

Fig. 1. Lung tissue with CMV infection stained by the immunohistochemical technique. The CMV-infected cells have a large size with voluminous nuclei occupied by nuclear inclusions, which stained a dark brown color (original magnification 400X).

Managing Editor, Gilles Lefebvre
Scientific Editor, Vinnie Della Speranza,
MS, HTL (ASCP) HT, MT

Introduction

Cytomegalovirus (CMV) is a member of the herpesvirus family. Human CMV was first isolated in 1956. A virus cannot live on its own or spread on its own; it needs human cells to live and grow. When healthy people are infected with a virus, their immune system usually helps control active disease. When the immune system is not working well, a virus like CMV can cause illness. It has been estimated by the Centers for Disease Control that one-third of all Americans are infected with CMV. This virus is transmitted through body fluids and blood transfusions and can cause diseases like colitis, esophagitis, myelitis, encephalopathy, and pneumonitis. The most common illness caused by CMV in AIDS patients is retinitis, which can cause blindness. CMV can spread throughout the body and infect different organs resulting in a variety of symptoms.

Techniques for the Detection of Cytomegalovirus

Bilques Bhatti, BS, HTL, QIHC
Gwendolyn Lusk, BS, HT

Histopathology Laboratory, The Methodist Hospital, Houston, TX

Abstract

Cytomegalovirus (CMV) infection is common in immunocompromised patients, including those with organ transplants. CMV is also the most common infection in AIDS patients. The CMV-infected cells typically show cytopathic effects including marked enlargement of infected cells (cytomegaly) and nuclear and cytoplasmic inclusions, which are composed of viral particles. The cells most often infected are endothelial cells. We used both avidin-biotin peroxidase complex (ABC) and direct immunofluorescence techniques applied to formalin-fixed, paraffin-embedded lung tissue after heat-induced antigen retrieval, to detect CMV-infected cells. For the ABC technique, a monoclonal antibody from Dako was used at a 1:20 dilution. For the direct immunofluorescence technique, a prediluted monoclonal antibody from Chemicon International was used. With the ABC technique, the infected cells showed positive nuclear inclusions by light microscopy. With the direct immunofluorescence technique, positive staining was characterized by bright apple-green fluorescence of the infected cell nuclei, in contrast to the dull red color of the uninfected cell nuclei. Both the ABC and direct immunofluorescence techniques can be used successfully to detect CMV-infected cells in paraffin-embedded tissue sections.

IN THIS ISSUE

Techniques for the Detection of Cytomegalovirus	25
Muscle Biopsy Basics	27
Concepts in the Routine Preparation of Eye Specimens	34
Does Formaldehyde Kill <i>Mycobacterium tuberculosis</i>?	37
A Revolution in the Making at the NSH Convention	38
Comparative Usefulness of Standard and Microwave-assisted Tissue Processing Methods	40
Mark Your Calendar	
Educational Opportunities in 2003	47

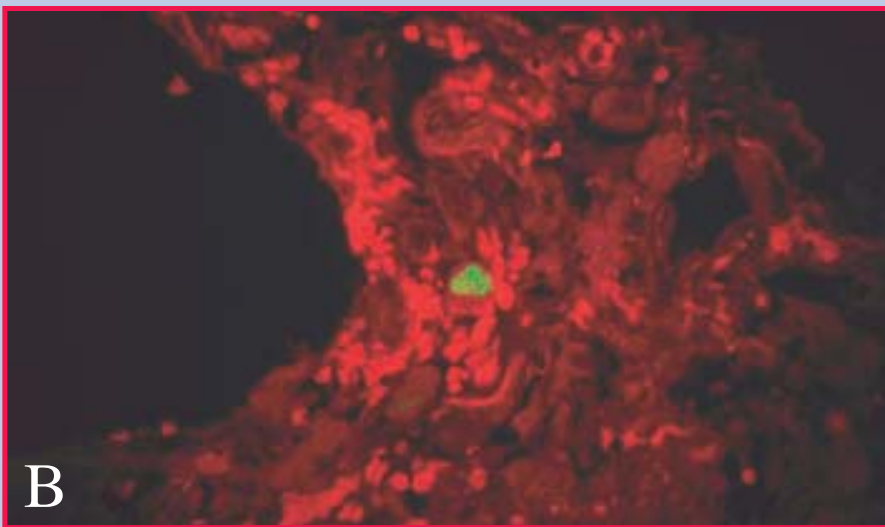
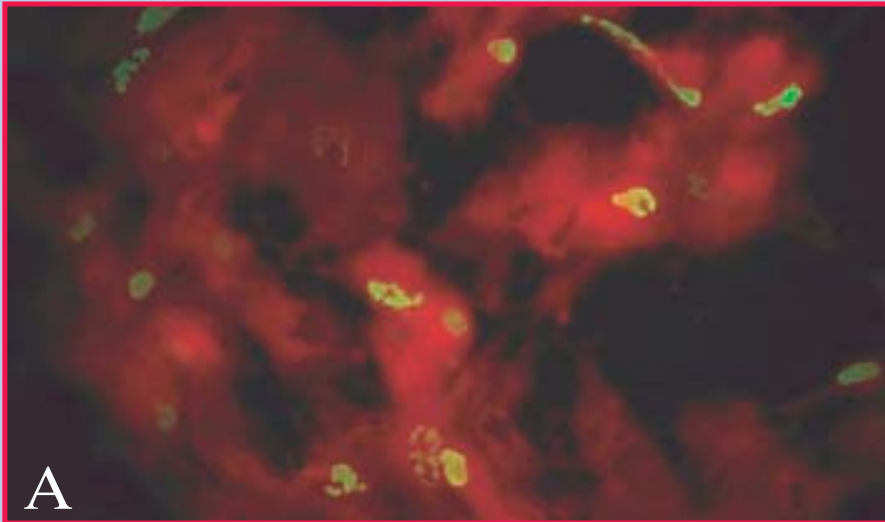


Fig. 2. Lung tissue with CMV infection stained by the immunofluorescence technique. (A) Positive control: cultured cells infected with CMV show positive nuclear staining with an apple-green fluorescence (original magnification 200X). (B) Lung tissue displays a rare infected cell with nuclear inclusion, which appears as apple-green fluorescence. The nuclei of the uninfected cells show a dull red color (original magnification 400X). Excitation wavelength 470-490 nm.

CMV-infected cells can be identified by light microscopy (LM) in routine tissue sections stained by hematoxylin and eosin. These cells characteristically show large size (cytomegaly) with typical nuclear and cytoplasmic inclusions. However, when the infection is mild or in its early phases, typical LM features are not seen and ancillary techniques are needed to confirm the diagnosis.

In this study, immunohistochemical and direct immunofluorescence techniques were used to detect CMV-infected cells in lung tissue. The results of these two techniques were compared.

Materials

1. Formalin-fixed, paraffin-embedded lung tissue infected with CMV
2. Automatic immunostainer, used with the Dako Detection System Kit, and a mouse monoclonal antibody directed against CMV antigens, clones DDG9 and CCH2 (Dako, Carpinteria, CA)
3. CMV Direct Immunofluorescence Assay, using a FITC-labeled monoclonal antibody directed against the CMV immediate early antigen IE1 and IE2 (Chemicon International, Temecula, CA)
4. Steamer and antigen-retrieval buffer solution

Methods

Immunohistochemical (IHC)

Technique:

Four-micron sections were cut from formalin-fixed, paraffin-embedded lung tissue infected with CMV. The sections were submitted for immunohistochemical staining as follows:

1. Tissue sections are deparaffinized and hydrated as follows:
 - Xylene, 3 changes, 3 minutes each
 - 100% alcohol, 3 changes, 2 minutes each
 - 95% alcohol, 2 minutes
 - 70% alcohol, 2 minutes
 - Deionized water, 3-4 rinses
2. Heat-mediated antigen retrieval:
 - Preheat working target retrieval solution (Dako #S1699, pH 6.0) until the temperature reaches 90°C or above.
 - Dip slides in hot water, place slides in the hot target retrieval buffer and steam for 20 minutes.
 - Remove slides after 20 minutes of steaming and allow to return to room temperature for at least 20 minutes or more. After cooling, rinse slides in 3 changes of deionized water.
3. Treat slides with 3% hydrogen peroxide for 8 minutes to block endogenous peroxidase in the tissue.
4. Immunostaining is performed using Dako anti-CMV primary antibody (Dako #M854) at room temperature for 20 minutes on an automatic immunostainer, followed by an avidin-biotin detection system (Dako #K4007).
5. Counterstain with hematoxylin for 1 minute and mount with a permanent mounting medium.
6. Examine stained sections with a light microscope.

Immunofluorescence Technique:

Four-micron sections were cut from formalin-fixed, paraffin-embedded lung tissue infected with CMV. The sections were submitted for immunofluorescence staining as follows:

1. Tissue sections are deparaffinized and hydrated as noted on page 26.
2. Heat-mediated antigen retrieval is done as in the IHC method outlined on page 26.
3. Slides are covered with ready-to-use anti-CMV FITC-labeled monoclonal antibody and incubated at 37°C for 30 minutes in a humid chamber.
4. Slides are rinsed thoroughly in PBS/Tween solution, then coverslipped with mounting fluid (Light Diagnostics, Chemicon International) and stored at 4°C.
5. Examine stained sections with a fluorescence microscope using excitation with ultraviolet light (470-490 nm).

Results

With the immunohistochemical technique, positive staining is demonstrated by brown color wherever the virus is located. CMV-infected cells were identified by nuclear inclusions, which stained a dark brown color (Fig. 1).

With the immunofluorescence method, positive staining was identified by an apple-green fluorescence. The CMV-infected cells were identified by nuclear inclusions, which displayed the apple-green fluorescence. The nuclei of the uninfected cells showed a dull red color (Fig. 2).

Conclusions

1. CMV can be identified in a routine hematoxylin and eosin stained section but there are other techniques available which are more specific for the diagnosis of CMV. Two commonly used techniques are immunohistochemical and immunofluorescence staining. (In situ hybridization is another accepted technique, but not discussed in this article.)
2. The immunohistochemical technique is commonly used for the detection of CMV in formalin-fixed, paraffin-embedded tissue sections. The direct immunofluorescence

technique is frequently used on frozen tissue sections, but it can also be applied to formalin-fixed, paraffin-embedded tissue sections after antigen retrieval.

3. Both the immunohistochemical and immunofluorescence techniques are specific for the identification of CMV-infected cells.

Muscle Biopsy Basics

Jean Mitchell, BS, HT (ASCP)
University of Wisconsin Hospital and Clinics
Department of Neurology
Neuromuscular Laboratory
Madison, WI

Abstract

Skeletal muscle biopsies present a unique challenge to the histologist. A review of the basics of skeletal muscle biopsies can begin to assist in meeting this challenge. Understanding why a patient is biopsied, how a patient is biopsied, and the methods for handling, processing, and staining the muscle biopsy are crucial to ensure that a quality panel of stains is obtained for correct patient diagnosis.

Introduction

Those nagging muscle aches and pains. Everyone has experienced them on one occasion or another. Whether those aches and pains are

derived from a strenuous workout or other extraordinary physical exertion, those symptoms are a normal response to a change in an individual's routine muscular activities. Normal aches will abate themselves within a short time frame. But what if those aches and pains don't go away over a period of time? If chronic muscle pain continues for an extended duration, or if a history develops and persists such as muscle cramping, weakness, stiffness, skin rash, consistently elevated blood serum creatinine kinase (CK) levels¹ (with normal CK values in the range of 38-174 units/L for males, 96-140 units/L for females),² or if there is a family history of muscular disorders, a skeletal muscle biopsy may be the diagnostic procedure to consider.

Skeletal muscle is the largest organ in the body; subsequently, there are many areas to choose when determining a site to biopsy. The biceps, deltoids, and quadriceps are the muscular areas of choice for this purpose. The optimal is to biopsy an area of the body that is moderately involved with the symptom complaints of pain, weakness, cramping, etc. A body site that has been greatly affected and displays wasting away of muscle components should be avoided. In all likelihood the results of such a biopsied site would be end stage muscle disease, which is inadequate for patient diagnosis.

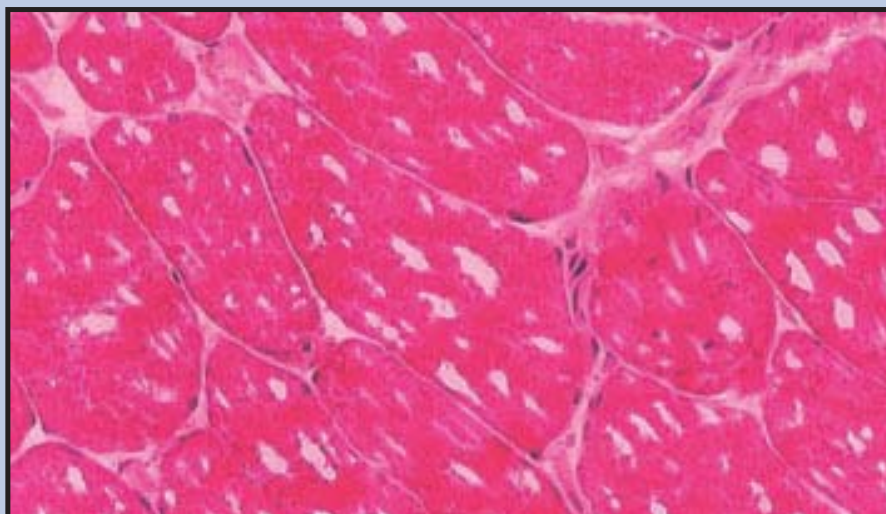


Fig. 1. H&E with freezing artifact. (20X)

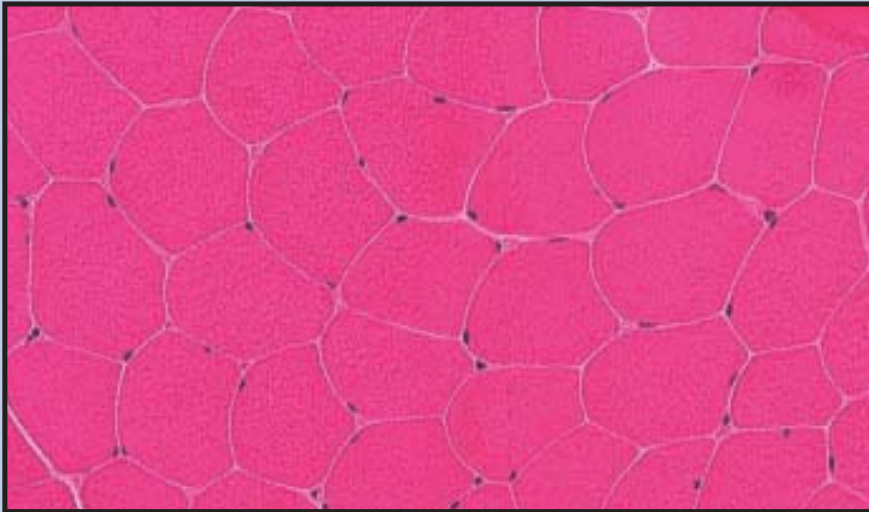


Fig. 2A. H&E normal muscle. (20X)

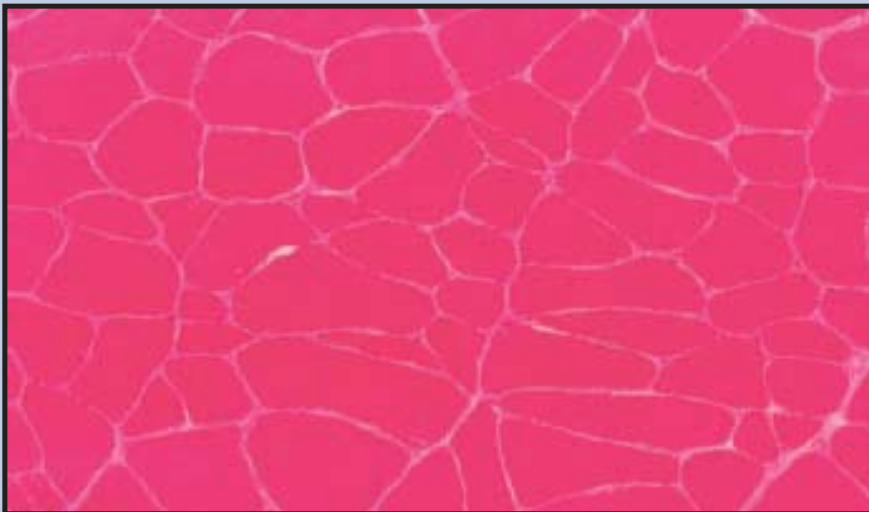


Fig. 2B. H&E abnormal variation of fiber size, atrophic and angulated fibers. (10X)

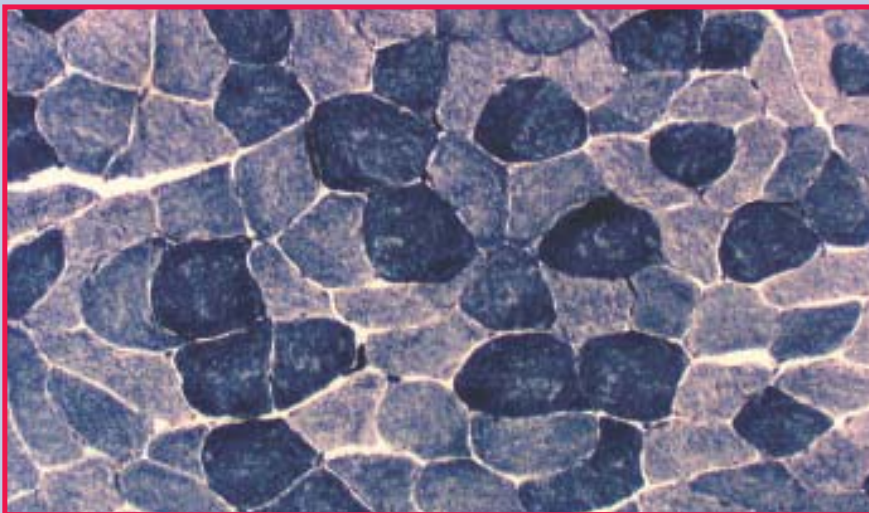


Fig. 3. NADH normal staining. Type I fibers appear dark, type II fibers appear light. (20X)

Once the area is chosen, muscle tissue can be obtained through an open biopsy surgical procedure or through a needle biopsy method. The open biopsy will procure an adequate tissue sampling but it is an invasive procedure that can require operating room time, cause discomfort to the patient, require stitches, and will leave a scar. The needle biopsy method requires a well-practiced physician to obtain needle core samples of muscle tissue. The patient in this case has few adverse affects, no stitches, little scarring, and the amount of tissue sample obtained is minimal in comparison to the open biopsy.

Processing muscle biopsies is unique to the world of histology. Instead of timely fixation in conventional fixatives, freezing the muscle sample is the method of choice for optimal results. Regular fixation, processing solutions, and heat may totally inactivate tissue enzymes,³ one of the most diagnostic features in muscle biopsies. Freezing muscle is the only method to ensure and maintain the ability to detect muscle enzyme activity accurately.

The technique that provides the best artifact-free frozen muscle sample is the “snap” freezing method of isopentane immersed in liquid nitrogen. Within 10-15 minutes, the liquid nitrogen will lower the temperature of a beaker containing isopentane to -160°C. A transversely mounted 1.0 cm x 0.5 cm x 0.5 cm piece of fresh muscle tissue can be sufficiently frozen in the prechilled isopentane in 15-20 seconds. A rule to remember: the lower the freezing solution temperature, the faster the freezing time and the less freeze artifact that will occur. If the freezing solution temperature is not low enough or the muscle sample has absorbed excess moisture prior to freezing, the result will be the formation of ice artifact on the frozen tissue specimen. This artifact will be reflected in the cryostat sections (Fig. 1). Ultimately, freezing techniques should be performed on

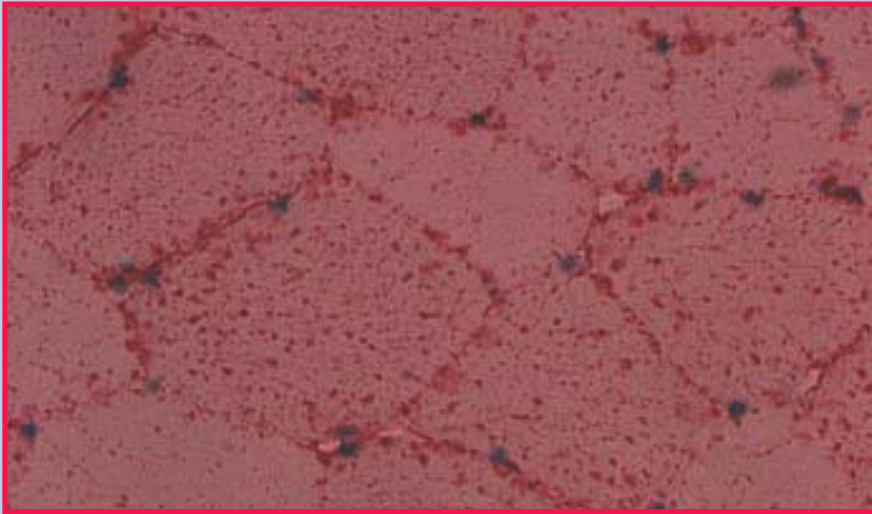


Fig. 4A. ORO normal lipid staining. Type I fibers have increased staining. (40X)

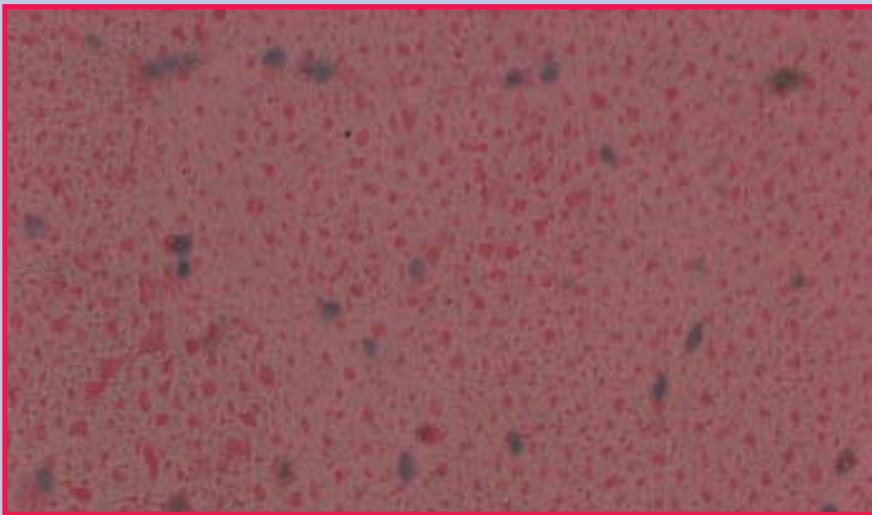


Fig. 4B. ORO abnormal lipid accumulation indicating a carnitine deficiency. (40X)

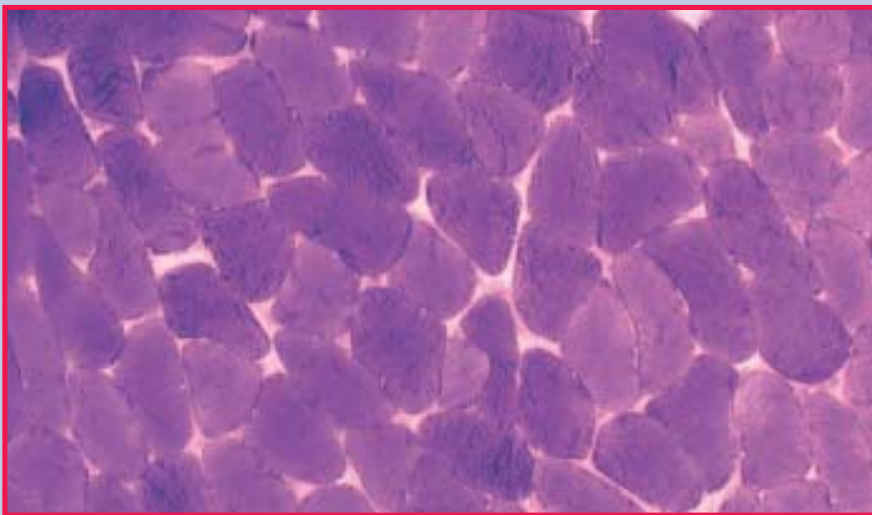


Fig. 5. PAS normal staining. Type II fibers slightly darker. (10X)

muscle biopsies as soon as possible following excision of the tissue sample. But studies have shown that a delay in tissue sample freezing (up to 48 hours) can still provide adequate nonenzyme and enzymatic staining results.⁴ Following “snap” freezing, the frozen muscle samples are sectioned in a -20°C cryostat at 8-10 microns. Sections can be mounted either on slides then stained in 50 ml Coplin jars or on 22x22 mm coverslips and stained in 10 ml mini-Coplin jars.

Skeletal muscle is divided into bundles called fascicles. These fascicles are in turn subdivided into individual muscle fibers. Normal muscle fibers are uniform in size and polygonal in shape, with an adult average diameter of 50 microns⁵ (Fig. 2A). Muscle fibers are further classified as type I and type II fibers (of which there are IIA, IIB, IIC). Type I muscle fibers are used for sustained body activity and receive their energy from mitochondria and lipids. Thus, in the mitochondrial stain nicotinamide adenine dinucleotide (NADH), the type I fibers stain prominently. The same will hold true for the lipid stain Oil Red O (ORO). The body employs type II muscle fibers for quick bursts of energy; their main energy source is glycogen. Consequently, for glycogen the periodic acid Schiff (PAS) reaction will be more distinct in type II fibers (Figs. 3, 4A, 5). The adenosine triphosphatase (ATPase) enzyme procedure is a stain specifically done to compare the number, size, and location of type I and type II fibers in relation to each other. Type I and type II fibers are distributed randomly throughout a muscle fascicle in a normal mosaic pattern. A disturbance of this normal mosaic pattern is termed “grouping,” which is a characteristic finding of several muscle disease processes (Figs. 6A, 6B).

With the aforementioned information on muscle stains taken into consideration, a hematoxylin and eosin (H&E) stain is not solely

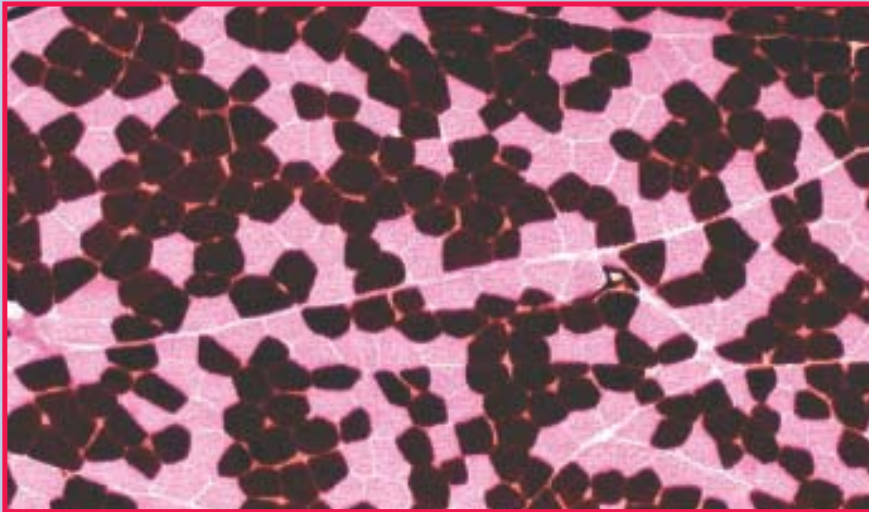


Fig. 6A. ATPase, pH 4.3 normal mosaic pattern. Type I fibers appear dark, type II fibers appear light. (10X)

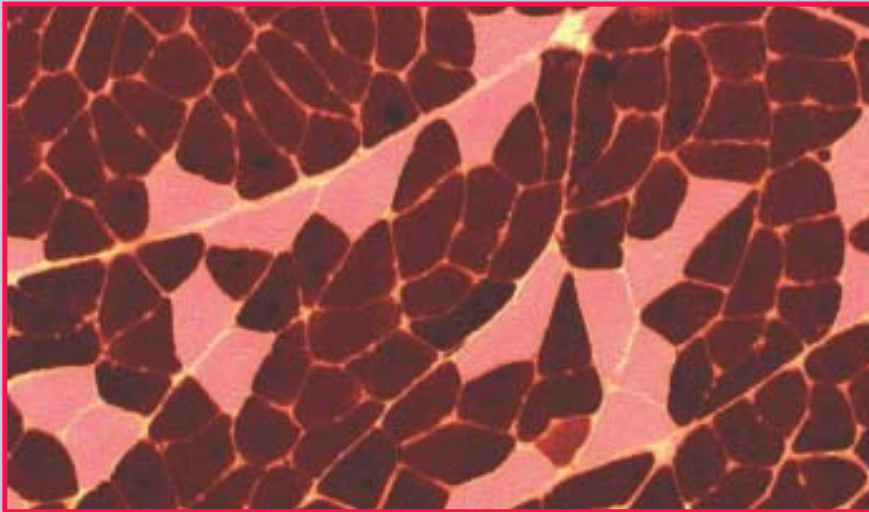


Fig. 6B. ATPase, pH 4.3 abnormal mosaic pattern, fiber-type grouping. Type I fibers appear dark, type II fibers appear light. (10X)

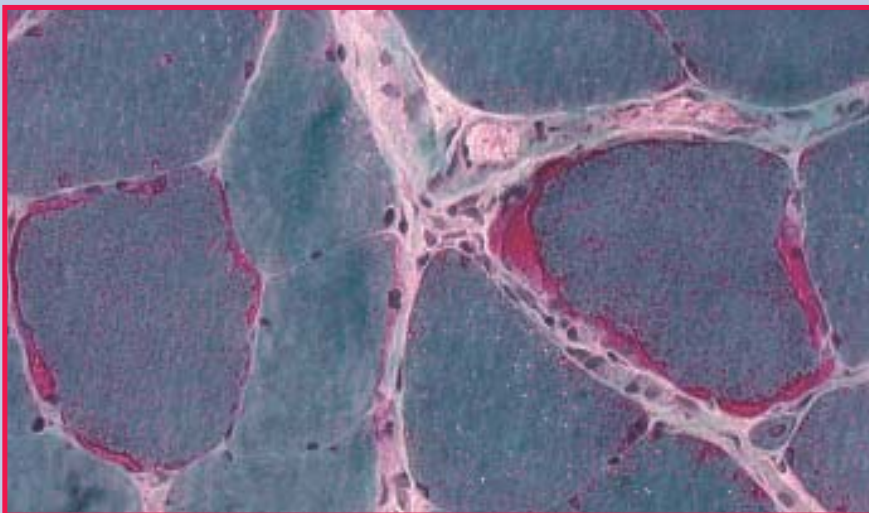


Fig. 7. One-step Gomori trichrome ragged red fibers indicating a mitochondrial myopathy. (20X)

sufficient to see the full pathologic picture of a muscle biopsy. A panel of stains should be performed on every frozen muscle sample acquired. Muscle biopsy protocols may differ with each institution but the requested staining panel should include nonenzyme stains as well as enzymatic staining methods. Examples of nonenzyme staining procedures that reveal valuable muscle features and abnormalities are H&E, one-step Gomori trichrome, PAS, and ORO. Enzyme stains such as NADH, ATPase (at varying pHs 4.3, 4.6, 9.4), acid phosphatase, esterase, and phosphorylase are invaluable enzyme staining procedures for diagnosing muscle diseases (Figs. 2-9). Viewing the completed panel of stains in its entirety is like putting puzzle pieces together and in the end you see the entire picture.

NADH

Purpose/Principle: This stain is an oxidation-reduction reaction with NADH employed as the substrate. The dehydrogenase enzymes in the muscle sections act on the substrate to release hydrogen (oxidation) → the hydrogen then reduces the tetrazolium salt (nitroblue tetrazolium). This results in deposition of blue-purple formazan pigment at the site of enzyme activity in mitochondria.

Type I fibers have greater enzyme activity (increased numbers of mitochondria) and consequently will have greater amounts of dye deposited and stain darker than type II fibers. This stain can also be used to indicate architectural changes in the muscle, such as central cores, whorled, lobulated, and moth-eaten fibers—all of which occur due to a displacement of the intermyofibrillar network and mitochondria.

Control: Internal

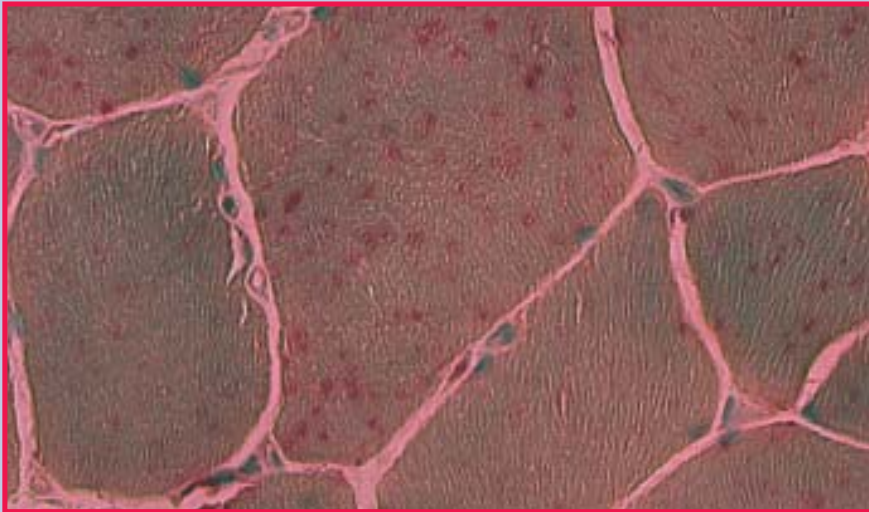


Fig. 8. Acid phosphatase with abnormal increased staining in a measles pattern indicating an acid maltase deficiency. (40X)

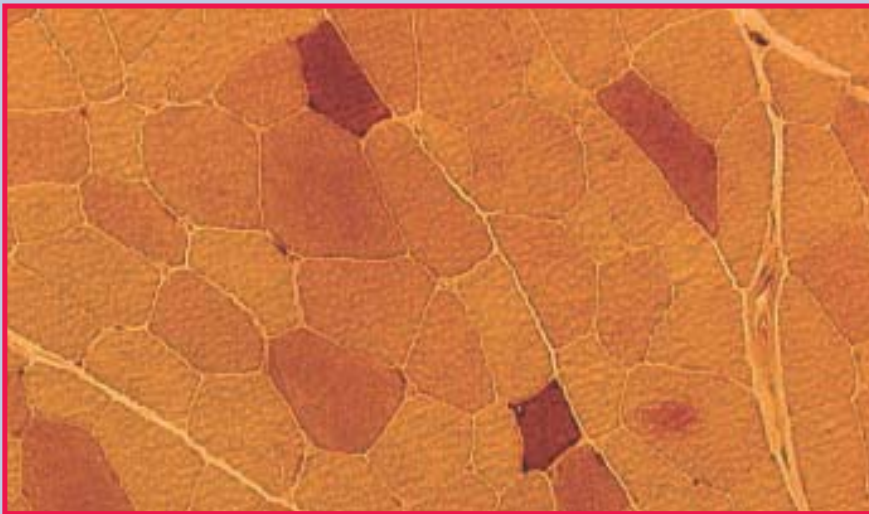


Fig. 9. Esterase with abnormal dark-staining angulated fibers indicating a denervation process. (20X)

Reagents:

0.2 M Tris Buffer, pH 7.2		
0.2 M Tris (hydroxymethyl) aminomethane	(2.42 g/100 ml dH ₂ O)	25.0 ml
0.2 M maleic acid	(2.32 g/100 ml dH ₂ O)	25.0 ml
0.1 M NaOH	(1.00 g/250 ml dH ₂ O)	50.0 ml
Double distilled water		150.0 ml

Nitroblue Tetrazolium Solution (50 mg/50 ml dH₂O)

NADH β-Nicotinamide Adenine Dinucleotide, reduced form. Store at -20°C.

Procedure:

Sections cut at 8-10 microns onto 2 coated coverslips and air dried a minimum of 20 minutes.

- | | | |
|------------------------------|-------------------|---------------------------|
| 1. 0.2 M Tris Buffer, pH 7.4 | 5.0 ml | } (mix all 3 & pH to 7.4) |
| Nitroblue tetrazolium soln. | 5.0 ml | |
| NADH | 2.0 mg | |
| 2. Incubate sections | (37°C) 60 minutes | |
| 3. Distilled water rinse | | |
| 4. Graded acetones | 1 minute each | |
| (30%, 60%, 90%, 60%, 30%) | | |
| 5. Distilled water rinse | | |
| 6. Air dry; mount | | |

Results:

- Type I fibers — dark blue
- Type II fibers — light blue

Variation/Remarks:

1. This stain is pH sensitive. Always calibrate your pH meter against a standard pH 7.0 buffer prior to stain pH adjustment.
2. Coverslips can be mounted with water-soluble mounting media directly after step 5 without drying.
3. Incubation media must be made fresh and used promptly.

NADH References:

Elias JM. The muscle biopsy. In: *Principles and Techniques in Diagnostic Histopathology*. Park Ridge, NJ: Noyes Publications; chap 5:231.

Engel WK, Brooke MH. Muscle biopsy as a clinical diagnostic aid. In: Fields WS, ed. *Neurological Diagnostic Techniques*. Chap 5.

Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis, Mo: C.V. Mosby Co; 1980:308.

ATPase and Reverse ATPase

Purpose/Principle:

The ideal is to perform ATPase (pH 9.4) and Reverse ATPase (pH 4.3 and 4.6) under identical incubation conditions. The difference is in a variance of pre-incubation media and its pH. Following preincubation, all sections are incubated in ATP substrate (adenosine 5-triphosphate) and calcium at pH 9.4. ATPase present in the muscle tissue hydrolyzes the substrate, forming ADP and orthophosphate. The released phosphate combines with calcium to form colorless calcium phosphate. Exposing the tissue to cobalt chloride exchanges cobalt for calcium and colorless cobalt phosphate is now present at the sites of enzyme activity. Developing with ammonium sulfide produces a black precipitate on the active sites.

Employing solutions of varying pH (4.3, 4.6, 10.4) in preincubation media will readily distinguish muscle fiber types I, IIA, and IIB. With this methodology, comparison and contrast of fiber types is easily demonstrated.

Control: Internal

Reagents:

0.2 M Acetate Buffer

(for Reverse ATPase preincubation reagent, pH 4.6 and 4.3)

0.2 M sodium acetate (4.1 g/250 ml dH₂O) 45 ml
0.2 M acetic acid (2.9 ml/250 ml dH₂O) 55 ml

Prepare only 100 ml at a time. Stable for Reverse ATPase for 1 month at 4°C.

ATP Stock Barbiturate Buffer

(for ATPase, pH 9.4 and preincubation reagent, pH 10.4)

0.1 M sodium barbiturate (2.06 g/100 ml dH₂O) 10 ml
2% CaCl₂ (2.00 g/100 ml dH₂O) 5 ml
Distilled water 35 ml

Stable for 2 months at 4°C.

Reaction Incubation Media (pH to 9.4)

ATP Stock Barbiturate Buffer 10 ml
ATP - Disodium Salt 25 mg (store at -20°C)

1.0% Calcium Chloride (CaCl₂) 2.5 g/250 ml distilled water

2.0% Cobalt Chloride (CoCl₂) 5.0 g/250 ml distilled water

Both stable for 6 months at 4°C.

Procedure—ATPase and Reverse ATPase:

Sections cut at 8-10 microns onto 4 coated coverslips and air dried a minimum of 20 minutes.

ATPase Sections

Preincubation reagent – Stock Barbiturate Buffer (sodium barbiturate with calcium chloride)
Adjust pH to 10.4

1. Preincubate 2 labeled coverslips at pH 10.4 for 10 minutes

Reverse ATPase Sections

Preincubation reagent – 0.2 M Acetate Buffer (sodium acetate with acetic acid)
Adjust pH of one mini-Coplin jar to 4.3 and another to 4.6.

1. No preincubation for 2 minutes
2. Preincubate individual sections at pH 4.6 and 4.3 for 5 minutes
3. Drain; add to pH 10.4 for 3 minutes

All sections should now be in pH 10.4 for 3 minutes. Drain and place all coverslips in Reaction Incubation Media containing the substrate (adenosine 5-triphosphate) at pH 9.4.

Reaction Incubation Media: Stock Barbiturate Buffer with 25 mg ATP disodium salt, pH to 9.4

1. Incubate all coverslips at 39°C 30 minutes
2. Wash–1% CaCl₂ 5 minutes
3. Wash–2% CoCl₂ 5 minutes
4. Water rinse
5. Develop–1% ammonium sulfide 1 minute (*Make fresh under hood; discard after use.*)
6. Water rinse
7. Split coverslips into separate carriers–pH 4.6 and 9.4 together, pH 4.3 and 9.4 together
8. pH 4.6 and 9.4 dehydrate; mount
9. pH 4.3 and 9.4, eosin–10 seconds, dehydrate; mount

Results:

pH 4.3—type I fibers will be dark and type II fibers will be light.

pH 4.6—type I fibers will be dark, type IIA fibers will be light, and type IIB fibers will be grayish.

pH 9.4—type I fibers will be light and type II fibers will be dark.

Remarks:

1. This stain is extremely pH sensitive. Always calibrate your pH meter against standard buffers of pH 4.0, 7.0, and 10.0 prior to stain use.
2. An increased amount of ATP disodium salt may be needed as the substrate ages.
3. Incubation media must be made fresh and used promptly.
4. Ammonium sulfide should be in a dark-colored bottle or stored in a dark place.
5. Ammonium sulfide should be ordered in small volumes to ensure its stability.
6. Ammonium sulfide is very odorous and should only be used under a hood!
7. Avoid distractions when running ATPs. Let the phone ring!

ATPase and Reverse ATPase

References:

- AFIP Muscle Biopsy Program.
Dubowitz V. *Muscle Biopsy: A Modern Approach*. 2nd ed. Lavenham Press; 1985.
Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis, Mo: C.V. Mosby Co; 1980:302-304.

Conclusion

A skeletal muscle biopsy is a valuable tool for the diagnosis of muscle disorders. The histologist must be well informed and prepared for proper tissue handling, freezing, sectioning, and staining techniques. Armed with this knowledge, a technician can find good results when working with this unique procedure.

Article References

1. Engel A, Franzini-Armstrong C. *Myology*. 2nd ed, vol 2. New York, NY: McGraw-Hill; 1994:1264.
2. <http://www.bloodbank.com/ranges.html>.
3. Sheehan D, Hrapchak B. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis, Mo: C.V. Mosby Co; 1980:294.
4. Goss G, Prayson R, Pavloski P. Delayed processing of muscle biopsy specimens: does it really compromise enzyme histochemistry? *J Histotechnol*. 1998;21(4):305-308.
5. Walton J, Karpati G, Hilton-Jones D. *Disorders of Voluntary Muscle*. 6th ed. New York, NY: Churchill Livingstone; 1994:5-7.

The Write Stuff.



Introducing



Tissue-Tek® AutoWrite™ Printers

Get the right tools to manage information flow without clerical errors with the new Tissue-Tek® AutoWrite™ Slide and Cassette Printers.

- High-resolution 1-D and 2-D bar codes, graphics, and alphanumeric characters
- Superior readability with reagent-resistant, smudge-proof ink
- Intelligent unloading of slide and cassette trays

Tissue-Tek® AutoWrite™ Slide Printer

- Capacity for 450 slides
- Outputs 12 slides per minute
- Flexibility to handle up to 3 different slide types at once

Tissue-Tek® AutoWrite™ Cassette Printer

- Capacity for 480 cassettes
- Outputs 15 cassettes per minute
- Automatic management of cassette colors
- Fast, convenient loading with prestacked cassettes
- Adaptable to Tissue-Tek® Uni-Cassettes® and Biopsy Cassettes with attached lids



Tissue-Tek® Uni-Cassettes® are available prestacked for easy loading.



Proven Reliability

Concepts in the Routine Preparation of Eye Specimens

Mary Georger, BS
University of Rochester
Center for
Cardiovascular Research

Editor's note: The author served as chief technician for the Ophthalmic Pathology Laboratory at the Univ. of Rochester for 10 years prior to her current assignment in Cardiovascular Research.

With the changing face of health-care in the wake of consolidation and competition, the routine histology lab is often called upon to handle tissues previously delegated to special interest facilities. One of the areas clouded with mystique is ophthalmic pathology, which encompasses both entire globes as well as orbital contents. It is my intent to offer some practical techniques to empower the routine lab when faced with the challenge of handling eye-related tissues. Most of the techniques carry over into the research setting for animal samples with just a few adjustments.

The first and often most important step to good eye histology is fixation. In the routine clinical lab, the standard fixative of 10% neutral buffered formalin (NBF) is quite adequate, keeping in mind that the optimal ratio of fixative to tissue is at least 10:1. Duration of fixation time needs to be a minimum of 24 hours for entire human eyes.

Injection of formalin into the globes to ensure penetration of fixative is unnecessary and can disrupt the retina when one tries to pierce through the sclera with a syringe. Often, eyes that have been fixed in 10% NBF are transferred into 70% ethanol prior to gross evaluation. Alcohol will restore natural color which can aid in external observations as well as in finding important vascular landmarks that enable proper orientation for grossing.

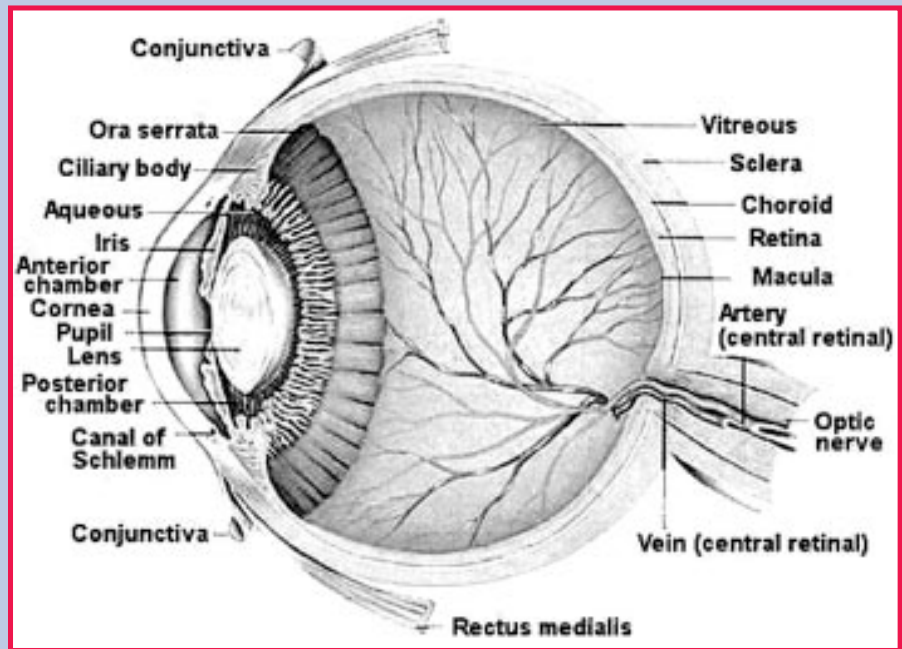


Fig. 1. Schematic representation of the anatomy of the human globe.¹

In very small infant eyes the sclera may be tissue thin. In this instance it is often helpful to place the eyes into several changes of ascending concentrations of ethanol up to 100% after initial fixation and prior to gross dissection of the globe. This will help harden the sclera and avoid complete collapse of the globe and subsequent detachment of the retina when the globe is opened for gross evaluation.

Another excellent fixative for intact globes is Davidson's Fixative.² This fixative contains formaldehyde, alcohol, and glacial acetic acid which stabilizes the sclera and prevents retinal detachment. This fixative is a great alternative for the research environment where there is more flexibility to optimize fixation. After initial fixation in Davidson's Fixative for 8-24 hours, depending upon the size of the eye, the specimens can be transferred directly to 70% ethanol and held for grossing.

The grossing of entire globes can be challenging, even in the best of circumstances! Residents are often told that trying to dissect a human eye is similar to cutting a basketball in half without collapsing it, and it can be just as frustrating. Prior to opening the

eye, it may be helpful to transilluminate the globe using a high-power fiber optic light in a darkened room. By placing the light adjacent to the surface of the eye you will be able to visualize any intraocular defects prior to cutting the globe open. This will assist in determining the best plane of dissection. The optimal plane for routine eyes is a transection across the horizontal, anteroposterior

Formula for Davidson's Fixative²

conc formaldehyde (37%):
2 parts or 500 mL
ethanol: 3 parts or 750 mL
glacial acetic acid: 1 part or
250 mL
tap water: 3 parts or 750 mL

plane, cutting parallel to the long posterior ciliary vessels and adjacent to the optic nerve. This technique produces a central tissue specimen that includes cornea, iris, lens, vitreous, retina, optic nerve, and sclera; it also ensures that the pupil, macula, and optic nerve are all in the same plane.³ This gross



Fig. 2. Transillumination of globe with fiber-optic light source.



Fig. 3. Transillumination reveals intraocular tumor.



Fig. 4. Gross dissection of human globe.

dissection technique can be utilized for larger animal eyes as well, however, smaller animal eyes are easily processed intact.

Processing of eyes, both human and animal, can be done on an automatic tissue processor, keeping in mind that the tissue is very dense but thin; therefore, prolonged time in any solution other than fixation can be counterproductive. The schedule I use is 6 hours in total duration which works well for most globes, but the time must be increased if the specimen submitted includes eyelids or orbital fat. It is also advisable to avoid using high vacuum or pressure until after the cycle has completed the last absolute alcohol to avoid further globe collapse and distortion. There is a wide range of paraffins available and making the selection is often a matter of preference. I prefer a harder paraffin without additives at a melting point of 56°-58°C. In my experience, softer paraffins do not adequately support the globe and subsequent microtomy may be difficult.

Eye microtomy has greatly benefited from the technological advances in all areas of histology. Upon completion of processing, it is most helpful to trim the eye blocks and soak them in ice water for several minutes prior to sectioning. The water facilitates the sectioning of the lens which can become very hard and brittle, even with the most gentle processing schedule. The lens can be most troublesome in animal eyes where it occupies a much larger volume of the eye cavity. After soaking, the eyes can be easily cut at 4 microns using a low-profile disposable blade. During sectioning, if the lens of the eye does not look clear and glass-like in the cut section, place the block back into the ice water for further soaking. A dry lens will not adequately adhere to the slide and will detach during staining.

After obtaining a ribbon, float it out onto a dish containing room temperature distilled water. Ease the section onto the slide, keeping the optic nerve end of the specimen

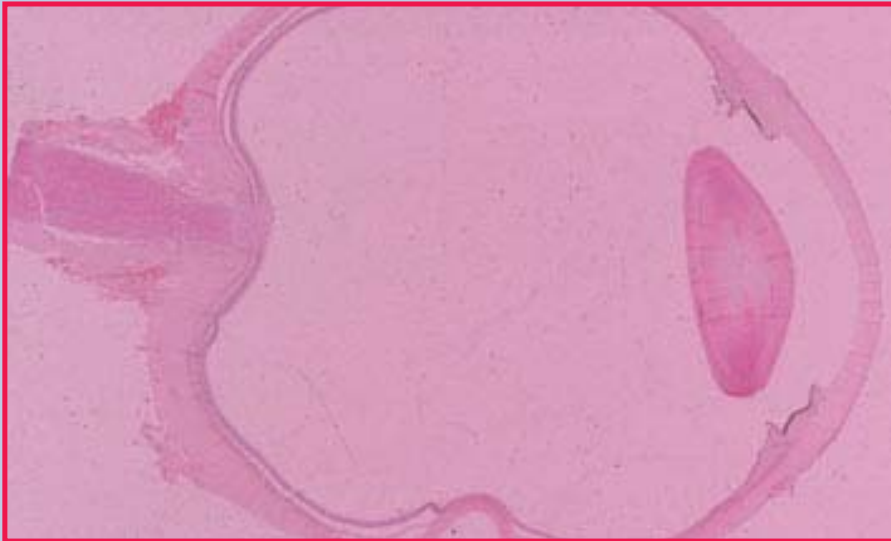


Fig. 5. Histologic section of human globe, H&E stain. Photo courtesy of John Metcalf, MD, Medical University of South Carolina.

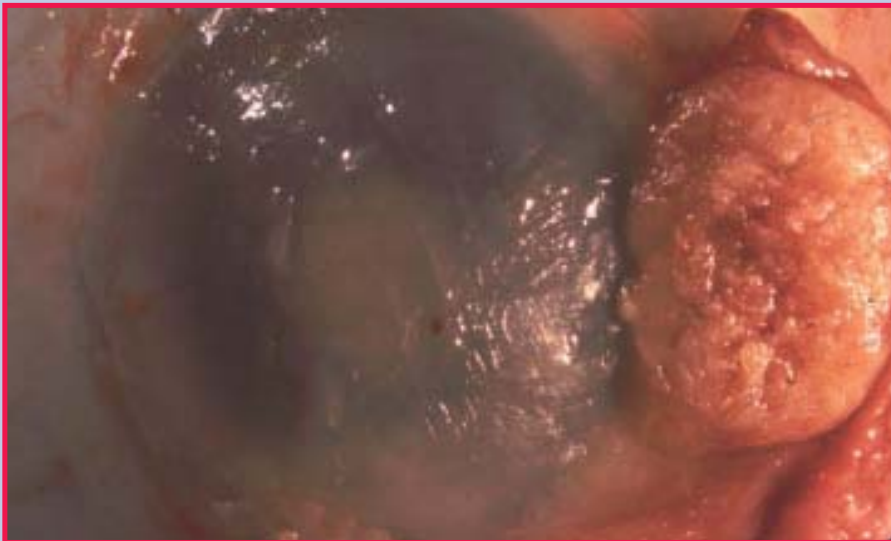


Fig. 6. Human globe demonstrating squamous cell carcinoma. Photo courtesy of John Metcalf, MD, Medical University of South Carolina.

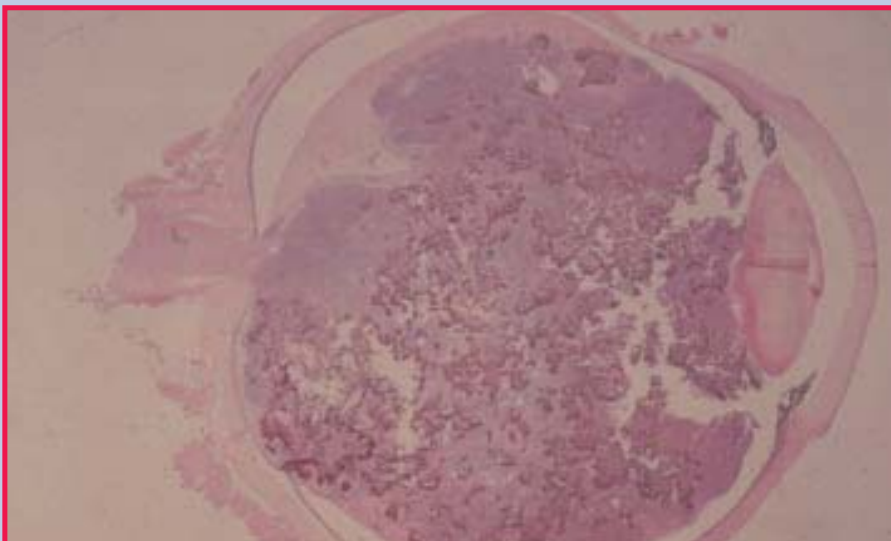


Fig. 7. Section of human globe demonstrating neuroblastoma, H&E stain. Photo courtesy of John Metcalf, MD, Medical University of South Carolina.

at the most distal portion of the slide nearest the label to ensure the least time in high-temperature water. After the section has been positioned, dip the slide into a tissue flotation waterbath set at between 45°-52°C. Beginning with the cornea portion of the eye and ending with the optic nerve end, gently stretch out the section on the face of the water, keeping one end of the paraffin adhered to the glass to act as an anchor. The sclera and lens should spread flat and the optic nerve should not blow out. This technique is the most difficult to regulate and requires patience, but once the proper temperature is found for the paraffin in use it is easily duplicated. Keep in mind that if the sclera is wrinkled you will need to raise the temperature of the waterbath, maintaining a watchful eye on the optic nerve which by nature is very sensitive to heat. Once your slides are complete, it is helpful to dry them in a 56°-60°C oven for at least 1 hour, optimally overnight. In routine cases it is not necessary to add any adhesive solutions to the waterbath, as the higher heat and overnight drying are adequate to ensure tissue adhesion.

Staining for eye specimens routinely includes hematoxylin and eosin, as well as periodic acid Schiff for basement membranes, and Gomori trichrome for collagen and muscle.

In closing, I would like to encourage all histotechnologists to enter into the arena of Ophthalmic Pathology with confidence, understanding, and enthusiasm for the challenges that await us in the changing face of our profession.

Acknowledgment: I would like to give a special thank you to Dr. Steven Scott Searl. Dr. Searl was my supervisor, teacher, mentor, and good friend during my 10 years in the Ophthalmic Pathology Laboratory at the University of Rochester, who encouraged me to learn, grow, and spread my wings.

References

1. Anatomy of the eye. Available at: <http://www.discoveryfund.org/anatomyoftheeye.html>.
2. Presnell JK, Humason GL, Schreiberman M. *Humason's Animal Tissue Techniques*. 5th ed. Johns Hopkins University Press; 1997.
3. Sheehan D, Hrapchak B. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis, Mo: C.V. Mosby Co; 1980:285-291.

Does Formaldehyde Kill *Mycobacterium tuberculosis*?

Vinnie Della Speranza
MS, HTL(ASCP) HT, MT
Mary Richardson, MD
Pathology Department
Medical University
of South Carolina
Charleston, SC

The inability of formaldehyde to neutralize the causative agent of Creutzfeldt-Jakob disease is well documented in the scientific literature.¹⁻³ Outside of this one exception, it is a widely held belief that 10% formalin, the most commonly used tissue fixative in the clinical setting, effectively neutralizes microorganisms encountered in surgical specimens. Fixatives are by design intended to kill bacteria in order to prevent their putrefactive action that would otherwise compromise the microscopic architecture of tissues we wish to study. Furthermore, the effective killing of tissue pathogens during fixation is an important consideration for the safety of those involved in gross tissue examination.

Recent discussions in our department and on the histology list server, Histonet, have brought to light anecdotal reports that the causative agent of tuberculosis, *Mycobacterium tuberculosis*, has been successfully cultured from formalin-fixed surgical or autopsy tissues. If true, this calls into

question the efficacy of formaldehyde in killing this tissue pathogen, which may be of some importance to those outside the pathology laboratory. Formaldehyde was at one time commonly used to disinfect dialysis machines and other medical equipment but was largely supplanted by glutaraldehyde in this role after it was determined that glutaraldehyde disinfection offered advantages over formaldehyde.⁴

Reports of increased incidents of TB exposure among autopsy personnel in the scientific literature are believed to be the result of exposure to aerosolized, viable, infectious bacilli during the removal and examination of fresh lungs.^{3,5-7} Yet if the tubercle bacillus can retain viability after formalin fixation, can exposure resulting from cuts or puncture wounds suffered while working with formalin-fixed tissues be completely ruled out? We conducted a Medline search to identify reports in the peer-reviewed literature of TB viability following formalin fixation and summarize this information below.

Available data is scant and contradictory. In guidelines written for high-risk autopsy cases, Orenstein states that “formalin does not effectively kill mycobacterium (it does reduce the infectivity),”³ although no reference is provided. Others agree with the commonly held belief that formalin is tuberculocidal.⁸ In the NCCLS (National Committee for Clinical

Laboratory Standards) guidelines for the Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissues (M29-A), it states that “10% buffered formalin in at least the normal 10:1 volume of 10% formalin:tissue will inactivate all important infectious agents except the agent of Creutzfeldt-Jakob Disease, Kuru (another prion disorder) and *Mycobacterium tuberculosis*.” This document goes on to state that “a tuberculocidal fixative may be prepared with 10% formalin in 50% ethanol.”¹

In correspondence to the *International Journal of Tubercle Lung Disease*, Gerston and Gafoor noted that of 25 formalin-fixed lungs they studied, 3 yielded live *M tuberculosis* bacilli that grew in culture. Those containing viable organisms were fixed for 4.5, 6.5, and 9 weeks, respectively. They found no correlation between the abundance of acid-fast bacilli in tissue sections or length of fixation time with the viability of organisms.⁹

Earlier reports date as far back as 50 years ago and were based largely on examination of embalmed tissues. Drawing any useful conclusions from this data is fraught with pitfalls as there was marked variation in the contents of the embalming fluids with no one ingredient common to all.⁵ In fact, the amount of formaldehyde varied considerably. In addition, the success of embalming is largely dependent upon the integrity of the vasculature, the volume of disinfectant, and of

Table 1. Summary of *M tuberculosis* Culture and Fixation Times from Historical Studies

Author	Date	Fixation Time*	Method	Culture	Cohort
Meade ¹⁰	1949	3–48 mo	embalming	0%	24
Weed ¹¹	1951	3–60 hr	embalming	88%	25
Johnson ⁵	1953	15–61 hr	embalming	64.7%	17
Smith ⁸	1986	1 yr	10% formalin	positive [†]	NR

Adapted from Kappel TJ, et al.⁸

* Fixation time refers to time from embalming to culture.

† Number of positive cases not reported.

course the time of exposure, among other factors. The possibility that many of these cases may have received antemortem antibiotic therapy makes prospective studies today problematic.

How might we account for the three cases reported by Gerston and Gafoor which contained viable organisms? For the reasons previously stated, the body of literature does not allow us to rule out the possibility that formaldehyde solutions are only partially effective in killing the tubercle bacillus. It is quite possible that some organisms of the species may well be resistant to this alkylating agent, possibly due to the lipid content of the organism's cell wall. We would be correct to question whether the grossing of the study's samples was adequate to allow the formalin complete access to the tubercle lesions contained within. We have all seen examples of large tissues immersed in formalin appearing to have unfixed areas when they were later cut down. Lastly, the challenges of successful TB therapy effectively illustrate how the body's inflammatory reaction leads to the isolation of the organism. The accumulation of living and dead macrophages, bacteria, and tissue cells in the area of infection contribute to the formation of the tubercle. Over time, a thick fibrous capsule may form around the tubercle, walling it off through the development of a granuloma that may contain necrotic material; often, this material is rich in lipid which may challenge the penetration of an aqueous fixative like formalin.

The scant body of literature makes it impossible to confirm or rule out anecdotal reports that the tubercle bacillus may resist the action of formaldehyde. Clearly this is an area that warrants further controlled study. In the absence of hard data, it would be prudent to exercise standard precautions when working with formalin-fixed tissues which may potentially harbor this organism.

References

1. NCCLS M29-A: *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissue*. Approved Guideline, December 1997:29.
2. Nolte KB, Taylor DG, Richmond JY. Biosafety consideration for autopsy. *Am J Forensic Med Pathol*. 2002;23(2):107-122.
3. Orenstein J. Guidelines for high risk and potentially high risk autopsy cases. *Pathologist*. 1984;35:33-34. Editorial.
4. Husni L, Kale E, Climer C, Bostwick B, Parker T. Evaluation of a new disinfectant for dialyzer reuse. *Am J Kidney Dis*. 1989;14(2):110-118.
5. Johnson B, David F, Jamison R, et al. Viability and chemotherapeutic sensitivity of tubercle bacilli isolated from pulmonary and other lesions in embalmed bodies at autopsy: case report and clinicopathologic correlation of cases studied to date. *Dis Chest*. 1953;23:686-692.
6. Templeton GL, Illing LA, Young L, Cave D, Stead WW, Bates JH. The risk for transmission of *Mycobacterium tuberculosis* at the bedside and during autopsy. *Ann Intern Med*. 1995;122(12):922-925.
7. Teppo L, Ojajarvi J, Brander E. The tuberculosis morbidity among pathologists in Finland. *Scand J Respir Dis*. 1974;55:257-261.
8. Kappel TJ, Reinartz JJ, Schmid JL, Holter JJ, Azar MM. The viability of *Mycobacterium tuberculosis* in formalin-fixed pulmonary autopsy tissue: review of the literature and brief report. *Hum Pathol*. 1996;27(12):1361-1364.
9. Gerston KF, Gafoor H. Viability of *Mycobacteria* in formalin-fixed tissues. *Int J Tubercle Lung Dis*. 1998;2(6):521.
10. Meade G, Steenken W. Viability of tubercle bacilli in embalmed human lung tissue. *Am Rev Tuberc*. 1949;59:429-437.
11. Weed L, Baggenstoss A. The isolation of pathogens from tissues of embalmed human bodies. *Am J Clin Pathol*. 1951;21:1114-1120.

A Revolution in the Making at the NSH Convention

Gilles Lefebvre, Managing Editor

The 2002 NSH Convention in Long Beach, California was a resounding success. A record-breaking crowd attended this year's NSH, not only for a first-rate educational program, but also for great activities. The main ballroom of the Queen Mary ship was the setting for the Sunday night banquet which offered a glimpse of the past splendors of a very romantic era. The clement weather allowed for the preceding cocktail hour to take place on the rear deck of the ship, providing a very picturesque view of Long Beach across the bay. Many people also took advantage of the unique venue to visit the various parts of this wonderful ship.

The Convention Center exhibit floor provided the attendees with a surprise preview of the Histology Laboratory of the future. Upon entering the exhibit hall, nobody could ignore the giant video wall located at the front of the Sakura Finetek booth.

This wall of light and sound invited everybody to discover two innovative instruments currently in the final stage of development at Sakura: the Tissue-Tek® Continuous Rapid Processor and the Tissue-Tek® AutoTEC™ Automated Embedding System. These two revolutionary instruments will dramatically reduce the turnaround time of specimen preparation in the histopathology laboratory. It will now be possible to process, review, and sign off most cases in the same day they are received. The Tissue-Tek® Continuous Rapid Processor is capable of processing up to 120 specimens per hour (Fig. 1). One basket of up to 40 cassettes can be loaded about every 20 minutes. The system consists of 1 loading station, 4 reagent retorts, and 2 unload stations.

A combination of a novel reagent system, patented microwave technology, and traditional vacuum infiltration technology is used to produce results that are comparable to or better than traditional processing. The proprietary reagents do not involve formalin or xylene, although the method is compatible with formalin-fixed tissues. The total volume of reagents used is reduced by about 80% as compared to the traditional method. Also, retort cleaning cycles are not necessary. Reagents are presented ready-to-use in disposable 1-gallon containers. The Tissue-Tek® Continuous Rapid Processor method¹ is based on a blend of very gentle reagents performing fast and efficient fixation, dehydration, clearing, and impregnation. Precisely controlled mechanical and thermal actions ensure optimal, standardized processing. Because there is less

variability in this method, results are more consistent. Biopsies and larger specimens can be run at the same time. The gentler process preserves DNA, RNA, and proteins in the block when specimens are processed fresh or pre-fixed in the Tissue-Tek® proprietary nucleic acid-safe fixative.

The Tissue-Tek® AutoTEC™ Embedding System is capable of embedding up to 120 specimens per hour, automatically (Fig. 2). Four magazines of up to 34 cassettes each can be loaded at a time. More can be added as the robotic system removes them from the loading station, allowing for continuous operation



Fig. 1. The Tissue-Tek® Continuous Rapid Processor operates at a rate of up to 120 specimens per hour and still provides results with morphology and staining characteristics similar to overnight processing.



Fig. 2. The Tissue-Tek® AutoTEC™ Embedding System uses the unique Tissue-Tek® Paraform® Cassette System to offer total automation of the embedding process at a peak rate of 120 blocks per hour. The throughput is not degraded even if only biopsy specimens are embedded.



Fig. 3. Biopsy and Standard Paraform® Cassette System.

throughout the day. The magazines can be used as baskets directly from the tissue processor. The Tissue-Tek® AutoTEC™ senses each cassette and automatically differentiates between biopsy and standard size cassettes. Each cassette is then placed in the proper size base mold, paraffin is precisely dispensed and then cooling is activated to form a block. The cassette is then moved to one of the four output doors. The doors can be detached from the system and used as convenient holding trays during sectioning.

Total automation of the embedding process is possible thanks to the patented Tissue-Tek® Paraform® Cassette System (Fig. 3). This sectionable cassette system is a true innovation in cassette design. It makes tissue preparation simpler and faster by at least 50% as compared to current techniques. The grossed specimen is inserted into the cassette and frame with the proper orientation. The lid is pushed down to secure the specimen between the lid and the cassette bottom, ensuring that orientation is preserved. Processing is then performed without the need to change the programs. After processing, the cassettes are embedded by the Tissue-Tek® AutoTEC™ Embedding System. Each cassette is automatically pushed down in a base mold. There is no need to open the cassette and handle the tissue. Orientation is preserved inside the special

Paraform[®] sectionable cassette and there is no paraffin to trim around the cassette prior to sectioning. The Paraform[®] material has sectioning characteristics similar to paraffin. It allows the user to section right through the cassette bottom without artifacts and without damage to the blade. The Paraform[®] material does not pick up stain and does not interfere with microscopic examination.

There is no doubt that these two instruments, along with the Paraform[®] Cassette System, will have a profound impact on the operation of the histology laboratory. For the first time, specimens can be processed, embedded, sectioned, and stained within 2 hours, without sacrificing quality and with no changes in microscopic morphology. The ability to prepare specimens continuously and to use significantly less reagents will have a positive effect on turnaround times, efficiency, operating costs, and better patient care. Both instruments are currently going through clinical trials. Full market release is expected by late 2003.

Reference

1. Morales AR, Essensfeld H, Essensfeld E, Duboue M, Vincek V, Nadji M. Continuous-specimen-flow, high-throughput, 1-hour tissue processing: a system for rapid diagnostic tissue preparation. *Arch Pathol Lab Med.* 2002;126:583-590.

Comparative Usefulness of Standard and Microwave-assisted Tissue Processing Methods

Azorides R. Morales, MD
Department of Pathology
University of Miami/Jackson
Memorial Medical Center
Miami, FL

Introduction

The steps used to prepare specimens for histology are well known and illustrated in Fig. 1. Tissue processing, which includes dehydration, clearing,

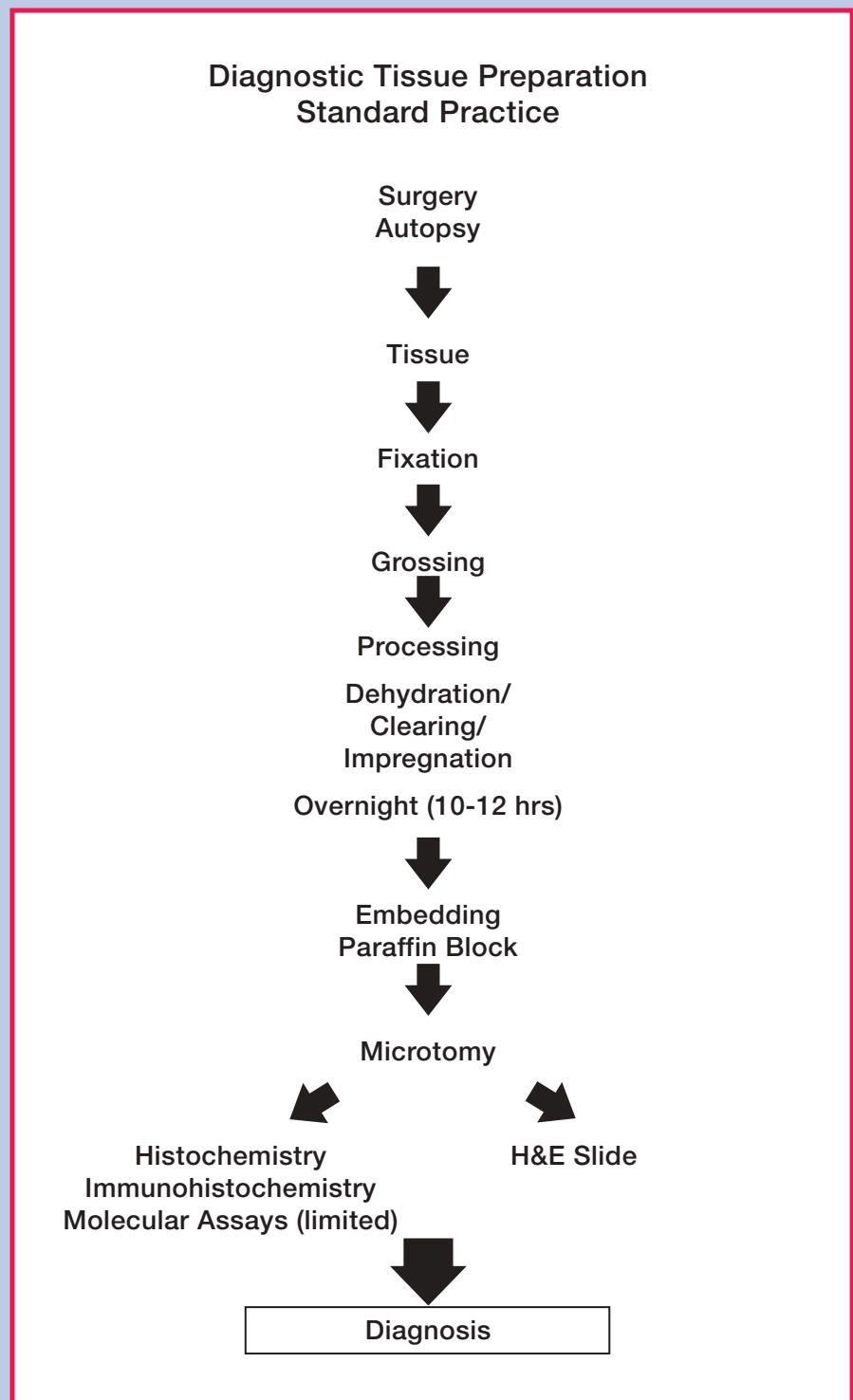


Fig. 1. This figure shows the steps required to prepare H&E stained slides from fresh tissue obtained following surgery or at the autopsy table.

and impregnation, is the lengthiest of these steps. It lasts 10 hours or longer, and is the major bottleneck in the workflow of histopathology laboratories. Typically after grossing, the samples are held in their respective cassettes for overnight

processing and embedded, sectioned, stained, and coverslipped in batches the next day. Batches of microscope slides are presented to the pathologist for review and diagnosis at the earliest, 1 day after grossing the specimen. This has been customary

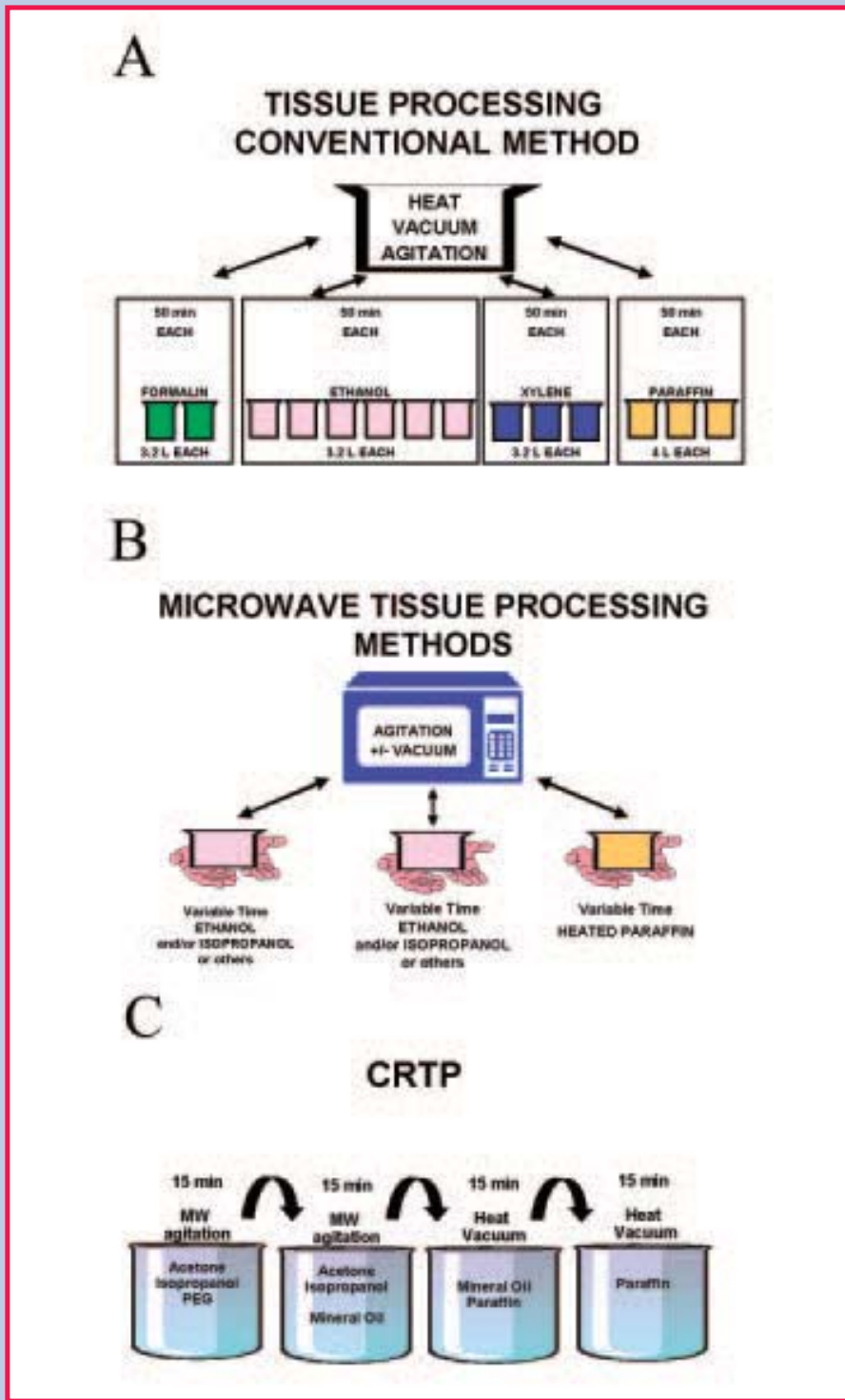


Fig. 2. Schematic representation of tissue processing methods. The major features in each method are highlighted: (A) standard; (B) common microwave-assisted; and (C) continuous rapid tissue processing.

for more than 5 decades. That practice is now increasingly challenged because of its shortcomings including its inability to meet the support required by current clinical demands and because there are now more efficient alternatives

such as microwave-based processing methods. The use of microwave technology in histology shortens tissue processing from hours to minutes, usually reducing by 1 day the time required to complete surgical pathology reports. In this

review, the utility of conventional and microwave-assisted tissue processing methods are compared.

Processing Methods

To illustrate conventional processing methods and instrumentation, a closed system processor, such as the Sakura VIP, is schematicized in Fig. 2A. In this system the samples are placed in a retort where the solutions are pumped in and out. During the processing cycle the tissues are exposed to the reagents and subjected to heat, vacuum, and agitation. In this procedure, it is customary to use formaldehyde for fixation, followed by incubation in a series of increasing concentrations of ethanol for dehydration, then xylene for clearing tissue of alcohol, before impregnation in paraffin. Open processing systems or rotary processors utilize similar reagents and exposure steps but instead of a single retort, every reagent is kept in different containers and the samples are transferred from one solution to the next by mechanical devices. Whether open or closed, the processing cycle in any of these systems lasts 10 hours or longer and therefore, it is almost always carried out overnight.

A number of different microwave applicators, such as Milestone's and Energy Beam's have been in use for a number of years. Their introduction in histopathology resulted from the creative undertakings of visionaries in the field, notably Boon,^{1,2} Kok,^{3,4} Leong,⁵ and a few others. The salient features of microwave-assisted processing methods are shown in Figs. 2B and 2C. As illustrated in Fig. 2B, the samples are manually carried in and out of the microwave chamber as many times as the steps require for the processing cycle. During each of these steps, the reagents bathe the samples for variable periods of time, usually depending upon the thickness of the sections. Reagents include an alcohol, either ethanol or isopropanol or both, or other undisclosed



Fig. 3. Shown in this photograph is the transfer of samples from a microwave chamber to a vacuum retort of the Continuous Rapid Tissue Processor. The cassettes with their respective tissue samples are shown in the basket, which is transported from one station to the next by a robotic arm.

reagent(s), and paraffin. Agitation, and in some systems vacuum, is applied during microwave irradiation of the tissue.

Depicted in Fig. 2C is the system developed and presently practiced at the Department of Pathology of the University of Miami/Jackson Memorial Hospital (UM/JMH). It is a modification of a previously reported method.⁶ It is a fully automated system that accepts specimens every 15 minutes. The tissues are processed through four stations. The specimens in their respective cassettes are placed in a basket and carried from one station to the next by a robotic arm (Fig. 3). Tissue samples are held in each station for 15 minutes, thus the entire processing cycle lasts 1 hour. In the first retort, the samples are immersed in a solution of acetone, isopropyl alcohol, and polyethylene glycol (PEG) and then subjected to microwave irradiation and agitation. A mixture of acetone, isopropyl alcohol, and mineral oil bathes the tissues in the second retort while they are irradiated with microwave

energy. In the third and fourth stations, the tissues are heated in a mixture of paraffin/mineral oil and paraffin, respectively, while subjected to heat and vacuum.

To examine the comparative usefulness of the above-mentioned processing systems, this review highlights the results, expediency, safety, cost, throughput, and versatility.

Quality of Results

Figs. 4A-C illustrate the quality of histology slides obtained by the various processing methods. These slides are composites of tissue processed by both the conventional and the UM/JMH microwave-assisted rapid tissue processing methods. The resulting sections were double-mounted in the same slide and stained with H&E, trichrome, and the estrogen receptor assay. Tissue structure and nuclear and cytoplasm characteristics are similar by these methods. Stronger staining in microwave methods is usually the case as demonstrated in the illustrations. This observation has

been made by other practitioners of microwave processing systems who report results with quality similar to those of conventional methods.

Expediency

Although a number of other benefits result from microwave-assisted tissue processing, its ability to save time led to its introduction into the histology laboratory. Those using microwave methods have all reported shortening the processing cycle to about 30 to 120 minutes.⁷⁻⁹ None of these reports, however, provides comprehensive data on the improvement in turnaround time of surgical reports due to the implementation of the microwave technology. At the UM/JMH, we began to phase in a microwave processing method late in September 1997 as previously reported.⁶ The original method, consisting of nine steps with three microwave stations, was simplified to four steps that include two microwave retorts, a practice that has been in effect for the last 2 years. With rare exceptions, all our surgical specimens are processed following this method. The impact on the turnaround time, shown in Fig. 5, was studied by reviewing the departmental records that track date and time of specimen accessioning and report completion. This turnaround time applies to all of the specimens examined. No attempt was made to segregate cases according to their complexity or whether immunohistochemistry or other studies supplemental to H&E staining were performed. The baseline to measure against was provided by the 1996 data, the year before the method started to be phased in. As Fig. 5 shows, there is major improvement in clinical responsiveness following implementation of our present practice. In fact, this turnaround time of surgical pathology reports is unmatched by those shown in published studies.^{10,11}

Safety

Those working in the field pursue reduction, or preferably elimination,

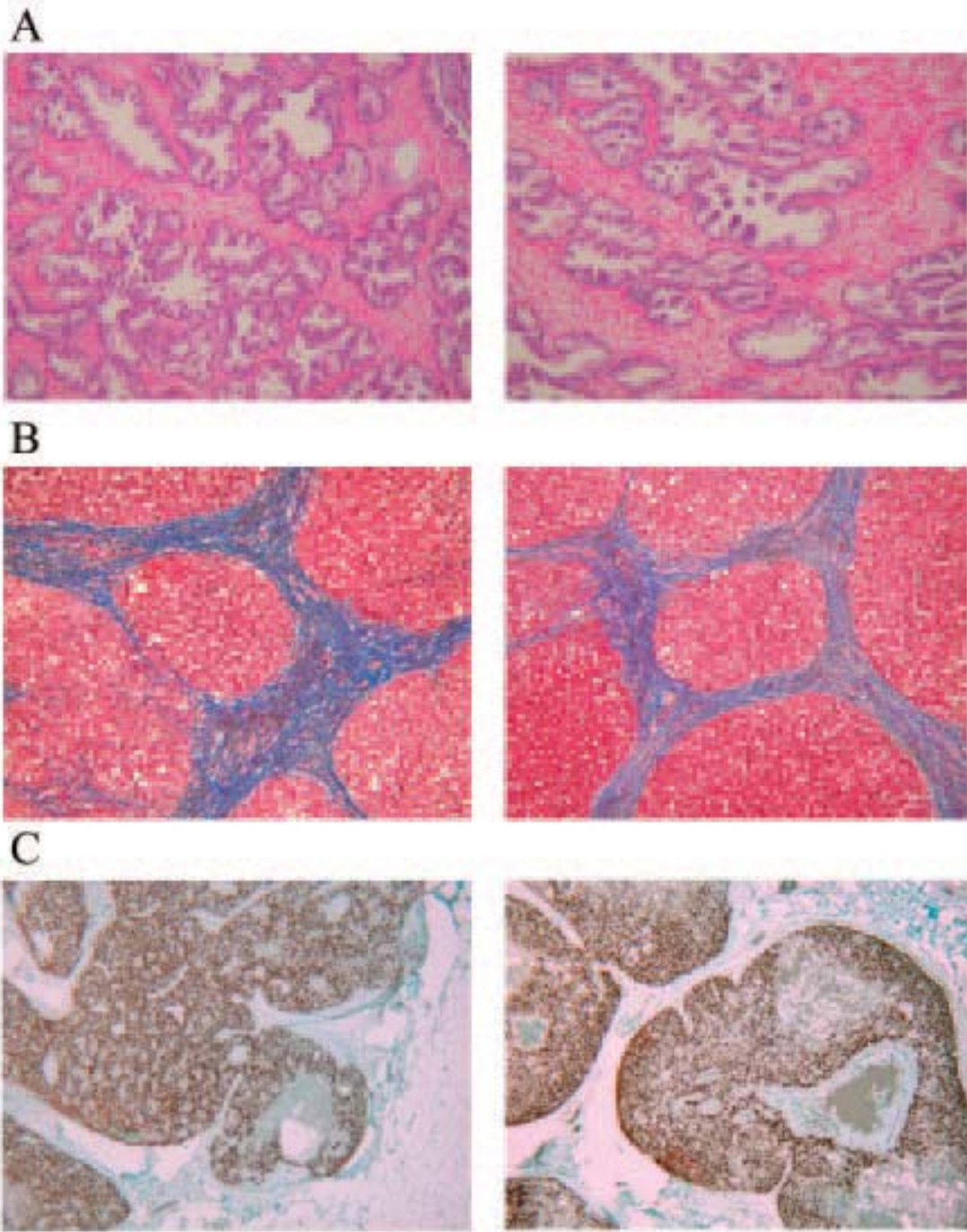


Fig. 4. (A) H&E stained slides of prostate hyperplasia, (B) cirrhosis of the liver stained with Masson's trichrome, and (C) estrogen receptor immunoreactivity in carcinoma of the breast. Sections on the right-hand side of this composite panel were processed by the conventional method and on the left, by the rapid tissue processing method. As can be appreciated, the CRTP-processed tissues have a slightly stronger affinity for the stains.

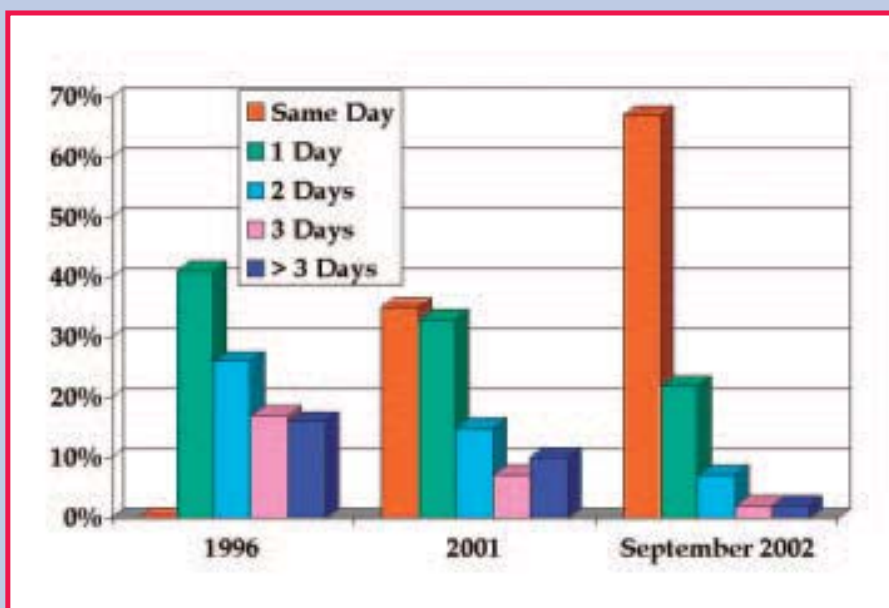


Fig. 5. This illustration shows the remarkable improvement in turnaround time of surgical reports following implementation of the rapid tissue processing method at the UM/JMH.

of toxic reagents from histopathology. Microwave procedures are conducive to that aim, as the volume of reagents used and the toxic exposure are significantly lower. In particular, replacing xylene with mineral oil⁶ or isopropyl alcohol for clearing,^{1,4,9} and excluding formaldehyde^{1,4,6,7,9} from processing via microwave methods is a welcome improvement in histology.

Cost

Microwave procedures utilize considerably smaller volumes of reagents than conventional methods, resulting in the reduction of purchase, storage, and disposal costs, which practically eliminates the need for recycling these chemicals. Published studies discuss this point but no quantitative data have been provided to substantiate the reagent savings provided by microwave methods. It is helpful to compare reagent consumption by examining a processing cycle in the VIP and the method that we are currently using, shown in Tables 1A and 1B. Every day we start with new solutions for each of the 4 retorts of the automated Continuous Rapid Tissue Processor (CRTP). These solutions are used during the entire day.

Because of the throughput of the instrument, it is possible to process up to 900 samples in an 8-hour shift with the same reagents. As can be appreciated in the tables, the savings of reagents vary according to the number of samples processed.

A significant factor affecting cost is automation, or the lack thereof. Conventional processing is carried out in automated devices requiring only the loading of specimens and reagent changes. Microwave processors, on the other hand, are labor intensive, as the samples need to be handled manually to proceed from one step to the next of the processing cycle. The single exception is the CRTP, developed by Sakura Finetek Inc., which utilizes microwave applicators originated at Microwave Materials Technology, Knoxville, TN. As previously described, this is a fully automated system where the samples are carried by a robotic arm. Reagents are placed in the instrument with ease and no technical attention during processing is required other than to load and unload the samples.

Throughput

Because commercially available tissue processors cannot be accessed with

additional samples until their processing cycle is completed, their throughput is limited. The number of samples that can be processed by any of these methods, conventional or microwave-assisted, is dependent upon the load permitted by their containers and retorts and the length of the processing cycle. Consequently, the throughput of the Sakura VIP-300 is one batch of 300 samples or less every 10 hours. For practical purposes, the samples are processed overnight and therefore, its throughput is limited to one batch per day. Shortening the processing cycle with microwave methods should theoretically increase the throughput. Unfortunately, there are no available data reported to support this notion. Visinoni et al reported a processing cycle load of up to 60 samples that can be processed in 30 to 120 minutes.⁷ The period of time required to process these samples is adjusted according to the thickness of the slices of tissue, a common practice in reported microwave methods. Moreover, throughput with these microwave methods is further limited by the requirement of tissue fixation prior to processing. Although at variance with these methods, we elected to standardize grossing and slicing of tissue samples no thicker than 1.5 mm. This may be considered a daunting task, but we have developed appropriate tools that make this easily possible. One of these tools was described in a previous publication.⁶ We believe that another distinct advantage of the system in practice at our institution is that both fixed and fresh tissues are amenable to processing. Further, the ability to introduce additional samples every 15 minutes allows for a continuous flow of specimens at a rate of 120 samples per hour. In fact, instead of accumulating tissue samples in batches, the system is available to process along with the grossing pace.

Versatility

The potential applications and versatility of microwave processing

We've got you covered.



Only Sakura gives you a choice.

Now you have a choice between two state-of-the-art instruments—one that's just right for your lab.

The Tissue-Tek® SCA™ Coverslipper, the *only* coverslipper that uses unique and proven film technology, is your choice for high-volume demands. A specially formulated film with precoated adhesive offers benefits unmatched by any other instrument. Unlike traditional methods, the drying time is substantially reduced for immediate slide review and for filing the same day! Coverslipping up to 1200 slides an hour, the Tissue-Tek® SCA™ Coverslipper produces slides that are clean and bubble free.

The Tissue-Tek® Glas™ Coverslipper is the perfect choice for lower volume labs. The instrument produces 400 slides per hour—ideal for labs where speed and turnaround time are not as critical.

Tired of downtime due to instrument failure and high maintenance costs? The Tissue-Tek® Glas™ Coverslipper features time-tested and proven Sakura robotics...and delivers the final word on performance.

For quality choices in automated coverslipping, there's really only one choice. Sakura.

*Proven
Reliability*



Sakura Finetek USA, Inc.
1750 West 214th Street
Torrance, CA 90501 U.S.A.
Phone: (800) 725-8723

Table 1. These tables illustrate the difference in volume of reagents consumed by the conventional and rapid tissue processing methods

	1 VIP*	1 CRTP**
Formalin	4	
Alcohol 80%	4	
Alcohol 95%	4	
Alcohol 100%	4	
Isopropyl ALC, Acetone & PEG		6.8
Xylene	4	
Mineral Oil		1.8
Paraffin	4	6.1
Total	24	14.7

	3 VIP*	1 CRTP**
Formalin	12	
Alcohol 80%	12	
Alcohol 95%	12	
Alcohol 100%	12	
Isopropyl ALC, Acetone & PEG		6.8
Xylene	12	
Mineral Oil		1.8
Paraffin	12	6.1
Total	72	14.7

* Sakura VIP-300 tissue processor.

** Sakura Continuous Rapid Tissue Processor.

methods are unattainable with conventional procedures. Rapid and continuous tissue processing as we presently practice at the UM/JMH, literally means no batching samples, no holding specimens, and stat capabilities. Processing is done in real time or concurrent with other histopathology activities, eliminating the need for midnight or other odd-hour shifts, or weekend duties. This method is ideal for surgical suites—we have done this in our institution, where specimens are brought directly from the patient to the

grossing station and the tissue processor, creating point-of-care surgical pathology. This practice seems ideal for surgicenters, and because of the high throughput, is convenient for both centralized hospital laboratories and independent reference laboratories with a high specimen volume. More importantly, it puts the laboratory in a unique position to study macromolecules directly from the paraffin block. Contrary to formalin fixation and conventional tissue processing, which destroy most macromolecules,

our preliminary studies show the feasibility of performing molecular assays on tissue processed by our microwave method.

Summary

Microwave-assisted tissue processing has brought a revolutionary improvement to histopathology. It is responsive to the patient and physician needs; improves utilization of reagents, while reducing or eliminating their toxicity; creates a personnel-friendly workflow; and places the laboratory in a better position to meet the demands of the rapidly expanding field of molecular medicine.

References

1. Boon ME, Kok LP, Ouwerkerk-Noordam E. Microwave-stimulated diffusion for fast processing of tissue: reduced dehydrating, clearing and impregnation times. *Histopathology*. 1986;10:303-309.
2. Boon ME, Wals-Paap C, Visinoni FA, et al. The two-step vacuum-microwave method of histoprocessing. *Eur J Morphol*. 1995;33:349-358.
3. Kok LP, Boon ME. *Microwave Cookbook for Microscopists: Art and Science of Visualization*. 3rd ed. Leyden: Coulomb Press; 1992.
4. Kok LP, Boon ME. Ultrarapid vacuum-microwave histoprocessing. *Histochem J*. 1995;27:412-419.
5. Leong AS-Y. Microwave irradiation in histopathology. *Pathol Annu*. 1998;23(pt 2):213-233.
6. Morales AR, Essenfeld H, Essenfeld E, Duboue MC, Vincek V, Nadji M. Continuous-specimen-flow, high-throughput, 1-hour tissue processing. *Arch Pathol Lab Med*. 2002;126:583-590.
7. Visinoni F, Milios J, Leong AS-Y, et al. Ultra-rapid microwave/variable pressure induced histoprocessing: description of a new tissue processor. *J Histotechnol*. 1998;21:219-224.
8. Rohr LR, Layfield LJ, Wallin D, Hardy D. A comparison of routine and rapid microwave tissue processing in a surgical pathology laboratory. *Am J Clin Pathology*. 2001;115:703-708.
9. Willis D, Hinschew J. Microwave technology in the histology laboratory. *Histologic*. 2002;35:1-5.
10. Zarbo RJ, Gephardt GN, Howanitz PJ. Intralaboratory timeliness of surgical pathology reports. *Arch Pathol Lab Med*. 1996;120:234-244.
11. Novis DA, Zarbo RJ, Saladino AJ. Interinstitutional comparison of surgical biopsy diagnosis turnaround time. *Arch Pathol Lab Med*. 1998;122:951-956.

Erratum

In the article by Joyce Hrad appearing in the Spring 2002 issue of *HistoLogic* entitled "Use of Commercially Clarified Methyl Green in the Methyl Green-Pyronin Stain," Figures 2 and 3 were reversed in error. We regret any inconvenience this may have caused the reader.

√ Mark Your Calendar! Educational Opportunities in 2003

January 15	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "Troubleshooting Special Stains for Amyloid" Speaker: Peggy Wenk, HTL(ASCP) William Beaumont Hospital, Royal Oak, MI Contact: NSH office (301) 262-6221</p>	May 16	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Cervical Cytology: The World's Best Screening Test" Speaker: Laura Mahood, MS, SCT(ASCP) Allegheny General Hospital, Pittsburgh, PA</p>
January 17	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Basic Principles of Fixation" Speaker: Barry Rittman, PhD, University of Texas Health Sciences Center at Houston</p>	May 16	<p>AMERICAN SOCIETY FOR CLINICAL PATHOLOGY Teleconference: 12:00 pm Central Time (800) 621-4142 x1260 Title: "The Theory and Practice of Heat-Induced Epitope Retrieval" Speaker: Russell Myers, PhD, Richard Allan Scientific</p>
February 19	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "Grossing to Embedding" Speaker: Suzanne Mahon, HT(ASCP) HistoSafe, Columbus, OH Contact: NSH office (301) 262-6221</p>	May 21	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "Mercury in the Histology Lab: Safety, Reduction and Disposal" Speaker: Eric A. Talley, MS Weill Medical College of Cornell University Contact: NSH office (301) 262-6221</p>
February 21	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Basic Immunohistochemistry" Speaker: M. Lamar Jones, HT(ASCP) University of Alabama at Birmingham</p>	June 6-7	<p>LOUISIANA SOCIETY FOR HISTOTECHNOLOGY Site: Kenner, LA Contact: Jane Goodman (504) 897-8830</p>
March 7-8	<p>ARKANSAS SOCIETY FOR HISTOTECHNOLOGY Site: St. Bernard's Regional Health Center, Jonesboro, AR Contact: Marty Finger (501) 686-6536 Email: FingerMarty@uams.edu</p>	June 18	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "Specimen Shipping Regulations" Speaker: Linda Durbin, EXXAKT Technologies Contact: NSH office (301) 262-6221</p>
March 7-9	<p>TENNESSEE SOCIETY FOR HISTOTECHNOLOGY Site: Gatlinburg, TN Contact: Mike LaFreniere (423) 495-6117 Email: Mike_Lafriniere@memorial.org</p>	June 20	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Handling Potentially Infectious Materials" Speaker: Maureen Doran, BS, HTL(ASCP) Southern Illinois University School of Medicine</p>
March 19	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "Role of the Histology Lab in a Bioterrorism Event" Speaker: Tim Morken, HTL(ASCP), EMT(MSA) Centers for Disease Control and Prevention Contact: NSH office (301) 262-6221</p>	July 16	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "HT Registry Readiness Workshop—The Practical Exam" Speaker: Robert Lott, HTL(ASCP) Baptist Health System, Birmingham, AL Contact: NSH office (301) 262-6221</p>
March 21	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Angiogenesis Research In Cancer Drug Discovery, Histology and Image Analysis" Speaker: Anne Lewin, BS, Bristol-Myers Squibb</p>	August 15	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Immunohistochemical Controls: Critical Evaluation of the Staining Characteristics" Speakers: David Hicks, MD, Sandra Dolar, BA, CT(ASCP) Cleveland Clinic Foundation</p>
March 28-29	<p>MISSISSIPPI SOCIETY FOR HISTOTECHNOLOGY Site: Raymond, MS Contact: Sally Jordan (601) 362-9851 Email: coffeemom@prodigy.net</p>	August 20	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "HT Registry Readiness Workshop—The Written Exam" Speaker: Robert Lott, HTL(ASCP) Baptist Health System, Birmingham, AL Contact: NSH office (301) 262-6221</p>
April 4	<p>AMERICAN SOCIETY FOR CLINICAL PATHOLOGY Teleconference: 12:00 noon Central Time (800) 621-4142 x1260 Title: "Color Your World—An Overview of Special Stains" Speaker: Kim Rhatigan-Drexler State University of NY at Stony Brook</p>	September 19	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "The Phenol Myth: New Developments in Acid Fast Staining" Speaker: Vinnie Della Speranza, MS, HTL(ASCP) Medical University of South Carolina</p>
April 5-7	<p>TEXAS SOCIETY FOR HISTOTECHNOLOGY Site: Houston, TX Contact: Donna Willis (817) 878-5644 Email: Dwillis@texashealth.org</p>	October 17	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Reductions of Medical Errors: Applications for the Histology Laboratory" Speaker: Terri Braud, HT(ASCP) University of Virginia Medical Center</p>
April 10-12	<p>GEORGIA SOCIETY FOR HISTOTECHNOLOGY Site: Atlanta, GA Contact: Connie Wavrin (404) 686-2385 Email: Connie_Wavrin@emoryhealthcare.org</p>	October 18-23	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY SYMPOSIUM/ CONVENTION Site: Louisville, KY Contact: NSH office (301) 262-6221</p>
April 11-12	<p>NSH REGION II Site: Hershey, PA Contact: Gloria Limetti (412) 647-8532 Email: glorialimetti@yahoo.com</p>	October 19	<p>OKLAHOMA SOCIETY FOR HISTOTECHNOLOGY Site: Integris Baptist Medical Center, Oklahoma City, OK Contacts: Angie Barnett (405) 748-9543 Theresa Schuldt (405) 736-0208 Email: tschuldt@rose.edu</p>
April 16	<p>NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE Title: "Immunohistochemistry in the Study of Alzheimer Disease and Parkinson Disease" Speakers: Charles White, MD, Christa Hladik, HT(ASCP) Univ of Texas Southwestern Medical School Contact: NSH office (301) 262-6221</p>	November 21	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "A Novel Spin on Making Histological Controls for Use When Staining Cytologic Samples" Speaker: Ethel Macrea, HT(ASCP), Q-IHC Ventana Medical Systems</p>
April 18	<p>UNIVERSITY OF TEXAS HEALTH SCIENCES CENTER/ SAN ANTONIO Teleconference: 12:00 pm Central Time (800) 982-8868 Title: "Taking a Look at Muscle Biopsy Procedures" Speaker: Jean Mitchell, BS, HT(ASCP) University of Wisconsin Hospital and Clinics</p>		
May 15-16	<p>ILLINOIS SOCIETY OF HISTOTECHNOLOGISTS Site: Peoria, IL Contact: Dana Spears (309) 342-2451 Email: HTLASCP@yahoo.com</p>		



Tissue-Tek® VIP™ Fixative

SAKURA



Eliminates routine warm water flushing



Salt precipitates form when 10% neutral buffered formalin is added to alcohol.

Tissue-Tek® VIP™ Fixative produces no precipitates in the presence of alcohol.

©2001 Sakura Finetek U.S.A., Inc.

A performance-proven alternative to neutral buffered formalin (NBF)

- For use with all tissue processors
- Contains Neutra-pHase, a stabilizing additive: no buffer salts added
- Compatible with—and can replace—10% NBF

Better results

- Proper ionic strength enhances cellular staining qualities in some procedures
- Increases cell wall rigidity, resulting in firmer cutting

Easy storage and disposal

- Compatible with Tissue-Tek® Neutralex™ (a formalin neutralizer) for down-the-drain disposal



SAKURA

Proven Reliability

Sakura Finetek USA, Inc.
1750 West 214th Street
Torrance, CA 90501 U.S.A.
Phone: (800) 725-8723
Phone: (310) 972-7800

Visit our web site at www.sakuraus.com

To receive your own copy of **HistoLogic**®, 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, **HistoLogic**® Editor, 165 Ashley Avenue, Suite 309, Charleston, SC 29425. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.



SAKURA

Sakura Finetek U.S.A., Inc.
1750 West 214th Street
Torrance, California 90501

PRESORTED
STANDARD
U.S. POSTAGE
PAID
PALATINE, IL
PERMIT #6

HISTOLOGIC®
Technical Bulletin for Histotechnology