Handbook of Practical Immunohistochemistry
Preface and How to Use This Book

How Did Immunohistochemistry Evolve to Our Current Practices?

Over the past 20 years, four key discoveries can be viewed as the cornerstones in the continuing evolution of immunohistochemistry (IHC). They include (1) the development of monoclonal antibodies, significantly increasing diagnostic specificity; (2) the introduction of heat-induced and proteolytic enzyme antigen retrieval methods, providing a foundation for the utility of IHC on formalin-fixed paraffin-embedded surgical specimens; (3) the use of a highly sensitive secondary detection system, allowing detection of trace amounts of proteins in formalin-fixed tissue with little background staining; and (4) the invention of the automated immunohistochemical stainer, providing a device to run hundreds of IHC slides on the same day, in the same laboratory, with highly reproducible results. In the near future, digital pathology will certainly take IHC to a new level of practice.

Because of these advances, immunohistochemistry has been smoothly integrated into the practice of modern surgical pathology and cytopathology with regard to diagnosis, differential diagnosis, prognosis, and targeted therapy. However, there is a massive body of knowledge of IHC and new antibodies emerge continuously, challenging the general pathologist to keep current in all subspecialty areas.

What Is This Book For?

In much the same way that both positive and negative immunohistochemical results offer valuable insight into a disease process, we would like to describe for you both what this book is intended to be and what it is not. It is not an exhaustive reference work detailing the science and theory of immunohistochemistry to be read cover to cover. Many of us already own and treasure excellent volumes for this purpose. As these books have grown in size and scope, recording our expanding experience with the proteome of disease, our group felt a need for simplicity. This book is intended to be a practical, quick reference for information related to using immunohistochemistry in clinical diagnosis.

How Do I Use This Book to Find What I Am Looking For?

The concept of this book was derived from the Frequently Asked Questions (FAQs) portions of web sites, a format that has become an established and successful part of the Internet. The table of contents and chapters are organ-based and designed in a question-and-answer format.
Within each chapter, the questions are grouped and ordered by relationship to one another, so adjacent questions may provide additional information relevant to your search. The goal is to enable the reader to quickly find the specific information he or she is seeking and get back to work. The book is available in paper and electronic formats, reflecting the transitional and hybrid nature of our current information age. Some readers may find that the search function of the electronic version of the book serves them well in navigating the pages.

What Information Does the Book Contain and What Are the Unique Features of This Book?

We are all familiar with the daunting task faced as residents of learning the nuances of subspecialty diagnoses and the time-consuming reading involved in staying current as generalists while managing diverse caseloads. We all have collected stacks of notes and articles reminding us of useful antibody panels we want to remember next time. We offer this book as a “curbside consult” of practical knowledge shared by colleagues who work with these organ-specific diagnostic questions every day. The unique features of this book can be summarized as follows:

1. Question-and-answer (Q&A) format with over 1,000 questions: This Handbook is designed to be practical, concise, and credible. Most chapters are written in a Q&A (question-and-answer) format to recapitulate daily practice in surgical pathology and cytopathology, and how we think and work as pathologists.

2. List of questions on first page of each chapter: The first page of each chapter lists the FAQs about that particular organ, which provides easy access for a user. For organ-based chapters, each question is addressed in a table to provide the best answer.

3. Suggested working antibody panels: When you examine each individual table, you will notice that some of the antibodies in the table are highlighted by color. The color-highlighted set of antibodies is the suggested panel for an initial workup. Brief notes are provided for many tables, in order to reiterate the most important diagnostic applications and pitfalls that one may encounter.

4. Color pictures from Geisinger Medical Laboratories (GML) IHC slides: A fairly representative set of color pictures and diagrams, if available, is included in each chapter to illustrate some of the key antibodies used in that particular chapter. There are over 500 color pictures taken from GML IHC slides using the recommended staining protocols contained in the appendices of this book.

5. GML data: In many tables you will see a column containing data from GML in comparison to data from the literature. This is a unique feature of this book. The reproducibility of antibodies reported in the literature is sometimes in question; to improve the reproducibility, we have undertaken the daunting task of testing the antibodies listed in the appendices using more than 5,000 TMA slides (close to 100 TMA blocks) and 1,000 routine slides. These TMA sections contain thousands of tumors from various organs and normal tissues in the GML archives. If your laboratory follows the protocols in the appendices, you should obtain similar results to GML.

6. IHC on normal tissues: Immunophenotypes of many normal tissues, which receive little or no attention in other surgical pathology and IHC books, have been included in many chapters, such as normal breast, lung, pancreas, ampulla, colon, stomach, small intestine, and kidney.

7. Antibody information: The lack of detailed information about staining protocols in the literature is quite frustrating, especially when trying to reproduce published results;
therefore, we have included detailed antibody information in this book. Most antibodies mentioned in this book have been routinely used at GML, or have at least been optimized in both the Dako and Ventana systems. You will find the appendices (Appendix A, Antibodies Tested in the Dako System, and Appendix B, Antibodies Tested in the Ventana System) in the back of this book that provide detailed information for each antibody, including vendor, catalog number, clone, antigen retrieval method, antibody dilution, in vitro diagnostic use (IVD) vs. analyte-specific reagents (ASR) vs. research use only (RUO) class, staining pattern, positive control tissue, and the contact information for each vendor. No one in our group has a financial interest in any of these companies.

8. **Data interpretation**: To standardize on one manual scoring system, the following recording system is applied throughout this book, unless otherwise specified:
   
   (a) $- =$ Usually less than 5% of cases are stained
   (b) $+ =$ Usually greater than 70% of cases are stained
   (c) $+ \text{ or } - =$ Usually more than 50% but less than 70% of cases are stained
   (d) $- \text{ or } + =$ Usually less than 50% of cases are stained
   (e) $v =$ Variable, or sometimes positive; data are somewhat inconsistent
   (f) $\text{ND} =$ No data available

9. **Automated IHC – perspective from industry**: As an additional helpful note, we have included some brief chapters listing answers to questions regarding managing an immunohistochemistry laboratory. Topics include practical advice on choosing and optimizing antibody titers and retrieval methods, making choices regarding automated platforms, ways to monitor the quality of your processes and personnel, and regulatory issues related to running a clinical immunohistochemistry laboratory. We have also requested contributions from vendors to share their perspectives on where they perceive the industry to be and where the future of automating immunohistochemistry may take us.

10. **Expert contributions**: Last but not least, many chapters also include contributions from an expert in his or her field.

**What Are the Points to Remember?**

When you navigate through the chapters, you will notice (or you already have) that no single antibody is entirely specific and absolutely sensitive to a specific diagnosis. As a general rule, those initially reported as highly sensitive and specific “hot” antibodies may lose their popularity following extensive testing in various tumors and organs. Therefore, a few points should be emphasized here: (1) use of a single antibody to make a diagnosis is discouraged; instead, a small panel of antibodies should be considered; (2) if an unexpected positive or negative result occurs, proceed with caution and expand your panel; (3) one should focus on the whole picture, including clinical information, histopathology, radiological findings, and the IHC results; and (4) when things do not fit completely, go back to your H&E slides, because morphology is still the most crucial information available to us!

Since this is the first edition and the book was written within a relatively short period of time by the authors while still carrying our normal caseload, we fully expect to have some
errors or even conflicting information. The editors sincerely ask for your understanding and also invite you to submit your feedback, suggestions, and comments to us (Flin1@geisinger.edu; Jwprichard@geisinger.edu; Hliu1@geisinger.edu; Mwilkerson@geisinger.edu; Cschuerch@geisinger.edu). With support from readers like you, we are confident that future editions will be even more complete and informative.

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