

# HISTO-LOGIC<sup>®</sup>

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## Vacuum Processing for Small Biopsies

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Provided below are the results of a study conducted to develop methods for rapid processing of small surgical specimens. They have proven useful in our laboratory. After processing and microtomy, the sections were stained with hematoxylin and eosin for evaluation.

Vacuum to speed fluid exchange and shorten tissue processing, was used as early as the late 1800's. The recent increase of small biopsies in our hospital has made it necessary for us to produce slides as rapidly as possible. The use of a tissue processor\* which incorporates vacuum, has made it possible for us to provide the pathologist with microscopic slides three hours after surgery.

Two fixatives are used: neutral formalin and Bouin's. Fixatives are heated to approximately 50° C. The fixative heating process is accomplished by the use of an extra paraffin pot at station #1 on the processor in place of a beaker.

The alcoholic formalin fixative used consists of the following: 80.0 ml of 10% aqueous formalin and 20.0 ml of 95% alcohol.

The vacuum was set at 11 inches of mercury.

### Method No. 1

Four (4) hour processing schedule for tissues which do not exceed 4 mm in thickness.

Processing Schedule Solutions	Exposure Time
1. Formal alcohol	55 minutes
2. 95% alcohol	10 minutes
3. Absolute alcohol	10 minutes
4. Absolute alcohol	10 minutes
5. Absolute alcohol	15 minutes
6. Absolute alcohol	20 minutes
7. Absolute alcohol	20 minutes
8. Absolute alcohol	10 minutes
9. Xylene	15 minutes
10. Xylene	15 minutes
11. Paraffin (56 to 58° C M.P.)	20 minutes
12. Paraffin (56 to 58° C M.P.)	40 minutes

**Results:** The specimens appeared well fixed and processed. They sectioned with no difficulty. The cellular detail was good in both morphology and staining quality.

### Method No. 2

Two (2) hour processing schedule for tissues which do not exceed 2 mm in thickness. Since very small pieces of tissue may be used with this procedure, it is suggested all specimens be placed in teabags for processing.

Solutions	Exposure Time
1. Formal alcohol	25 minutes
2. 95% alcohol	5 minutes
3. Absolute alcohol	5 minutes
4. Absolute alcohol	5 minutes
5. Absolute alcohol	5 minutes
6. Absolute alcohol	10 minutes
7. Absolute alcohol	10 minutes
8. Absolute alcohol	5 minutes

9. Xylene	10 minutes
10. Xylene	10 minutes
11. Paraffin (56 to 58° C M.P.)	15 minutes
12. Paraffin (56 to 58° C M.P.)	15 minutes

**Results:** Similar to those obtained with Method No. 1.

### Method No. 3

This two (2) hour processing schedule differs primarily in its use of Bouin's fixative instead of formal alcohol. The tissue specimens should not exceed 2 mm in thickness. As in Method No. 2, the specimens should be placed in teabags for processing.

Solutions	Exposure Time
1. Bouin's	25 minutes
2. 95% alcohol	5 minutes
3. Absolute alcohol	5 minutes
4. Absolute alcohol	5 minutes
5. Absolute alcohol	5 minutes
6. Absolute alcohol	10 minutes
7. Absolute alcohol	10 minutes
8. Absolute alcohol	5 minutes
9. Xylene	10 minutes
10. Xylene	10 minutes
11. Paraffin (56 to 58° C M.P.)	15 minutes
12. Paraffin (56 to 58° C M.P.)	15 minutes

**Results:** Specimens fixed in Bouin's appear to be well fixed and well processed. They sectioned with no difficulty. As expected, the H & E staining reaction was intensified in the finished slides. We prefer this method since the stained slides were more intense in staining reaction. Otherwise, there was little difference noted when this method was compared with methods No. 1 and No. 2.

\*Trimatic Tissue Processor, Lipshaw Manufacturing Co., Detroit, Michigan 48210.

**Editor's Note:** Please refer all questions concerning this article to the author.

## Histo Hazard

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THIS KNIFE YOU USE  
PLEASE DON'T ABUSE  
BE ALMOST AFRAID TO USE IT

IF THE HAND YOU'VE GOT  
FUMBLES A LOT  
IT WON'T BE LONG BEFORE YOU LOSE IT

SO BE CALM — RELAX  
WHEN YOU PLAY WITH THE WAX  
BUT WHEN IT'S TIME TO CUT - REMEMBER

YOU CAN EMBED THE FLOOR  
COVERSLIPPING'S A BORE  
BUT A MICROTOME KNIFE CAN DISMEMBER

## Histology: A Many Faceted Gem

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In the early 18th century, Anton Loeuwenhoeck (1632-1723) made simple but efficient microscopes through which he was able to observe microorganisms. This discovery opened up entirely new insights into many fields of medical study, particularly those of Bacteriology, Hematology, and most important of all to us, Histology.

Histology was the first of these fields to evolve and become an accepted way to scientifically investigate the secrets of disease and cellular structure. Histology is an old and honored aid of medicine. This "gem" has rightly earned its place as a highly regarded medical specialty.

Often, histology has been pushed aside in our schools and colleges of allied health as being of minor importance, something any dolt can master. We histotechnologists know how hard we must work to improve and polish the many techniques that I choose to call the gem of histology. Our individual expertise and collective knowledge is essential in many areas of medicine. Many laymen have heard of the vital role histology plays in the diagnosing of surgical biopsies for cancer determination. Few are aware of the many other facets of histology. Histotechnologists, are you familiar with a sliding microtome? Are you familiar with a Minot, or Lipshaw, or a Baush & Lomb? Have you heard of a Shiefferdecker-Becker microtome or the Minot that was designed by Dr. C. S. Minot and was intended for both celloidin and for paraffin work? This sliding microtome was new and original, its knife remained fixed and the object holder moved back and forth. This was unheard of in the late 19th century. Today's rotary microtome still carries these same features of a fixed knife and movable block of tissue. Now automated laboratories are taken for granted, the ultra microtome, the ultra processors as well as a battery of other sophisticated equipment are deemed indispensable.

Let's back up a little to my statement concerning the laymen's acquaintance with histology. Not only do laymen have a limited view of histology, but the same is true of many histotechnologists. W. D. Foster in his book "A Short History of Clinical Pathology" refers to the establishment of clinical laboratories in the 1880's and 1890's in England as a necessity in all hospitals. In the hospitals which established these laboratories, histological examinations of specimens led all other testing. We must note that sections were cut freehand with just a straight shaving razor. When rotary microtomes were first invented and finally manufactured, many histologists refused to use them as they were deemed too slow. Tissues were first fixed in "spirits," soon other fixatives followed. Paraffin embedding was introduced in 1869 by Edwin Kleps. No infiltration was used, just dipping and embedding into block form. With the dawn of the 20th century the technique of paraffin embedding was perfected.

Drs. Ehrlich, Virchow, Wright, Mallory, Lillie, etc., are names familiar to histotechnologists as innovators of various methods and stains. Today's leaders such as Cowdy, Preace, Luna and Sheehan are just as familiar. How did they reach their positions of leadership? Only through hard work and perseverance.

Anyone who has the will, manual dexterity, and determination can succeed. Today's histotechnologist must be skilled in more than just one aspect of histology. It is no longer enough to be proficient in microtechnology and hematoxylin and eosin staining. Now, you must have the very best of skills and experience, starting with a background in anatomy, physiology, bacteriology, chemistry, physics, mathematics, management and administration. All of these are the basics which should be included in educating the histotechnologist. Although approved schools of histology have been accredited, all other teaching laboratories can update their programs to fulfill the needs of their students. Great rewards in both monetary and scholastic achievements await those who truly wish to advance.

Once your educational requirements have been completed many histology specialties await your talents. Ophthalmic pathology is but one facet, requiring exact and meticulous attention to every phase of processing for the preparation of microslides. The delicate structures

of the whole orbit, lens, cornea, optic nerves, muscles, lids and ducts of the eye are the anatomical structures included in ophthalmic pathology. Many special stains and techniques have been individualized for this specialty.

Orthopedic pathology is yet another facet of histology. Here decalcification and gross sectioning of the long bones are the greatest stumbling blocks to the histotechnologist. Bone marrow aspirations and frozen sections of bone material are included in orthopedic research and study. We must remember that the skeletal system is a framework of support for the soft tissues of the body and the protector of vital organs housed in the skeletal cavities. The skeleton, therefore, plays a passive but essential role in the life of man. The skeleton is composed of 206 bones, some of whose function is the production of red blood cells. How many of these bones can you identify? To properly do their jobs, good orthopedic histotechnologists have to know all the bones and their functions.

Let's move on to neuro-anatomical histopathology, the study of the brain, spinal cord and nerve centers. This system has often been compared to a huge computer with the brain as the key, receiving and transmitting incoming and outgoing data. The neuro-system monitors motor reflexes, sensory stimuli, comprehensive discernment as well as the breathing control centers, chemical control centers, etc. On no other system in the body, with the exception of the circulatory system, does so much of the body depend.

The handling of this delicate material requires great skill and finesse; you either know what you are doing or you botch it. Histochemistry is now utilized to complete the study of the neuro-system. Celloidin processing of the large brain areas is still the best and only way to prepare large brain slides. A sharp eye for detail and infinite patience are a must. If neurohistology is not for you, let's move on to a different view of our gem.

The preparation of museum specimens has made many revolutionary changes in recent years. Here the histotechnologist often joins forces with the artist. Life-like plaster casts of human organs complement the use of slides and wet specimens in teaching the medical student and allied health student alike. These forms are also used to educate the public in the functions of the body. Cataloging, updating and displaying are also special skills necessary here. By next year, who knows what new techniques await us. Let's not forget the glamour fields in electron microscopy and drug testing. Many papers and books have been written in the past ten years dedicated to these two fields.

Now I would like to highlight my own specialty: Otolaryngeal Pathology. The 1950's and 1960's not only heralded the advent of the Korean and Vietnam wars, but also the age of environmental "awareness." The conservation of man's natural resources became a critical issue in the United States. The effects of air pollution have become a definite hazard to both the old and the young. The results of air pollution have had far reaching consequences. These are: sinusitis, upper respiratory illness and the upsurge of lung cancer. These difficulties are increasing daily.

The histotechnologist as well as the cytologist plays a broad role in the diagnosing and treatment of upper respiratory illness for the otolaryngologist. Lung brushings, vocal cord biopsies as well as embryological studies are the employ of the otolaryngologist. The determination to perform radical surgery often depends upon the results of these tests. Fifty years ago lung cancer was a rare disease. Today it is estimated that 60,000 men and women per year in the U.S.A. will die of this disease.<sup>1</sup> Chronic obstruction of the bronchopulmonary

### Tissue-Talk



tree refers to three common respiratory ailments: chronic bronchitis, asthma, pulmonary emphysema. These diseases are crippers of men, women and children. Many victims are forced to retire during their most productive years because of these diseases. Exposure to excessive atmospheric pollution and occupational dusts often work together with cigarette smoking to increase the number of cases and deaths from respiratory disease.<sup>3</sup>

The otolaryngologist has mainly relied on the celloidin preparation of whole larynges to give him the true anatomical study of the larynx. This is a very slow and time consuming method of preparation, taking from 6 to 8 months per specimen. The density of the tissue has always dictated the method of preparation. With the upsurge of radical surgery, a faster method was needed. The E.N.T. Department of the Armed Forces Institute of Pathology, along with the Chevalier Jackson Clinic Research of Temple University and The Laryngeal Laboratory at the Hahnemann Medical College and Hospital, have all begun to utilize the paraffin technique of embedding whole larynges. This method has enabled histotechnologists to process and complete sectioning of a whole larynx in 6 to 8 weeks versus 6 to 8 months for celloidin. This method also gives as fine a result to cellular detail as the celloidin method.

As you see from the above mentioned facets of histology, it is up to you, the individual histotechnologist, to determine what aspect of this specialty you wish to pursue. Whatever you do, polish your skills and add the luster of continuing education through your professional local societies, state societies and national societies. No one will ever see the gem of histology in all of its facets unless each and every histotechnologist lets their light shine bright. IT TOOK YOU YEARS OF BLOOD, SWEAT AND TEARS, IT TOOK WORK, PAIN, SACRIFICE AND TIME, TO REACH THIS POINT AT LEAST AND TO SAY, "THIS IS MINE".

HISTOLOGY, MY FIELD HELD IN ESTEEM,  
I KNOW IT, I LOVE IT, I'M PART OF A TEAM.  
I'LL LET MY LIGHT SHINE BRIGHT,  
I SHALL NOT HIDE THIS GEM.  
A BEACON I WILL BECOME,  
NO ONE MY LIGHT MAY DIM.  
NOW, WHEN THIS GOAL BECOMES YOUR DREAM,  
DON'T SKIP A STEP OR TWO,  
TAKE TIME TO DO IT RIGHT MY FRIEND,  
NO MATTER HOW LONG IT SEEMS.  
AND THEN THE DAY WILL COME,  
A STUDENT WILL COME AND SAY,  
"JUST LIKE YOU I WANT TO BE,  
A HISTOTECHNOLOGIST, TOO".

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## Microtomy of the Eye, Lens and Other Friable Tissues: Application of Glycerin

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Paraffin techniques for processing and microtomy of the eye and lens have been described.<sup>1-3</sup> In these procedures, the paraffin-embedded eye or lens is cut and oriented to expose a desired area. The cut surface of the block is then soaked with cotton saturated with warm water for several minutes,<sup>2-4</sup> or coated with 3% celloidin<sup>1-3</sup> prior to chilling the knife and block for sectioning. The water soak or celloidin support minimizes or prevents shattering of the lens, blood or other exudates within the globe.

We have devised a variation of the former microtomy procedure that has been used successfully in our laboratories for almost ten years. The oriented cut surface of the block is coated with glycerin before applying cotton saturated with warm water. The eye or lens is soaked for 3-15 minutes. Eyes that have been preserved in Zenker-acetic or Bouin's fixative require a longer soak time to prevent the lens from shattering. Prior to sectioning, the knife is chilled as usual, but the block is chilled by applying ice over the cotton to avoid fracturing the embedding medium.

The glycerin-warm water soak is useful for sectioning most hard, friable or fragile paraffin-embedded material including embryos, decalcified bone and teeth, calcified or hemorrhagic areas, exudates, hoof, nail, keratin and chitin of insects.

We have found that application of glycerin enhances frequency and quality of intact sections for light microscopy evaluation.

A glycerin-alcohol soak is especially useful for microtomy of silver impregnated material, such as for the Levaditti method for spirochaetes. Unlike Cuevas and Torres,<sup>5</sup> the exposed glycerin coated block remains in position on the microtome, and is covered with cotton saturated with 80% alcohol.

When it is necessary to interrupt serial sectioning, the exposed block may be coated with glycerin to protect the tissue components from the air.

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## The H.T. Practical Exam

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The following items were written by the author while a student at Hurley Medical Center, School of Histology Technology, Flint, Michigan. The first was written the day she began work on the Practical Examination conducted by the ASCP Board of Registry. The second was written the day the slides were mailed to the Board for grading.

HAIL REGISTRY! FULL OF WOE.  
HAIL KNIFE! FULL OF KNICKS, YOU KNOW,  
HAIL TEACHER! YOU HELP US ALONG,  
YOU HOLD OUR HAND AND KEEP US FROM WRONG.  
HAIL TECHNICON, GOING 'ROUND AND 'ROUND,  
PLEASE, OH PLEASE, DON'T LET US DOWN,  
HAIL TO GMS, BROWN AND BRENN, AND AFB,  
THOSE LITTLE BUGS IN THERE, OH, SAY CAN YOU SEE?  
HAIL TO RESIN AS WE MOUNT OUR SLIDE,  
OUR HANDS ARE QUICK, OUR FINGERS GLIDE.  
HAIL PATHOLOGIST, SO SWEET AND KIND,  
PICK OUT THE BEST OF OUR SLIDES, THE MOST FINE.  
NOW, TO WIT, OUR PART IS HALF THROUGH.  
HAIL ASCP, IT'S ALL UP TO YOU,  
WHETHER OUR PRACTICAL WE PASS OR FAIL,  
WE PATIENTLY WAIT, AFTER LONG TRAVAIL.

OH LORD, GOD ON HIGH,  
MOST MERCIFUL AND ALMIGHTY,  
THOU HAST MADE BLINDED EYES SEE,  
NOW HOW'S ABOUT MAKIN' SEEING EYES *BLIND!*

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## HELPFUL HINTS

### Softening Hard Keratin in Specimens for Microscopic Sections

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Upon occasion, it is necessary to prepare microscopic sections from specimens with very hard keratin such as nails or cutaneous horns. The horn is often difficult to cut on the microtome when processed and embedded in paraffin in the routine manner. Since much of the

toughness of such hard keratins is due to the disulfide crosslinks, it seemed logical to attempt to disrupt such bonds prior to sectioning.

The simple expedient of using readily available materials designed specifically for cleaving cystine has proved eminently satisfactory in this laboratory for the past few years. I refer to either permanent wave or depilatory lotion, the former usually containing thioglycolate, the latter either thioglycolate or inorganic sulfide. Soaking the paraffin block, face down, in a shallow vessel containing either of these two types of commercial cosmetic preparations for about one hour, sufficiently softens the hard keratin to permit cutting technically fine sections without difficulty. None of the epithelial or connective tissue structures is apparently damaged by this technique.

(Shapiro, L. Amer. J. Clin. Path., 54:773, 1970. Reprinted with permission of the editor, A.J.C.P.)

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.