Manual of Histopathological Staining Methods

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Frederick A. Putt
Department of Pathology
Yale University School of Medicine

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FOREWORD

The production of a histological section requires teamwork. Usually the person who interprets the final product also selects the tissue sample, does the initial trimming, and starts the fixation. These are, of course, critical steps and their importance to the usefulness of the final product cannot be minimized. Between sending the fixed sample to the histology laboratory and receiving the finished stained slide, however, the average pathologist or researcher may give little thought to what is happening to his sample. The histology technician is entrusted to handle "routinely" a thousand details of utmost importance. Valid interpretation of the final product depends not only on the skill of the interpreter but also on the quality of the slide. The quality must be very good. The fairly thin, fairly well mounted, fairly well stained, and fairly well cleared slide is a murky forest laced with quicksand traps and treacherous pools. The excellent preparation may not provide all the answers, but at least it puts the investigator on firm ground. This alone justifies the efforts of a hard-working, skilled technician, but there is also a bonus. A fine section is a joy to see. What esthetic pleasure, what pure fun can come from looking through a microscope to discover crisply sharp images in bright clear colours!

For several years I have had the good fortune to have slides prepared under Frederick Putt's watchful eye. Many technicians have prepared these sections, as there have been many students trained by this master. That the extremely high quality has been sustained by all is testimony to Mr. Putt's skill as a teacher. The intricacies of preparation of any given stain are many. The chemical structure of the dye (when that is known) and the exact way it functions (when that is understood) are quite interesting and important. They are explained, discussed, and debated in many large volumes. But the histology technician's problem can sometimes be more immediate—he needs to know how to make a stain "work" right now. This manual concerns itself with how to make a stain work. The

small details and little tricks that make the difference are all included. Reading from this text is the next best thing to having a master at one's elbow.

I know from experience how useful this manual can be. My copy of the first draft proved to be invaluable when I was faced with setting up a laboratory in a new medical school four years ago. Since that first draft was finished each page has been read and reread by beginners as well as experienced technicians. This volume has thus been subjected to extensive bench trials. I am confident that student technicians who are just beginning to learn their trade and experienced technicians who want to refresh their memories will find this to be a manual of unequaled value in the laboratory. I predict that very few copies will rest long in office or library bookcases, but I do hope that its publication will enable me to keep a copy near my desk. It is most aggravating to discover that my copy has once again migrated back to the lab every time I wish to consult it.

Charles B. Carrington, M.D.
Associate Professor of
Pathology and Director of
Autopsy Services
Yale University School of
Medicine

PREFACE

The histopathological staining methods presented in this manual are those with which we have had personal experience Most have been evaluated for consistency and reliability by members of the technical staff and have proved practical in routine application.

They include routine, special, and histochemical procedures as carried out in the histological laboratory in this department. Some have been modified to suit our own particular needs; others have originated from this laboratory. Classical methods which are still requested have been retained. A discussion of the all-important accessory procedures to staining, fixation, decalcification, dehydration, embedding, and sectioning is also included.

In order that a routine histological laboratory function efficiently, certain basic procedures common to various staining techniques, usually carried out in sequence, must remain fairly constant. In keeping with the needs of such a laboratory, the majority of staining methods outlined in this book can be completed during the course of a routine laboratory day. In general, the less complicated and more reproducible staining method has greater possibility of being accepted as a routine procedure. Methods that are unpredictable and that have been successful for only one or two individuals are soon rejected.

Many staining procedures share solutions in common and these could be listed together in a table. We have found it more practical, however, at the expense of repetition, to precede each method by the stated fixative of choice and the solutions necessary to complete the procedure. The reader who actually uses this manual in the laboratory will soon learn to appreciate this arrangement.

Routine, special, and histochemical methods to demonstrate related structures or tissue components are grouped together, but not in any given sequence. In some cases, more than one selection is presented in order that a choice can be made and results compared. In this manner, experience can be gained in a practical way. When checking a new or unfamiliar staining procedure, relevant control material is advisable. Because of their special applica-

tion, specific metallic and selected neuropathological staining methods are presented separately in Chapter 12. Many require special fixatives and are among the more difficult techniques to master for consistent results. A modified method to mount whole organs on paper as outlined by Gough and Wentworth is given in Chapter 14.

Very little automation has crept into the histological laboratory thus far. Preparing tissue for microscopic examination is still a practical and exacting art. Many staining methods can still be considered empirical, since the chemical basis on which many staining reactions takes place is not fully understood. Histochemical technique is based on specific reactions between chemicals or dyes and tissue components. Variation of procedure or staining method becomes necessary at times to bring about the desired results. This will depend more on experience and individual skill than on didactic description in a text.

Choice of tissue and proper fixative depends mostly on the pathologist; producing acceptable histological preparations depends on the technician. Cooperation is essential. Shoddy preparations, inadequate in detail for diagnostic purposes, are a constant source of frustration to the pathologist, all the more so if lectures, student teaching, or illustrations for publication are involved.

It would be quite impossible for any one individual to keep abreast of and evaluate current staining and related procedures as they appear in the literature. The subject is also too extensive for one text book to cover. For those inclined to pursue the subject further, a list of histological and related textbooks as well as references to the original publications consulted in the preparation of this manual are included.

Much of the content of this manual was formerly available in the form of mimeographed notes. It is hoped that assembling the material in book form will result in a basic and helpful textbook for both the students and technicians for who it is primarily intended.

In describing the various procedures and staining techniques, I have taken into consideration most of the difficulties that I have personally encountered. I have tried to present procedural details clearly and concisely in order to avoid pitfalls. I have also benefited from questions pertinent to a given procedure which have been raised by members of the staff. I wish to express my appreciation to the pathologists, technicians, and students whose stimulating questions and suggestions have been most rewarding.

I am especially grateful to Dr. Charles Carrington for his helpful criticism and advice, and to Dr. Klaus Bensch and Dr. Roy Barnett for their interest and encouragement.

I am greatly indebted to Mrs. Felicia Naumann for typing the manuscript. I also wish to thank Miss Dorothy Hyatt, Mrs. Judy Daly, and Mrs. Gail Bliss for their technical assistance. A textbook such as this is in great part based on the research and experience of many authors. I acknowledge my indebtedness to authors and publishers who have so kindly allowed me to make use of or extract material from their publications.

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