Handbook of Practical Immunohistochemistry

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Editors

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Frequently Asked Questions

Third Edition

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Preface to the Third Edition

As in the second edition, the third edition of the *Handbook of Practical Immunohistochemistry: Frequently Asked Questions* is written in a question and answer (Q&A) format and intended to be a practical, user-friendly quick reference for information related to using the most up-to-date immunohistochemistry and in situ hybridization in clinical diagnosis. The new edition demonstrates a significant revision and improvement over the second edition in many ways summarized as follows:

1. More chapters: Five new chapters have been added: (1) "Immunohistochemistry: Leica's Perspective"; (2) "Immunohistochemistry: Maixin Perspective"; (3) "RNA In Situ Hybridization: Applications in Anatomic Pathology"; (4) "Applications of Rapid Immunohistochemistry on Frozen Tissue Sections During Intraoperative Pathologic Diagnosis"; and (5) Cutaneous Lymphomas.
2. RNA in situ hybridization: This important topic has been included in the third edition. We predict that this novel technology will increasingly become popular and eventually become an indispensable adjunct technique for anatomic pathologists in the coming years. Using this new technique for HPV detection in head and neck squamous cell carcinoma has been well accepted. However, our experience and the literature have demonstrated that there are many other potential applications in anatomic pathology, including (a) tumor characterization, such as detection of albumin in hepatocellular carcinoma and intrahepatic cholangiocarcinoma; (b) use when antibodies are not commercially available, such as KIM-1 in detection of clear cell and papillary renal cell carcinoma; (c) detection of gene fusion/amplification, such as ALK and MDM2, which is traditionally done by FISH and can potentially be replaced by RNA ISH; (d) kappa and lambda light chain detection in lymphomas without plasmacytic features; and (e) detection of other infectious agents, such as Merkel cell polyomavirus (MCPyV), EBV, BK, and CMV.
3. Extensive additions and changes: Significant additions and revisions have been made to many chapters. For example, in Chap. 2 ("Standardization of Diagnostic Immunohistochemistry"), we recommended using cultured cell lines as positive IHC controls to provide better quality IHC positive control blocks and to save invaluable tumor blocks for other clinical applications. We shared some very valuable internal data on testing over 100 cancer cell lines from Geisinger's IHC Laboratory and recommended a set of specific cell lines to construct positive control blocks for selected biomarkers.
4. More questions and answers: Over 150 new questions and answers have been added to the third edition. All chapters have been updated to include relevant new questions, new markers, more refined IHC panels, representative pictures, and current references.
5. More refined working antibody panels: When you examine each individual table, you will notice that some of the antibodies in the table are highlighted. The highlighted set of antibodies is the suggested and refined 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.
6. More new diagnostic and predictive biomarkers: Many recently described antibodies in clinical IHC laboratories have been added. These include both diagnostic markers and

predictive biomarkers such as INSM1, OTP, BAP-1, NUT, BCOR, CAMTA1, ETV4, H3K27me3, FOSB, SMARCA4, SMARCA2, PAX3, STAT6, NKX2.2, PAX7, PLZF, 5-hmC, NXK3.1, ATRX/DAXX, SDHB, MYB, pan-Trk, PD-L1 (SP142), PD-L1 (SP263), and albumin by RNA ISH.

7. More high-quality color pictures from Geisinger Medical Laboratories (GML) IHC slides: An extensive set of high-quality color pictures and diagnostic algorithms, if available, is included in each chapter to illustrate some of the key antibodies, including many recently discovered and substantiated antibodies used in that chapter. There are nearly 1000 color pictures, a significant increase over the second edition, taken from GML IHC, RNA ISH, and FISH slides using the recommended staining protocols contained in the appendix of this book.
8. More GML data: In many tables you will see comparisons of data from GML 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 appendix using more than 10,000 TMA slides and 2,000 routine slides. These TMA sections contain thousands of tumors from various organs and normal tissues in the GML archives. If your lab follows the protocols in the updated appendix for automated staining procedures, you should obtain results like ours.
9. Updated appendix: The updated appendix contains detailed antibody information for automated immunostaining procedures for over 250 antibodies available at Geisinger IHC Laboratory.
10. 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.
11. More current references: A large number of current references have been included in each new and updated chapter.
12. Better index: A significant amount of time and effort was devoted to creating a better and more useful index in the second edition, which got good feedback and was well accepted. Therefore, the index in the third edition remains the same, encompassing an extensive list of common and rare diagnostic entities; frequently used, rare, and newly described antibodies; and many keywords that a practicing pathologist may use to search for a specific question.
13. Updated IHCFAQ.com website: An updated free companion IHC website (IHCFAQ.com) containing additional stain images and staining protocols for many new antibodies will be available following the publication of this new edition.
14. Data interpretation: To standardize our manual scoring system, unless otherwise specified, the following criteria are applied throughout this book:
    - − = usually less than 5% of cases are stained
    - + = usually greater than 70% of cases are stained
    - + or − = usually more than 50% but less than 70% of cases are stained
    - − or + = usually less than 50% of cases are stained
    - V = variable, or sometimes positive; data are somewhat inconsistent
    - ND = no data available
15. More expert contributions: Last but not least, many chapters also include contributions from an expert in their field.

After publication of the first and second editions, we received overwhelmingly positive feedback and valuable suggestions and comments from readers, and we would like to take this opportunity to express our very sincere thanks to all of you. As always, the editors ask for your understanding of any potential errors in this new edition 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). With continued support from readers like you, we are confident that each future edition will be even more complete and informative than the previous one.

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Acknowledgments

It has been nearly 10 years since the publication of the first edition. Producing the third edition of this IHC Handbook was as challenging as publishing the second edition for our department, and we wish to acknowledge the assistance and tremendous support we received from our staff. Diana Kremitske, Vice President, Operations, Diagnostic Medicine Institute, supported and encouraged this project from conception through completion. Sandy Mullay, Operations Director of Anatomic Pathology, always ensured we had the technical, secretarial, and clerical support needed for all phases of the project. Nicole Papp served as our project coordinator, collecting hundreds of new references, scheduling numerous meetings, running CoPath searches to identify thousands of suitable cases for TMA block construction, keeping us organized, and moving the project forward. Kathy Fenstermacher was invaluable in editing, formatting, and polishing book chapters, creating an EndNote library for thousands of references, as well as producing many diagrams. Without Nicole's and Kathy's tireless efforts, it would not have been possible to meet the third edition submission deadline. John Shaw constructed many TMA blocks for testing and studying many new antibodies. Angela Bitting and Jianhui Shi are responsible for bringing you the hundreds of optimized IHC protocols available in the appendix of this book. Tina Brosious, Laurie Kneller-Walter, Deanna Ward, Beth Reigle, Denise Petrucelli, Kris Bricker, and Kathryn Powell, immunohistochemical technologists, performed IHC stains on numerous tissue microarray sections and routine slides. Mary Beagle spent countless hours pulling and refiling slides and paraffin blocks, keeping everything well organized. Christy Attinger provided expert secretarial support. Kristen Brown, System Manager for Anatomic Pathology, helped with scheduling and coordination of technical staff. In addition, we would like to thank Dr. Megan Lim, Professor and Director of Hematopathology at the University of Pennsylvania, for her thorough review and invaluable comments on the lymph node chapter. Finally, we are in debt to our families and close friends for their understanding that we have again buried ourselves in this project for such a long period of time. We are very fortunate to have your love and incredible support.

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