucins in these special stains contrasts markedly with the " empty appearing" goblet cells seen with routine hematoxylin and eosin staining (Fig. 1).

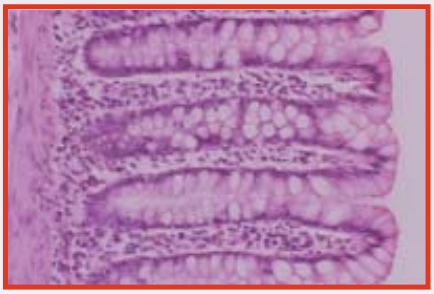

Fig. 1. In this hematoxylin & eosin stained section of adult distal colon, the goblet cells lining the crypts are " empty appearing."

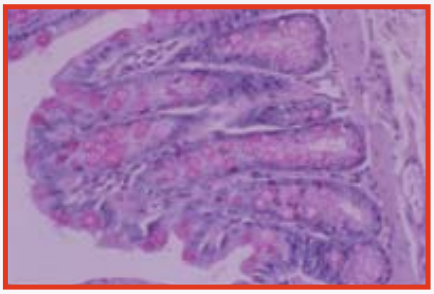

Fig. 2. After PAS staining, the mucins in the colonic goblet cells are variable shades of magenta.

To understand the staining properties of the goblet cells, it is necessary to have a basic knowledge of the chemistry of their secretory product, the mucins. Mucins are high molecular weight glycoproteins

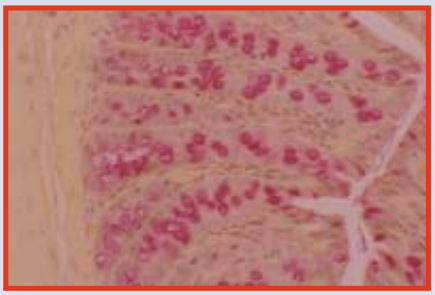

Fig. 3. The goblet cells in these colonic crypts stain a rose-pink after staining with mucicarmine. The tartrazine counterstain contrasts and delineates these cells clearly.

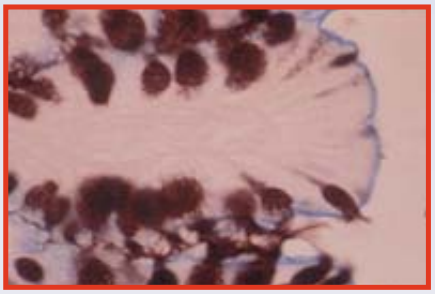

Fig. 4. This is a higher magnification of the surface and upper crypt cells in the adult colon. The goblet cells are intensely HID positive and they appear brown/black.

synthesized within the rough endoplasmic reticulum, and glycosylated (sugars added) in the Golgi. The sugars (carbohydrates) are sequentially added to the protein core, ultimately forming a series of side chains of variable length and complexity. Each side chain is typically 0-linked via a galactosamine residue to a serine or threonine on the protein core. In the human colon these heterogeneous side chains constitute 60%-70% of the mucin molecule.[4] Despite this chemical complexity, only five different sugars- galactose, fucose, glucosamine, galactosamine, and sialic acid- are incorporated into the goblet cell mucins. Of these, it is the **sialic acid** moieties that are responsible, at least in part, for the staining properties of goblet cells. Sialic acids are actually a family of nine carbon ketone sugars, many of which are derivatives of neuraminic acid. There are two histochemically reactive features associated with sialic acids that are important to the staining of colonic mucins. First, there is a *carboxyl group* located at position $C_1$. This functional group (COOH) can be ionized depending upon the pH of the staining solution, and when it is ionized (COO-) it

carries a net negative charge and will stain with cationic dyes. The second feature is the presence of *vicinyl diols* located at positions $C_7$-$C_8$ and $C_8$-$C_9$. In the PAS technique, these diols are oxidized by the periodic acid and then bind with the Schiff's reagent to give a positive reaction (Fig. 2). Of course, other sugar moieties on the mucin molecules, (eg, fucose), also have vicinyl diols and will contribute to a PAS positive reaction.

Another consideration for the histochemical staining of mucins is that the carbohydrate side chains may be sulphated, generally on the galactose moieties. As in the case of the sialic acids, **sulphate** functional groups may be ionized ($SO_4^{-2}$) *depending on the pH* of the solution, and this fact is the key to understanding several special stains. Consider the use of Alcian blue. This copper phthalocyanin dye carries a net positive charge and will bind with high affinity to anionic sites, such as the sialic acid and/or suphate groups present in colonic mucins. Furthermore, since the pKa, (the dissociation constant), of sulphate is lower than the pKa of the carboxyl group present on the sialic acid, the sulphate will remain ionized at a lower pH than the carboxyl group. More specifically, both groups are ionized at a pH of 2.5, while only the sulphate is ionized at a pH of 1.0. Therefore, if a stain sequence on a colonic biopsy utilizes a cationic dye such as Alcian blue at a pH of 2.5, both groups will bind the dye and stain, whereas if the same dye is applied at a pH of l.0, only the sulphated mucins will stain. This differential staining can be used clinically to distinguish the **sialomucins** from the **sulfomucins.** Since the abundance and distribution of these two types of mucins are altered in disease states, this stain sequence can be a valuable diagnostic tool. Be careful, however, as you make the Alcian blue solutions since there may be significant differences in the dye concentration among different lots. Always check the dye content and adjust (mathematically) for differences. Also, be sure to use Alcian blue 8GX and check that it has been certified by the Biological Stain Commission.

One disadvantage of the Alcian blue procedure described above is the need to stain multiple sections and/or sequential slides. Another approach that avoids this difficulty is the sequential application of dyes of contrasting colors at different pHs. This is the basis for the HID/Alcian blue staining protocol.[5] The slide is first stained with the HID at a low pH, generally 1.2-1.5, followed by staining with Alcian blue at a pH of 2.5 (Fig. 4). The resulting tissue shows sulfomucins as black, and sialomucins as blue. Though it is not necessary here to review the caveats of interpretation with this or the Alcian blue staining, it is worthwhile mentioning that one needs to be cautious in interpreting the results. For example, the black color of the HID may mask the presence of blue-staining sialomucins. Or what about the possibility of other anionic substances in the epithelial cells? And what about other secretory cell types in the colon, such as the vacuolated cell or the deep crypt secretory cell?[6]

In conclusion, it is evident that the application of special stains such as Alcian blue, PAS, and High Iron Diamine can provide important information about the colonic mucosa. Of course this is the key to using special stains in the first place. But equally important is the basic knowledge of the chemistry and/or principles underlying the staining process. This requires an understanding of both histology and some chemistry. If you master this information it is easier to troubleshoot problems encountered in any staining procedure. And finally, do not forget the pH of your dye solutions. When was the last time you took a pH of hematoxylin or eosin? Do you even know the proper pH for maximizing staining?

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# Memoirs of a Self-Made Histotech (Part Two)


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*(Continued from Part One Vol. XXXII, No. 1, May 2000)*


After six years at Barnabas, I moved to a busy 500-bed general hospital on the south shore of Long Island (Southside Hospital). Here was a place that performed everything from neurosurgery to complex head and neck cases, as well as orthopedics and pediatric surgery- the full range of surgical material. My collection grew by leaps and bounds, especially after digging into the archives at this place, where many rarities lurked. Usually, it was another full-time histotech and me processing 50-150 blocks per day with a cytotech who did her own prep work. The three docs were wonderful, and I became sort of a pathologist's assistant, but they continued to gross their own specimens- I might search for nodes in an axillary fat pad while they did the rest. I did regrosses for them and often, at the behest of impatient surgeons, dissected frozen section specimens for them when they came late to work in the morning.

During this time, I had become quite skilled and fast at microtomy, and histology in general. In the greedy 1980s, I became an automaton, moonlighting for numerous private labs- a general and gyn lab, a dermpath lab, and a veterinary path lab. I also processed specimens of rat lungs and rabbit intervertebral discs for the local SUNY Stony Brook University clinical research departments. I produced commercial control slides, staining 50 AFBs, GMS, and such at one time for the boxed sets. I'd visit each lab and pick up the specimens. The veterinary lab had a facility and I was able to use this and the hospital to process some 200 (occasionally 400) blocks per day on average, for three to five clients every week. This all hinged on providing the best and fastest service to my pathologists at the hospital, and I can say that they never lacked for anything because of my sidelines. Except for one thing.

My director, Dr. Milton Dana, used to make a one word request starting in the mid to late seventies- "IMMUNOPEROXIDASE" he'd moan at me. I'd nod my head and say something like "It's not FDA approved and it's too expensive for a small place like this." I was too busy making money, but I did attend an early workshop at SUNY SB under Dr. Jules Elias, where we performed LCA stains on tonsil sections in the old prefab buildings that served as a pathology department before they built University Hospital. This piqued my interest, but I was still too busy earning and taking care of the busy lab to get into immuno. In 1983, I was grandfathered into sitting for the HTL exam. During the 1980s, I also became adept at assessing those newfangled fine needle aspirations, often spending hours in the radiology suites since the pathologists did not care to wait around, so they delegated this to me.

After twelve years of cranking out slides for everyone who owned a microscope, I had bought a nice house by the beach and decided to retire to it for a while and smell the roses. In 1988, I resigned from the hospital and all of my labs and took a few months off. That winter I worked in another busy hospital up island (Central General Hospital for EJ Felderman), but quit in Spring to go out east again. I became a gardener and salesperson in a garden center, then an estate caretaker, weeding a millionaire's gardens, cleaning out his chicken coops, and taking care of his dogs, cats, frogs, and turtles. I played drums in a band, worked as a doorman at a local nightclub during the summer, and also rented rooms out in summer. It was a living.

After a year of weeding and dogwalking, my new career became tiresome, and I was lucky to get a job at the local hospital in Southampton starting in 1990. The pace there was much more leisurely, and I began, to the delight of my pathologists, to dabble in immunostaining, using Avidin Biotin detection kits and pre-diluted antibodies. We established a general diagnostic panel including cytokeratin AE1.AE3 and CAM 5.2, vimentin, smooth and common actins, desmin, LCA, T-cell, B-cell, ER and PR, chromogranin, PSA, etc. I developed an interest in soft tissue tumors, and began to study the differentiation markers used to characterize these spindle cell tumors.

I'm a nonconformist and I like to stir up trouble. The nosologic term "fibrohistiocytic" is currently controversial, especially when used in conjunction with the prototypical fibrohistiocytic malignancy, the malignant fibrous histiocytoma, or MFH, which one noted authority thinks doesn't exist. Based on a study of normal, reactive, and neoplastic tissues, I advanced a theory that the term fibrohistiocytic referred not to one cell type showing dual differentiation pathways, but rather to interactive growth of two distinct cells present in normal connective tissue- fibroblasts that express the progenitor cell antigen CD34, and histiocytes that express coagulation factor XIIIa. This composition is conserved in a diverse group of fibrohistiocytic tumors in many organs. Tumors are, after all, not cell cultures of a malignant cell clone, but tissues that are composed of more than one cell type. In fibrohistiocytic tumors, the fibroblasts are the truly neoplastic cells while the histiocytes (or tissue cells!) are essential building blocks necessary for the growth of the tumors (see references). This has received a rather cool reception in some quarters of the pathology community thus far, but interest is rising slowly. I have presented papers in the US, Canada, and Europe, and it has been the thrill of my life. In 1996, I successfully prepared the Qualification in Immunohistochemistry of the ASCP.

I still work in our community hospital, and have been honored to work for nine years now as part of a closely knit team of dedicated clinicians and technicians, taking care of my friends and neighbors while trying to advance medical science. What better calling could there be? I've been doing histology now for 28 of my 47 years.

To all those working in histology, or those just starting out, I can only advise you to be inquisitive and helpful, as most pathologists welcome "extracurricular interest," and this can only serve to enhance your skills, enthusiasm, and professionalism. Your passion for learning is your most important attribute- whether it be on your own or in a formal educational setting. Histologists occupy a unique position that offers a ringside seat to witnessing the wonders of nature. Stop and take a look.

*Reprinted from a 1972 issue of* ***Histo-Logic***

# A Modified Movat Pentachrome Stain

**Jeffrey S. Silverman**
**Westbury, New York 11590**

**Fixation**

Bouin's fluid or 10% buffered neutral formalin.

NOTE: If formalin fixative is used, decerated tissue sections must be mordanted in Bouin's fluid for one hour in a 50° C oven. Wash well in running water to remove picric acid deposits.

**Microtomy**

Paraffin sections at 4-6 micra

**Solutions**

**1% Alcian Blue**

| | |
|---|---|
| Alcian blue | 1.0 g |
| Distilled water | 98.0 mL |
| Acetic acid, glacial | 1.0 mL |

**Ammonium Hydroxide**

| | |
|---|---|
| Ammonium hydroxide, 58% | 5.0 mL |
| Ethyl alcohol, 95% | 95.0 mL |

**Orcein-Verhoeff Stock Solutions**

**Solution A**

| | |
|---|---|
| Orcein | 1.0 g |
| Ethyl alcohol, 70% | 498.0 mL |
| Hydrochloric acid, concentrated | 1.0 mL |

**Solution B**

| | |
|---|---|
| Hematoxylin | 8.0 g |
| Ethyl alcohol, 100% | 160.0 mL |

**Solution C**

| | |
|---|---|
| Ferric chloride | 9.6 g |
| Distilled water | 90.0 mL |

**Solution D**

| | |
|---|---|
| Iodine | 1.0 g |
| Potassium iodide | 2.0 g |
| Distilled water | 97.0 mL |

**Orcein-Verhoeff Working Solution**

Immediately before use, combine above solutions in the following order:

| | |
|---|---|
| Solution A | 25.0 mL |
| Solution B | 8.0 mL |
| Solution C | 5.0 mL |
| Solution D | 5.0 mL |

Use working solution once and discard.

**Woodstain Scarlet-Acid Fuchsin Stock Solutions**

**Solution A**

| | |
|---|---|
| Woodstain scarlet, NS concentrated | 0.l g |
| Distilled water | 99.5 mL |
| Acetic acid, glacial | 0.5 mL |

**Solution B**

| | |
|---|---|
| Acid fuchsin | 0.1 g |
| Distilled water | 99.5 mL |
| Acetic acid, glacial | 0.5 mL |

**Woodstain Scarlet-Acid Fuchsin Working Solution**

| | |
|---|---|
| Solution A | 40.0 mL |
| Solution B | 10.0 mL |

**0.5% Acetic Acid Water**

| | |
|---|---|
| Acetic acid, glacial | 0.5 mL |
| Distilled water | 100.0 mL |

**5% Phosphotungstic Acid**

| | |
|---|---|
| Phosphotungstic acid | 5.0 g |
| Distilled water | 100.0 mL |

**6% Saffron Solution**

| | |
|---|---|
| Saffron (Spanish) CI. No. 75100 | 6.0 g |
| Ethyl alcohol, 100% | 100.0 mL |

To allow proper dye extraction, solution must be placed in an airtight bottle in a 56°-60° C oven for 48 hours.

**Staining Procedure**

1. Decerate section and hydrate to distilled water.
2. Stain in 1% alcian blue solution for 20 minutes.
3. Dip five times in distilled water.
4. Place an ammonium hydroxide solution in a 56° C oven for 10 minutes.
5. Wash in running tap water for 2 minutes.
6. Stain in Orcein-Verhoeff working solution for 2 hours.
7. Wash in running tap water for 3 minutes.
8. Stain in Woodstain scarlet-acid fuchsin working solution for 2 1/2 minutes.
9. Place in 0.5% acetic acid water for 30 seconds.
10. Differentiate in 5% phosphotungstic acid for 5-10 minutes. Well differentiated sections demonstrate colorless collagen and blue-green mucopolysaccharides.
11. Rinse in 0.5% acetic acid water for 30 seconds.
12. Three changes of 100% ethyl alcohol, 1 minute each.
13. Stain in 6% Saffron solution for 8 minutes.
14. Dehydrate in 100% ethyl alcohol, two changes.
15. Clear in xylene, three changes.
16. Coverslip.

**Results**

| | |
|---|---|
| Nuclei | black |
| Cytoplasm | red |
| Elastic fibers | purple to black |
| Collagen and bone | yellow |
| Mucopolysaccharides | blue-green |
| Muscle | red |

Cross striations and intercalated discs in cardiac muscle are clearly stained.

NOTE: The author wishes to thank Mr. Richard J. Schroeder for helpful criticism and guidance during the development of this technique.

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# Paraffin in the Histology Laboratory: An Infiltrating and Embedding Medium

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A common thread in histology laboratories is the piquant odor of formaldehyde and the slippery nature of paraffin. The use of these two products stands as a trademark to both skin and surgical pathology laboratories.

This short monograph will clarify concepts in the composition and use of paraffin as infiltrating and embedding medium essential in the microscopic preparation of tissue specimens.

A hackneyed question among histotechnicians revolves around the best type of paraffin to be used in histology. Skin and surgical pathology laboratories must decide on the type of paraffin best suited for their applications. For example, fat and collagen in the dermis of skin have the tendency to collapse during microtomy. Paraffin with a melting point of 60°-62° C contains high levels of polymers which provide excellent support to these tissue elements during sectioning, but unfortunately the high heat promotes coagulation of proteins; samples acquire a sclerotic or morphea-like artifact in the dermis. In this case, shorter infiltration time in paraffin with a lower melting point (56°-58° C) would yield a more favorable result in skin. Surgical samples such as uterus and other highly fibrous tissue however, are best infiltrated in paraffin with a high melting point (60°-62° C). This harder medium provides the needed support to prevent dense samples from "popping out" of paraffin blocks during sectioning. Furthermore, fibroid tissue is more resistant to denaturing or coagulation by prolonged exposure to heat. The chart below provides a guide to the type of paraffin best suited to your laboratory.

The meteoric rise in the volume of skin samples submitted to surgical pathology laboratories prompted the use of paraffin with a melting point in the mid-range temperatures of 56°-58° C. This type of paraffin has an adequate amount of polymers to facilitate the cutting of surgical and skin samples at 4-5 microns with little or no icing.

New technology in the manufacture of paraffin has changed simple paraffin wax into compounds put together in complex mixtures. The advent of these new brands of paraffin has provided an edge to technicians during microtomy; delicate, thinner, and uncompressed sections (2-5 microns) are now routinely attainable by rotary microtome.

Before we can study the chemical composition and function of embedding and infiltrating media, let's examine briefly precepts germane to *melting point* and *polymerization* as applied to paraffin.

Melting point and polymerization, vis-à-vis heterogeneous crystallization, are factors set in paraffin through processes of refinement and purification. Cutting paraffin blocks into thin ribbons is more effective when solidification of the embedding medium occurs in a uniform matrix of homogeneous crystals; concurrently, the innovation of more advanced media gave rise to "soft and hard" paraffin, each manufactured with specific melting points.

The physical change of solid paraffin into a molten state is basically the disruption of a uniform crystalline lattice into a more random organization of particles. The change from solid to liquid occurs at specific temperatures when the kinetic energy of particles is great enough to overcome the binding forces that hold them together.

Paraffin is a "non-ionic" compound, which means that carbon molecules are linked to each other by covalent bonds. Covalent bonds share electrons between atoms, unlike ionic bonding which requires the electrostatic transfer of electrons between atoms- ionic bonds are more stable and can only be broken down at high temperatures. In contrast, covalent bonds are unstable and easily disrupted by the high kinetic energy of particles, brought about by increases in temperature.

| Temp. | Amount of Polymer | Cutting Thickness | Hardness | Infiltration Time | Type of Tissue |
|---|---|---|---|---|---|
| 52°-53° C | Least | 5-6 microns | Soft | 1-2 hours | Soft tissue, Blood vessels, GI, Brain |
| 56°-58° C | | | Intermed | 2-4 hours | Surgicals, Skin, Fat, Enzyme stains |
| 60°-62° C | Most | 2 microns | Hardest | 4 hours | GYN Proc., Bone |

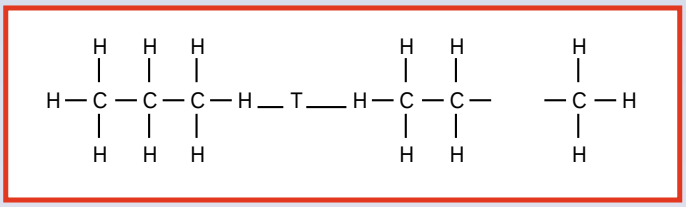

Fig. 1. Illustrates the covalent bonds between carbons easily disrupted by increased kinetic energy.


Fig. 2. Schematic illustration of polymers with molecular chains varying in length [A-C]. Individual monomers are connected by covalent bonds [D].

***Polymerization:*** A *polymer* is a long chain molecule with repeating smaller units called *monomers*; monomers are held together by single covalent bonds.

Polymers are chemical compounds that increase the hardness of paraffin, and as a consequence, improve support during cutting; polymers reduce shrinkage (of the media and tissue) and prevent fluctuations of melting points.When mixing polymers in paraffin, manufacturers follow specific guidelines of compatibility and solubility between the two compounds; polymers and paraffin must have similar melting points to allow uniform blending, and also to be mixable with solvents such as xylene.

Adequate paraffin infiltration is influenced by yet another factor known as viscosity. The term viscosity in general chemistry is defined as "*the resistance to flow.*" Liquids with high resistance to flow (oils and molten paraffin), exhibit high viscosity, whereas liquids with little resistance to flow (water), have low viscosity. High and low viscosity are relative to strong or weak intermolecular attractive forces, respectively. Strong forces render the liquid highly viscous while low viscosity is characteristic of liquids with weak intermolecular binding forces.

Other factors affecting the viscosity of paraffin are the size and molecular weight of each polymer. Shorter chains have low molecular weights and in turn, lower viscosity; paraffin with short polymers are quicker to penetrate tissue but consequently offer low margins of support in microtomy. The reverse is equally true, where long chain polymers with higher molecular weights are slow infiltrators but provide greater support during cutting because of their higher viscosity and harder consistency. In brief, paraffin hardness, consistency, and melting points are factors regulated by the blend of different polymers. The mixtures of multiple polymers must have similar molecular chain lengths and similar, if not equal, molecular weights. Cutting paraffin blocks free of flaws and artifacts is only possible with a homogeneous mixture of polymers in the embedding medium.

The new design of paraffin is an amalgam of the following compounds:

1. Paraffin wax
2. Hydrocarbon polymers (resins)
3. Rubber polymers
4. Microcrystalline wax and natural bees wax
5. DMSO (Dimethyl sulphoxide)

Let's take a closer look at these constituents.

***1. Paraffin Wax:*** Paraffin wax alone was the most common media used in the early days of histotechnology. This incipient infiltrating and embedding medium was made up of a mixture of solid hydrocarbons with simple, straight molecular chains joined by single covalent bonds easily broken at various temperatures. Since paraffin wax by itself has no strong bonds and no specific melting point(melting point ranges between 39° and 69° C), it created a conundrum to histotechnicians during cutting-microtomy was an arduous task with sections commonly breaking apart on flotation baths, even though temperature was kept at low levels. Paraffin wax, void of additives such as plastic and rubber polymers, resulted in compressed sections and overlays because of poor expansion of sections.

As a result of variations in the length of the molecular chains, the unstable covalent bonds, and the occurrence of melting over a wide range of temperatures, paraffin wax acquired a crystalline form, each crystal varying in shape and size. This crystalline structure gave the paraffin block a " grainy" texture, which was ultimately responsible for knife marks and scores when crystals were pushed through the tissue section by the microtome knife. Manufacturers of paraffin soon discovered that sectioning is best achieved in a hydrocarbon matrix of homogeneous molecular composition.

Parenthetically, simple paraffin wax shrinks up to 15% in volume during solidification *along with the tissue sample embedded in it.* Decompression and expansion of the paraffin sections occur quickly when floated on a heated water bath, whereas tissue segments do not recover as easily, remaining compressed with folds and overlays.

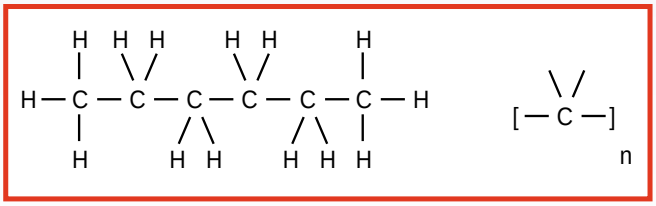

Fig. 3. Paraffin wax is a simple chain of repeating hydrocarbons held together by covalent bonds.

2. ***Hydrocarbon Polymers:*** Hydrocarbon polymers are resins incorporated in paraffin in exact proportions; they are thermoplastic, or stable at high temperatures, and resistant to discoloration.

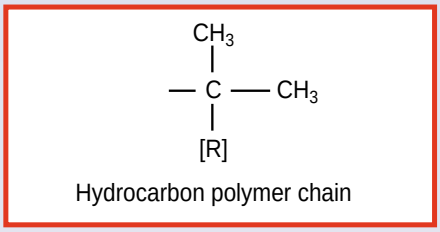


Fig. 4. Hydrocarbon polymers

3. ***Rubber Polymers:*** There are several advantages and disadvantages to the addition of rubber polymers to paraffin, with both factors obvious in microtomy. Advantages include:
   1. Increased adhesion of sections allows for better handling of ribbons.
   2. Rubber polymers prevent sharp fracturing of paraffin during trimming or facing of blocks.
   3. Rubber polymers prevent compression of tissue sections by increasing elasticity of ribbons.

   The disadvantages of rubber polymers include:
   1. Rubber polymers oxidize at temperatures 10 to 20 degrees above the melting point of paraffins. A yellowish tinge and a soap-like consistency are indications of the medium's deterioration.
   2. Rubber polymers increase the "stickiness" of paraffin, making sections difficult to separate with forceps.

4. ***Microcrystalline Wax from Petroleum and Natural Bees Wax:*** Both polymers have branch hydrocarbon chains responsible for increasing strength and hardness of paraffin. These two additives also help regulate the medium's melting point.

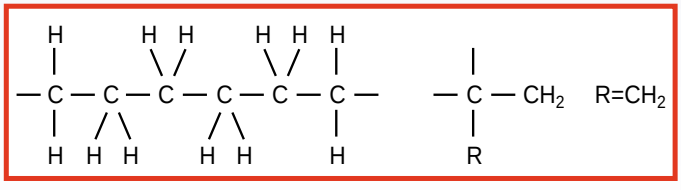


Fig. 5. Microcrystalline wax from petroleum and natural bees wax.

5. ***DMSO (Dimethyl Sulphoxide):*** This additive in some brands of paraffin is used as a propellant. DMSO is a hygroscopic compound (attracts and absorbs moisture) that improves infiltration of paraffin into tissue. Unfortunately, DMSO is a carcinogen, and it is also efficient in penetrating the skin of the technicians handling it. My recommendation is to check the safety data supplied by manufacturers and follow safety measures when using paraffin with this additive.

It is now clear that the addition of polymers brings important, yet drastic changes to paraffin, both as an embedding and infiltrating medium. Infiltration time, temperature of flotation baths and convection ovens, section thickness, and available time to deparaffinize in staining, are all parameters that need to be considered when choosing the type of paraffin used in the laboratory. A good place to start is by examining the coloration and consistency of the paraffin.

Color: After filtration and purification, paraffin becomes clear, almost transparent; the addition of specific types and amounts of polymers may bring changes to the color of the media. Large amounts of polymers result in a white, "milky" and harder paraffin, which allows microtomists to achieve thinner, more delicate sections.

Clear paraffin will yield 5-micron thick sections

White paraffin will yield 2-micron thick sections

Temperature: Polymers increase the overall melting point of paraffin. It is then necessary to be vigilant of areas of tissue preparation that require frequent adjustment of temperature. For example, fluctuations of temperature in flotation baths and melting ovens must be kept at a few degrees above or below these values.

Infiltration: The penetration rate of paraffin into tissue is proportional to the size and molecular weight of the polymers it contains. The longer the polymer, the slower the penetration of the media, but the greater the support during microtomy. Paraffin void of additives infiltrates faster because simple carbon chains penetrate tissue faster. In this case, penetration of the media is efficient, but the support during microtomy is minimal. The addition of branched carbon polymers increases infiltration time with improved support during the sectioning of paraffin blocks.

One of the functions of solvents in a processing cycle is the removal of cellular and interstitial material from within tissue. The substitution of this material with mixtures of molten paraffin and polymers is known in histology as infiltration. After solidification, paraffin infiltrated in tissue provides the necessary support for sectioning by microtome. Plastic polymers incorporated in paraffin facilitate sectioning of skin into thin ribbons, which are then laid on a flotation bath and positioned on a slide.

The contamination of infiltrating media (paraffin) with residual xylene is a common deficiency in processing that results in inferior sections and poor staining. Traces of xylene are unavoidably carried over to infiltrating paraffin from previous steps of clearing. Large specimen

loads and infrequent changes of the media contributes to the buildup of even higher levels of xylene contaminants. The presence of xylene in the infiltrating medium results in the following artifacts:

**Poor infiltration:** Contaminated paraffin, irrespective of the type, can no longer penetrate and support tissues effectively during microtomy. Skins, for example, infiltrated in less than optimal medium will invariably lack cohesion, particularly in the papillary dermis, where collagen is thin and fragile; the subcutis becomes mangled during cutting and " blown up" when sections are laid on a flotation bath.

**Poor staining:** High traces of xylene mixed in paraffin affect the quality of microscopic preparations; examples are legion but the loss of the tissue affinity for hematoxylin & eosin dyes, poor reactions in special stains, and nonspecific immunohistochemical staining are consequences of contaminated infiltrating media. The solvent contaminant in the heated, molten paraffin may cause the destruction of stain binding sites and antigens in the cell membrane. The reduction in the number of binding sites leads to loss of staining intensity, " washed-out" stains and nonspecific immunoreactions.

Fig. 6. The influence of xylene contaminants in the infiltrating medium manifested in poor staining.

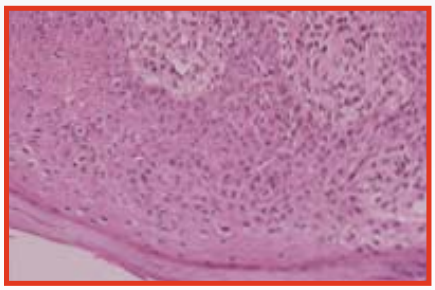

Fig. 7. The H&E section was cut from tissue infiltrated in clean paraffin.

An even greater impact of xylene contamination of paraffin is the destruction of tissue morphology; exposure of tissue to heated molten media with high levels of xylene causes severe shrinkage and *alteration* of epidermal keratinocytes in skin.

Fig. 8. Severe shrinkage of epidermal keratinocytes is a flagrant example of xylene-contaminated medium.

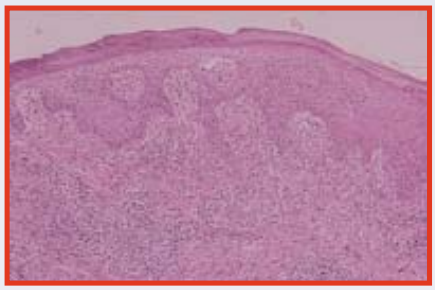

Fig. 9. Normal epidermal keratinocytes.

Faulty processing equipment with extreme temperature fluctuations is yet another setback in infiltration resulting in the breakdown of paraffin- overheated paraffin causes degradation and breakdown of polymers in the media which results in the lack of support during microtomy.

**Deparaffinization:** Paraffin sections with high levels of polymer additives require longer exposure to xylene in the deparaffinization steps of staining. Complex chains of polymers are more resistant to breakdown into the smaller monomers. It becomes necessary to increase the time sections are exposed to xylene to at least 10 minutes.

In closing this discussion I have included two useful recommendations you can implement in the daily monitoring of your processing, specifically in the infiltration cycles; these steps will ensure a complete and more effective penetration by the embedding medium:

1. Elimination of contaminants, such as xylene, in the paraffin stations by the carryover from previous clearing steps. Rotation of paraffin stations is a simple and economical way to resolve this problem, where the last station is moved back to second place; the empty station is then replenished with clean paraffin. This quality control step is crucial for laboratories processing large specimen volumes.

2. Frequent temperature readings are crucial for the general quality control of the laboratory. But close scrutiny of temperatures during infiltrating cycles is tantamount to excellent tissue processing. I suggest recording these values on temperature charts as a source of information when troubleshooting processing problems; a pattern of abnormal temperature values can easily pinpoint specific deficiencies during sectioning or staining. This measure is especially important in preserving tissue antigenicity in laboratories performing immunohistochemical procedures.

In sum, I trust that readers of this short essay can begin to understand and appreciate the value of this well-engineered compound and its relevance to the preparation of integument. Attention ascribed to the type and applications of paraffin will bring improvement to the overall quality of tissue sections.

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1. Hot wax? It depends on the snow conditions. Irene Downs, BA HTL(ASCP), Polysciences,Inc. Pa; 2000.
2. Bancroft JD, Stevens A. *Theory and Practice of Histological Techniques.* 3rd ed. New York, NY: Churchill-Livingston; 1990.

# All In a Day's Work

**Peggy A. Wenk, HTL(ASCP)**
**Histotechnology Program Director**
**William Beaumont Hospital**
**Royal Oak, MI**

*"Can't we have 12 students, one for every month?"*

Diana Maroudis [l] (this year's HT student) and Van Ho [r] (HTL graduate class 2000) practice microtoming in immunohistochemistry lab.

That was the remarkable response I received when I asked the histotechs at William Beaumont Hospital (WBH), Royal Oak, Michigan, if they would be willing to have six students rotate through their lab for one month each, instead of the current four students.

Eva Odish (HTL graduate class 2000) [l] showing Terry Setto [r] (this year's HTL student) slides with multi-headed microscope in residents' room.

At a time when many hospitals are dropping their med tech, cytotech, and histotech training programs, the histotechs, pathologists, and administration at our hospital agreed to add on an additional program.

WBH is committed to education. The School of Histotechnologists was established in 1976, and was affiliated with Oakland University, Rochester, Michigan. This was the first baccalaureate histotechnology program in the country. Dorothy Cummings, MT(ASCP), came home to Michigan to live, after successfully running a histologic technician (HT) program at the University of Chicago, Illinois. She approached Jay Bernstein, MD, Director of Anatomic Pathology, about the possibility of starting an HT program. The pathology department already had a medical technologist (MT) and a cytotechnologist (CT) program, both at the baccalaureate level. Dr. Bernstein suggested setting up a baccalaureate level histotechnologist program, to be equivalent in education and training to the MT and CT programs. Our program was started 4 years *before* the ASCP Histotechnologist (HTL) exam was first offered!

The four HTL students that we accept each year have a heavy background in the sciences - anatomy & physiology, immunology, hematology, histology, microbiology, molecular pathology, genetics, organic chemistry, biochemistry, and physics - as well as math, medical terminology, and computer. There is a 3+1 tract, where the students take the required courses their first three years, and spend their senior year at the hospital, earning college credits. There is also a 4+1 tract, where the students graduate from college after taking the required courses, then spend an additional year at the hospital, without earning college credits.

The practicum is very intense. There is a separate student classroom in the department, set up just like a histology lab. Each student has his own microtome and water bath. There are hoods, balances, a pH meter, hot plates, microscopes, a computer, and even an ultramicrotome! Much of this equipment is second-hand, but in very good condition. There are also all the chemicals and dyes needed for the histology stains, however, we have to borrow the reagents for immunology and enzyme stains from the department labs. And no, we don't have an electron microscope in the student lab. We have to use the one down the hall.

Please note: Van (photo 1) and Eva (photo 2) have taken the HTL(ASCP) exam in August 2000, but are awaiting their results.

The HTL students start in September, and stay in the classroom for the first 7 months, receiving one to three lectures a day, plus one or two labs. There are additional homework assignments, videos, slides, and other learning tools. They work on tissues that already have a diagnosis and are to be disposed. That way, there is no harm to the patient if the student makes a mistake. The students also embed, section, and stain the autopsies, once they become proficient. During this time, the HTL students learn the theory and techniques of histology, immunohistochemistry (immunofluorescence and immunoperoxidase), electron microscopy, enzyme histochemistry, and molecular pathology.

For the last 5 months (April through August), after they have passed all the exams and demonstrated proficiency in all the techniques, the students rotate through the various labs, one month each. They work side by side with the techs, working on patient tissue that has NOT had a diagnosis. The students know that if they make a mistake, either a diagnosis may not be able to be made, or a wrong diagnosis could be made. The students take this responsibility very seriously. They rotate for one month each through histology, immunopathology, electron microscopy, and molecular pathology. They also spend one week in surgical pathology, and several days in the cytology prep area, and at WBH histology lab in Troy, Michigan.

In addition, the students each do a research project, such as comparing a variety of neurofibril stains for quality, cost, and safety, or trying to

Jenny Lehmann [front] (this year's HTL student) at student computer with [back] Peggy Wenk, HTL (ASCP) (Program Director, and HTL graduate class 1980) in student classroom/lab. Note student library and board for lectures.

determine if length of decalcification is causing false-negative iron staining in bone marrow biopsies. They present their research findings in a lecture to the pathologists, residents, and techs in the department, so they have to take microphotographs and prepare a PowerPoint presentation and handouts. They have to write up their research in the format of a journal article. Additional studies include management techniques and education methodologies.

In 1990, Dorothy retired and I was fortunate enough to become the new director of this wonderful program that taught me when I was a student (graduated in 1980). One of the things I did was to bring in newspaper want ads to help the students find jobs. I realized that some of the positions didn't need a baccalaureate level HTL. However, the nearest HT program was 1 hour away, and they were thinking about closing.

In 1992, I talked with Ali-Reza Armin, MD, the HTL medical director, about the possibility of setting up an HT program, to be run at the same time as the HTL, but for only the histology lab portion. It would be for students with an associate degree, with some biology (anatomy, physiology, microbiology), a couple of chemistry courses, plus medical terminology and math. They would be in the program only about 6 months, and would rotate only in the histology lab, with smaller rotations through surgical pathology, cytology, and WBH-Troy.

Before we talked with our pathologists or administration, I wanted to find out how the histotechs felt about this. They are the ones who have to work with the students at the bench. They are responsible for keeping an eye on the students and making sure the quality of their work is good. They are the ones who teach the students the little tricks, improve their ability to coordinate doing many stains, and answer their questions about sectioning a difficult tissue. They have to trust the students and be patient so as to let the student figure out how to correct a mistake. All these things take time, and in a hospital like ours that did 57,000+ specimens last year, techs don't have time to be slowed down by students.

I told the histotechs about the idea of accepting two HT students a year, who would be doing their rotations in histology in February and March. The lab would then have students for six months of the year instead of four. I was so delighted when the first question was, " Can't we have 12 students, one for each month?"

Several community colleges became interested, and HT associate degrees were established, with the students earning college credit for the six-month practicum. The first two students started our new HT program in September 1994.

Rachel Kropf [back] (HTL graduate class 1998) shows Jason Adams [front] (this year's HTL student) molds of prostate macrosections at embedding center in histology lab.

Again, WBH was willing to be a front runner. We became one of the few hospital-based HT programs at that time to require an associate degree. And we became the first institution in the U.S. to have both an HT and an HTL NAACLS-accredited program.

I recently polled the histotechs to learn what they found to be the advantages and disadvantages of having students in the lab. They could list many *advantages*:

- Students come well prepared for the rotations, and help with the workload
- Students work well independently, so the techs don't have to watch them a lot, and can do other tasks
- Students keep them up-to-date, since they always ask questions
- Students are in awe of techs, since techs are faster and better in histology techniques. It feels good to have someone think that what you do is great
- If the workload is *very* heavy, or if there are many techs out sick, the school rearranges lecture schedules, and the students help out in histology, sometimes just labeling and coverslipping, sometimes sectioning or staining, so that the slides get out on time
- Students can be hired as contingents, for an hour or two before or after school, sometimes as a lab assistant making solutions or filing slides, or as a histotech later in the year
- If there is an opening, the histotechs prefer to hire one of our students, as the student already knows all the Beaumont procedures, and knows where everything is. Also, the techs have a say in which student to hire, as they know the quality of work each student does, and their personalities
- Students perform safety checks to prepare for the CAP inspections
- Fresh gossip. The techs have worked together for so many years, they know everything about each other. The students are someone new to learn about, with new topics of conversation

When I asked for *disadvantages* of having students, no one could come up with one. I suggested maybe the students slowed them down too much, or asked too many questions, or made too many mistakes. The techs all said no, that the students were well prepared to work in the lab and they enjoyed having the students work with them.

I'm proud of the caliber of the techs that work in our department. They work hard, they do many procedures that most labs don't do, they work with a volume that is unbelievable and at a pace that is incredible. And yet they are willing to share this knowledge, experience, and technical ability with students. And to make it even more astonishing, the techs ACTUALLY ENJOY working with students!

# The Coding Nightmare: Making Sense of It All

**Debbie J. Siena, HT(ASCP) QIHC**
**Baylor University Medical Center**
**Dallas, Texas 75246**

" Fraud and Abuse," " upcoding," " unbundling," " compliance," and " CPT codes" have all become buzzwords of the new millennium. Government investigations are on the increase and health care administrators everywhere tremble in their boots at the thought of an audit. In 1996, an Office of Inspector General (OIG) audit of the Health Care Financing Administration (HCFA) revealed errors in 30 percent of all claims paid by HCFA for that year. These errors account for approximately $23.2 billion annually, or 14 percent of total Medicare fee-for-service payments. As a result of these audit findings, providers have seen an increased effort by the federal government to prevent, identify, and punish health care fraud and abuse.

What does this mean for you, the histology director, supervisor, or bench tech? What can you do to help reduce the chance of your employer becoming a target of a fraud investigation?

The first thing that you can do is to become more familiar with the CPT codes and the principles of CPT coding.

The American Medical Association's (AMA) Current Procedural Terminology (CPT™) coding system was developed in 1966 to describe the professional medical, surgical, radiology, laboratory, and anesthesiology services of physicians. The first edition contained primarily surgical procedures, with limited lab and radiology procedures. The second edition, CPT2, was published in 1970, and presented an expansion of the terms and codes to designate diagnostic and therapeutic procedures in surgery, medicine, and the specialties. The second edition introduced a five-digit coding system to replace the former four-digit classification. The fourth edition, published in 1977, and in use today, provided significant updates in medical technology, a system to review, and periodic updating to help keep pace with the rapidly changing medical field. In 1983, the CPT code was adopted as part of the HCFA Common Procedure Coding System (HCPCS). The Medicare system adopted the CPT coding system for reporting professional services provided to Medicare beneficiaries. Many private insurance companies also pay claims based on reporting services using CPT.

CPT is maintained by the CPT Editorial Panel (16 members), which is authorized to revise, update, or modify CPT. There are specific procedures in place for addressing suggestions to revise CPT, adding or deleting a code, or modifying existing nomenclature. Once a change is submitted, it requires two years from time of petition to the assignment of a new code. Laboratory technology, however, continues to outpace coding updates, making billing of new services difficult. Medical specialty societies, individual physicians, hospitals, third-party payers, and other interested parties may submit materials for consideration by the Editorial Panel. The panel meets quarterly and addresses nearly 350 major topics a year. The AMA welcomes correspondence, inquiries, and suggestions concerning old and new procedures.

January 1 is generally the date that new CPT codes go into effect. The annual AMA update is sent out in late fall of each preceding year. The AMA offers several publications to help with CPT coding, " CPT 2000," " CPT Assistant," and " CPT Companion." There is also a new publication, " Principles of CPT™ Coding," available to members at the AMA web site, http://www.ama-assn.org. Resources for non-AMA members to purchase publications about CPT coding include Amazon.com and St. Anthony Publishing, Inc., P.O. Box 96561, Washington D.C. 20090 (800-632-0123), or from their web site at http://www.st-anthony.com.

In the CPT 2000 code book, you will find the Pathology and Laboratory Guidelines in section 80000-89999, with Anatomic Pathology in section 88000-88099, Cytopathology in section 88141-88158 and Surgical Pathology in section 88300-88399.

Some of the Medicare billing ground rules are:

1. Medicare law places the responsibility on the provider to select the code(s) that most closely matches the test(s), procedure(s), or specimen(s) submitted and/or services provided by the laboratory.

2. In surgical pathology, the specimen is the unit of service. A specimen is defined as tissue or tissues that is/(are) submitted for individual and separate attention,

requiring individual examination and pathologic diagnosis. Service codes 88304-88309 are specifically defined by the specimens.

*Note: A specimen is not equal to a container. Any means such as a clip, ink, suture, shape, size, etc, can be used to establish a specimen's identity. Example: Twin placentas submitted in the same container—twin A's cord has no clamp, twin B's cord is clamped—this may be counted as two specimens and charged accordingly (88307 × 2).*

3. There are certain specimens that have to be bundled together, even if submitted in separate containers, at different times, and they are:
   - One or more lymph nodes with larynx
   - One or more lymph nodes with colon or radical/modified radical mastectomy
   - Fallopian tube with ovary
   - Adenoids with tonsils
   - Right or left adnexa with uterus
   - Inflammatory nasal sinus polyps
   - hemorrhoids
   - two or more fingers from the same hand or two or more toes from the same foot

4. Services 88300-88309 include accession, examination, and reporting. They do not include the services designated in codes 88311 (decalcification) through 88365 (in-situ hybridization), which are coded separately when performed.

5. Service code 88300 (gross exam only) is used for any specimen that in the opinion of the pathologist can be accurately diagnosed without microscopic examination. ***Never charge 88300 + 88302-88309 for the same specimen.***

6. Service code 88302 is used when gross and microscopic examination is performed on a specimen to confirm identification and the absence of disease (eg, fallopian tubes).

7. Frozen sections, 88331 (pathology consultation during surgery, with frozen section(s), single specimen) are charged per separately identified specimen subject to intraoperative rapid microscopic diagnosis. Any additional tissues sent for frozen section on that specimen are charged 88332 (each additional tissue block with frozen section(s)). Intraoperative consultations (88329) are charged per separately identified and diagnosed gross only OR consult specimen. Notation in report with specimen name and diagnosis must be made along with a notation for the basis of diagnosis. There is no technical component for an intraoperative consultation.

8. Special stains are either 88312 (for microorganisms) or 88313 (other than for microorganisms), or 88314 (special stain on frozen material). Immunohistochemical stains are reported with 88342, 88346 for immunofluorescent stains (direct method), and 88347 for indirect immunofluorescent stains. Each stain is orderable once per specimen, regardless of the number of blocks submitted for examination. However, a combination stain may be charged (88313 x 2), (eg, PAS with and without diastase and an Alcian blue pH 1.0 and 2.5). ***Note: Each stain must be mentioned in the Final Surgical Pathology report and must be correlated to the specimen to which it relates.***

9. Decals (88311) are another area where you may only charge once per separately identified specimen regardless of the number of blocks submitted for decalcification.

10. Any unlisted specimen should be assigned to the code that most closely reflects the physician work involved when compared to other specimens assigned to that code. ***Remember that you may not alter CPT code for any specimen that is unequivocally identified. Upcoding based on amount of work is prohibited.***

There are three ways to bill most procedures in pathology, although not all codes have the ability to be billed in this manner. There is global billing, where the professional and technical components are billed together (when the pathologist is an employee of the hospital or special arrangements have been made to reimburse the pathologist). Certain procedures are a combination of both a physician and a technical component. When the physician component is reported separately, the service may be identified by adding the modifier - 26 to the usual procedure number or the service. When the technical component is reported separately, the service may be identified by adding the modifier - TC to the usual procedure number or the service.

In the end, laboratory compliance policies should ensure that the CPT or HCPCS code that is used to bill Medicare or Medicaid accurately describes the service that was ordered and performed. A model compliance plan for a clinical laboratory may be found at http://www.os.dhhs.gov/progorg/oig/modcomp/cpcl.html if you have any questions regarding what elements a clinical laboratory compliance plan must cover.

In closing, I would like to state that there is help available through regular discussions on the Histonet (send an email message to: histonet@pathology.swmed.edu and in the subject line, type the word subscribe and you will be added to the listserver); National Society for Histotechnology (http://www.nsh.org), Legislative Committee Chair (214-820-2465, Debbie Siena); and through CAP Division of Government and Professional Affairs by calling 800-392-9994.

# Mark Your Calendar!
# Educational Opportunities in 2001

January 17 **NATIONAL SOCIETY FOR HISTOTECHNOLOGY**
Teleconference: 1:00 pm Eastern Time (301) 262-6221
Speaker: Vinnie Della Speranza, MS, HTL(ASCP)HT
" How to Avoid Hiring the Wrong Person"

January 19 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Glenda Hoye, BS, HT(ASCP)
" Connective Tissue Stains"

February 16 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: I-Tien Yeh, MD
" Pigments, Crystals and Crystalloids: A Guide to Microscopic Identification"

February 21 **NATIONAL SOCIETY FOR HISTOTECHNOLOGY**
Teleconference: 1:00 pm Eastern Time (301) 262-6221
Speaker: Peggy Wenk
" Preparing for the HT/HTL Written Exam"

March 9-10 **IOWA SOCIETY FOR HISTOTECHNOLOGY**
Site: Sheraton Four Points Hotel, Cedar Rapids, IA
Contact: Debra Foster (319) 244-5665

March 10 **ARKANSAS SOCIETY FOR HISTOTECHNOLOGY**
Site: Jefferson Regional Medical Center, Pine Bluff, AR
Contact: Louellen McLemore (870) 541-7541
Email: mclemore@crseik.net

March 16 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Anne Horstmann, HT(ASCP)
" Continuous Quality Improvement and Management"

March 16-17 **KENTUCKY SOCIETY FOR HISTOTECHNOLOGY**
Site: Louisville, KY
Contact: Renee Matherly (502) 852-5587
Email: rmath0516@aol.com

March 17-19 **NSH REGION II & MARYLAND SOCIETY FOR HISTOTECHS**
Site: Radisson Hotel Cross Keys, Baltimore, MD
Contact: Terri Decarli (410) 787-4546
2394 Grant 73
Sheridan, AR 72150
(W) 870-541-7541 (H) 870-942-4483

March 30 **AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS**
Teleconference: 12:00 pm Central Time 1-800-621-4142
Speaker: Robert Reddick, MD
" Electron Microscopy: History to the New Frontier"

April 6-7 **NSH REGION I**
Site: Royal Sonesta Hotel, Boston, MA
Contact: Barbara Morey (617) 376-4082
Michael Fredrickson (617) 726-5153

April 20 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Sheron C. Lear, HTL(ASCP)HT
" Advantages and Disadvantages of Epitope Enhancement in Immunohistochemisty"

April 20-21 **NSH REGION VII**
Site: Children's Hospital, Denver, CO
Contact: John McGinley (303) 239-3464
Email: mcginleyj@amc.org

April 21-22 **NEBRASKA HISTOLOGY SOCIETY**
Site: Mahoney State Park, Ashland, NE
Contact: Janice Mahoney (402) 398-5661
Email: jmahoney@alegent.org

April 26-28 **NEW JERSEY SOCIETY FOR HISTOTECHNOLOGY**
Site: Central New Jersey
Contact: Barbara Bujak (609) 252-3548

April 28-29 **OKLAHOMA SOCIETY FOR HISTOTECHNOLOGY**
Site: Westin Hotel, Oklahoma City, OK
Contact: Angie Barnett
Email: bbarnett@socket.net

May 3-6 **FLORIDA SOCIETY OF HISTOTECHNOLOGY**
Site: Westin Innisbrook Resort, Tampa, FL
Contact: Tonia Breckenridge (850)469-5155
Email: tabrecken@aol.com

May 4-5 **NEW YORK STATE HISTOTECHNOLOGICAL SOCIETY**
Site: Wyndham Hotel, Syracuse, NY
Contact: Ellen McAvoy / Sandy Cummings
Email: emcavoy@twcny.rr.com or toosandyd@aol.com

May 11 **AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS**
Teleconference: 12:00 pm Central Time 1-800-621-4142
Speaker: David Tacha, HTL(ASCP)
" A Holistic Approach to Antigen Retrieval"

May 15 **AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS**
Teleconference: 12:00 pm Central Time 1-800-621-4142
Speaker: Dan Jones, MD, PhD, FASCP
" T-Cell Lymphoproliferative Disorders: Using Flow Cytometry and Immunohistochemistry to Aid in Diagnosis"

May 17-20 **CALIFORNIA SOCIETY FOR HISTOTECHNOLOGY**
Site: Radisson Hotel Harbor View, San Diego, CA
Contact: Linda McGlothlen (916) 635-3240
Email: lindamcgloth@gateway.net

May 17- 18 **ILLINOIS SOCIETY FOR HISTOTECHNOLOGISTS**
Site: Hawthorne Suites, Champaign, IL
Contact: Rae Ann Staskiewicz (309)344-2451
Email: raestask@galesburg.net

May 18 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Dean Troyer, MD
" The Renal Biopsy: Light Microscopy and Immunofluorescence Techniques"

June 8-9 **LOUISIANA SOCIETY FOR HISTOTECHNOLOGY**
Site: Le Meridien Hotel, New Orleans, LA
Contact: Kathy Coats
Email: kat@acadiacom.net

June 15 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Robert Skinner, BS, HTL(ASCP)
" Concepts for Producing High Quality Slides from Decalcified, Paraffin Embedded Bone"

June 16 **NEW MEXICO SOCIETY FOR HISTOTECHNOLOGY**
Site: Tricore Reference Lab, Albuquerque, NM
Contact: Pat Barnes (505) 938-8824
Email: queensd54@yahoo.com

July 16 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Steven E. Slap, MA, M.Phil.
" How is a Laboratory Microwave Oven Useful in Electron Microscopy?"

August 17 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Theresa Schuldt, HTL(ASCP)HT
" Internet Education Resources for the Histotech"

September 8 **NEBRASKA HISTOLOGY SOCIETY**
Site: Nebraska Health System, Omaha, NE
Contact: Janice Mahoney (402) 398-5661
Email: jmahoney@alegent.org

September 21 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Rodney Miller, MD
" Immunohistochemistry on Cytologic Specimens and Previously Stained Slides"

**September 22-27** **NSH SYMPOSIUM/CONVENTION**
**Site: Charlotte, NC**
Contact: NSH Office
4201 Northview Drive, Suite 502
Bowie, MD 20716-2604
301-262-6221
Fax: 301-262-9188
Email: histo@nsh.org
Web page: www.nsh.org

October 19 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Nancy L. Shelhorn, BA, HT(ASCP)
" Immunohistochemistry: Application, Troubleshooting and New Technologies"

November 10 **NEBRASKA HISTOLOGY SOCIETY**
Site: Lincoln, NE
Contact: Janice Mahoney (402) 398-5661
Email: jmahoney@alegent.org

November 16 **UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Speaker: Gwen Goss, HT(ASCP)
" Essential Cost Savings for Anatomic Pathology"

## Sakura Salutes Customers- all VIPs- at NSH

Sakura chose this century's first National Society for Histotechnology (NSH) meeting, held in September in Milwaukee, Wisconsin, to stage a special salute to the very important people who have supported the Tissue-Tek® VIP® Vacuum Infiltration Processor through the years.

A colorful, multipaneled **Tissue-Tek® VIP WALL OF FAME** stretched throughout the Sakura booth. The VIP WALL OF FAME featured photographs of more than 1,000 loyal VIP® customers - shown with their Tissue-Tek® VIP® instruments in their respective hospitals, clinics, and laboratories. And these photos represent just a small portion of the VIP family!

All visitors to the Sakura booth received complimentary picture frames. Visitors whose photographs were featured on the VIP WALL OF FAME also received a duplicate of their VIP® photo.

Also on display were pictures of **VIP CUSTOMERS IN TRAINING**, individuals who chose to make a trip to the Sakura headquarters in Torrance, California to attend special two-day workshops organized to assist them in gaining a greater understanding of the extensive capabilities of their new instruments.

## The Next Generation

Under the banner of **Tissue-Tek® : THE NEXT GENERATION**, Sakura Sales Specialists introduced customers to the next generation of two histology instruments recognized the world over as *laboratory standards* for their proven reliability: **Tissue-Tek® VIP® 5 Vacuum Infiltration Processor** and the **Tissue-Tek® TEC® 5 Tissue Embedding Console.**

*Tissue-Tek® TEC™ 5*
*Tissue Embedding Console*

To reward customers and reinforce the reliability and accuracy of its fifth-generation Tissue-Tek® instruments, Sakura held a raffle for 12 Reliable Time Projector Clocks.

The reliability and accuracy of these unique clocks are tied to the special 60 kHz frequency time signal generated by the U.S. atomic clock in Boulder, Colorado, the most accurate clock in the United States.

*Tissue-Tek® VIP™ 5*
*Vacuum Infiltration Processor*

To receive your own copy of ***Histo-Logic***, or to have someone added to the mailing list, submit home address to: Sakura Finetek U.S.A., Inc., 1750 West 214th Street, Torrance, CA 90501.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Vinnie Della Speranza, ***Histo-Logic*** 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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