Leong's Manual of Diagnostic Antibodies for Immunohistology

Third Edition

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It is with much nostalgia that we dedicate the third edition of this book to Professor Anthony S.-Y. Leong, BBS, MD, FRCPA who passed away in 2011. In honor of Professor Leong's memory we have decided to entitle the book *Leong's Manual of Diagnostic Antibodies for Immunohistology*. This is a befitting tribute to Tony's brainchild, which was first published in 1999. More importantly, we are deeply indebted to his wife Wendy and children Joel and Trishe for their unconditional support to publish this third edition.

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Preface to the first edition

The rapid acceptance and entrenchment of immunohistochemistry as an important and, in some cases, indispensable adjunct to morphological examination and diagnosis has imposed the necessity for anatomical pathology laboratories to be proficient in immunostaining procedures. However, for immunohistochemical stains to be meaningful, technical competence must be accompanied by a familiarity with the characteristics and specificities of the reagents employed. In particular, the medical technologist and pathologist must have knowledge of the sensitivity and specificity of the primary antibody employed, the nature of the epitope demonstrated by each antibody and its sensitivity to common fixatives. They should be equally conversant with protocols for tissue processing as well as the various methods of antigen/epitope retrieval which are appropriate for the demonstration of the specific protein sought for in the tissue section or cell preparation.

The versatility and contributions of immunohistochemistry to diagnostic pathology, particularly in the areas of tumor diagnosis, lineage identification, prognostication and therapy are largely dependent on the ever-increasing range of antisera and monoclonal antibodies that are commercially available. However, this latter feature is a two-edged sword. While the extensive spectrum of antibodies allows the identification of a wider and wider range of cellular antigens, the user must also be familiar with the properties and characteristics of each of these many antibodies.

This book provides a comprehensive list of antisera and monoclonal antibodies that have useful diagnostic applications in tissue sections and cell preparations. Various clones, which are commercially available to detect the same antigen, are listed and the sensitivities and specificities of the antibodies are discussed. Importantly, our own experience with these reagents is provided together with pertinent references. While as many available sources of antibodies are provided, it is acknowledged that the listing cannot be exhaustive and only major sources are covered. A brief coverage of the diagnostic approach to the general categories of the poorly differentiated round cell and spindle cell tumors in various anatomical sites using panels of selected antibodies is provided in the form of tables. Staining protocols and antigen/epitope retrieval procedures including those employing enzymes, microwaves and heat are also given in detail.

It is hoped that this compendium will provide a source of useful and practical information to both the diagnostic as well as research laboratory.

Anthony S.-Y. Leong
Kumarasen Cooper
F. Joel W.-M. Leong

Preface to the second edition

Since the publication of the 1st edition of this book in 1999, the role of immunohistology in diagnostic pathology has consolidated further and continued to expand. Immunohistology has rapidly become an integral part of microscopic examination and diagnosis, occupying a position of importance only next to the hematoxylin and eosin section and usurping many of the diagnostic roles of histochemical stains and electron microscopy. There is widespread use of immunohistology in diagnostic services throughout the world and in the field of oncologic pathology and as many as 25% of tumors require immunostaining for accurate diagnosis. The percentage is considerably higher in the case of anaplastic and pleomorphic tumors where morphologic features to allow accurate typing of the tumor are absent. With the introduction of Herceptin as the first antibody treatment for cancer, immunolabeling for the Herceptin or c-*erb*B-2 antigen has empowered immunostaining even further. The detection of other antigens such as CD117 (c-*kit*) provides equally important information that predicts response to the specific therapeutic agent SYT-571. Immunostaining is a requirement for the examination of sentinel node biopsies in most treatment protocols. The role of immunostaining in cancer pathology continues to increase so that it now is employed not only for diagnosis but also for other parameters including prognosis, microscopic tumor staging, prediction of response to therapy, and for the selection of specific therapeutic agents. Immunostaining also provides invaluable information for the understanding of tumorigenesis and the identification of carrier states through the identification of gene products.

Automation in immunohistology is well established with a variety of machines employing a number of different patented techniques for antibody incubation. Such instruments have the main advantage of consistency over manual techniques rather than cost or labor savings. Many autoimmunostainers suffer from the disadvantage of rigid incubation times so that overnight incubation of primary antibodies at room temperature or at 4 °C is impossible or difficult to perform as an automated procedure. Optimal conditions for antigen detection have thus given way to expediency.

The threshold of antigen detection continues to be lowered as more sensitive detection methods are developed, the tyramide signal amplification or catalyzed reporter deposition system being a notable example. Modifications of amplification systems that employ multiple antibody layers such as the mirror image complementary antibody (MICA) detection system also continue to be introduced. Such systems increase the cost of the procedure, making it prohibitive to use routinely or in high volumes. Furthermore, they sometimes produce undesirable background staining if not optimally employed.

Undoubtedly, a major milestone in immunohistology was the introduction of heat-induced antigen retrieval, which lowered antigen detection thresholds sufficiently that most proteins of diagnostic interest could be demonstrated with standard detection systems. A variety of methods to generate heat for retrieval have been introduced with minor differences in efficacy so that the method of choice has been very much influenced by familiarity or convenience of use. This was so until the recent demonstration that superheating to 120 °C, attained under pressure, appeared to lower detection thresholds further than conventional methods. This is in keeping with the concept that both time and temperature are major influences in heat-induced antigen retrieval. The pH of the retrieval solution is another major factor, and it has been demonstrated that the majority of antigens are better enhanced following retrieval at alkaline pH. The breaking of protein cross-linkages induced by aldehyde fixatives is the prevalent concept in heat-induced antigen retrieval. However, the true mechanism of antigen retrieval continues to elude us, particularly as it has been shown that ultrasound, which generates negligible heat, can be an effective form of antigen retrieval. Furthermore, microwave heating also enhances immunostaining of tissues fixed in a non-cross-linking fixative such as ethanol,

suggesting that heating may unmask epitopes by mechanisms other than through the breakage of cross-linkages.

While the great majority of diagnostic antibodies are highly sensitive, highly specific antibodies are few and far between. Other than those raised to specific gene products most antibodies, at best, are only tissue-selective and their effective usage is to some extent dependent on knowledge of the range of tissues that the reagent may label, specific or otherwise. As often is the case, with the progression of time and usage, newly developed antibodies are found to stain an increasing range of cells besides what they were intended for. Much of this 2nd edition is devoted to this aspect of antibody properties as well as to newer applications. Pertinent references are updated and new diagnostic panels are provided. Also, newly produced antibodies are discussed, particularly as there are now a number of antibodies available that have been raised against specific gene or fusion gene products that are of diagnostic importance.

Anthony S.-Y. Leong
Kumarasen Cooper
F. Joel W.-M. Leong
October 2002

Preface to the third edition

Much of what was written in the prefaces of the first two editions is still pertinent to the practice of immunohistology today, albeit 16 years later, and will not be repeated. Runjan Chetty and Allen Gown are welcome and refreshing additions to the editorship, each bringing his own brand of expertise to this compilation, alongside contributions from many new authors. The format of the first two editions has been retained, with an alphabetic listing of antibodies enabling quick and easy reference, followed by updated tables covering common neoplasms and targeted differential diagnoses. With the burgeoning expansion of available commercial antibodies for immunohistology, this new edition has been enhanced by the addition of several new antibodies, and we are very grateful to the numerous contributors for their valued efforts. In order to maintain the size and weight of this tome, while retaining its usefulness as a manual, the number of quoted references has been restricted to a select few.

Recent years have seen an exponential growth in molecular technology, along with the development of an expanded range of targeted therapies, establishing an enhanced level of standard of care. Key to this molecular evolution, an accurate and complete surgical pathology reporting of neoplasms, supported by equally proficient and excellent immunohistology, remains the diagnostic mainstay leading to efficacious state-of-the-art clinical management of patients. The role of immunohistology as a diagnostic, prognostic, and predictive tool, and, more recently, in the identification of gene products, is still critical for patient care since this technology evolved in the 1970s.

We trust that this compendium of antibodies for the detection of tissue and cellular antigens will be practical and useful for trainees, as well as for both recently initiated and experienced pathologists.

Kumarasen Cooper
Runjan Chetty
Allen M. Gown

Note

Sources/clones lists are not exhaustive. They all refer to antibodies reactive against human epitopes; the search was performed at www.biocompare.com and www.antibodyresource .com, last accessed on November 14, 2014, from Philadelphia, USA.

Introduction

This book discusses diagnostic antibodies and antisera in alphabetical order and provides the background, applications, and diagnostic pitfalls of each reagent, together with pertinent references. Common clones of diagnostic relevance and some sources are listed, but this is not intended to be exhaustive; furthermore, it is mainly antibodies shown to be immunoreactive in fixed paraffin-embedded tissue sections that are discussed, as paraffin sections remain the mainstay of diagnostic histopathology.

Diagnostic approach

Diagnostic antibodies should not be employed in isolation, but always as part of a panel of antibodies directed to the entities considered in differential diagnosis. As the latter is derived from the cytomorphologic appearances of the tumor, it is evident that immunohistochemical diagnosis is morphology-based. For this reason we favor "immunohistology" over the more established term "immunohistochemistry," as it emphasizes the relationship of immunostaining to morphology and that immunostaining is not a test procedure but an integral component of microscopic diagnosis. To assist with the diagnostic process, both antibodies to markers recognized as being expressed by the tumor in question and those associated with entities considered in differential diagnoses should make up the panel. As markers are almost never tissue-specific, the application of a panel of antibodies will generate an immunophenotypic profile comprising both positive and negative findings which, in combination, produce the most accurate results. By defining the immunophenotype of the tissue tested, the errors of false-positive and false-negative staining will be reduced and the highest diagnostic yield obtained. For example, anaplastic large-cell lymphoma has carcinoma and melanoma as morphologic mimics. Anaplastic large-cell lymphoma may express epithelial membrane antigen (EMA) in about 45% of cases, and may fail to stain for CD45 (leukocyte common antigen) in as many cases. These findings, taken in isolation, may be mistaken for those of a carcinoma. However, if antibodies to vimentin, broad-spectrum cytokeratin, S100, and HMB-45 (melanoma-associated antigen) are also employed, the error will be averted as the profile of EMA+, CD45−, VIM+, CK−, S100−, HMB-45− fits best with that of anaplastic large-cell lymphoma, in the context of the differential diagnoses. In some situations it may be necessary to perform the immunostaining in two stages. A primary panel of antibodies provides the major categorization of the tumor, and a secondary panel allows further subtyping. For example, positivity for CD30 will be useful for the confirmation of the diagnosis of anaplastic large-cell lymphoma and lineage typing can be further performed.

As an alternative, the algorithmic approach may be adopted, but whichever approach is favored, it is important that antibodies directed to all entities considered in differential diagnosis be employed. Of course, exceptions to this rule include the application of immunostaining for prognostic markers and the identification of infectious agents in tissue sections.

Standardization and optimization of immunostaining

Much has been discussed about standardization in immunohistology, but this goal is difficult or impossible to achieve simply because fixatives, durations of fixation, and methods of tissue processing employed by laboratories are different. The ability to demonstrate various tissue antigens is very much dependent on their preservation and therefore on the method of fixation and processing employed. With the vastly different practices in laboratories throughout the world, it is clear that standardization as a goal may be impossible to achieve.

It would be more appropriate to aim for optimization of immunostaining within the individual laboratory. This means consistency, reproducibility, and the ability to obtain

the optimal results with the method of fixation and processing employed. To this end, it is necessary for each laboratory to adopt a method of fixation and tissue processing which will allow the optimal antigen preservation and yet not compromise cytomorphological preservation. It may be appropriate to examine each fixation and processing step and adjust for optimization, remembering that antigen preservation may also be influenced by the surgeon or physician who has responsibility for placing the excised specimen into the fixative, to avoid the effects of cold ischemic time on immunohistochemistry.

Antigen retrieval

It is imperative to test every new antibody on tissue blocks processed in your own laboratory. While reagent dilutions and tissue preparation instructions provided by the manufacturers are useful guides, they are universal recommendations and not individualized. It is necessary to evaluate various methods of antigen retrieval and to determine, by titration, antibody concentrations that are optimal for tissue processed in your laboratory. The introduction of the heat-induced epitope retrieval (HIER) procedure has contributed significantly to our ability to optimize immunostaining procedures, and HIER must be evaluated for each new antibody used. With very rare exceptions, we have not found HIER to be deleterious to the majority of diagnostic antigens and recommend that it be applied as a routine before any immunostaining is performed. The combination of HIER with enzymatic digestion should also be explored for some antigens. A variety of methods for HIER have become popular, including the use of microwave irradiation, pressure cooker heating, steaming, wet autoclaving, and simple boiling. While there continues to be debate on the actual mechanism of antigen retrieval induced by heating of deparaffinized tissue sections and the role of microwave irradiation, there is general agreement that the threshold of antigen staining is largely dependent on both temperature and the duration of heating. Superheating to 120 °C, attained under pressure, produces the most effective antigen retrieval.

Controls

Diagnostic interpretation in immunohistology includes the assessment of internal positive control cells or tissues. Many test sections contain normal tissue that expresses the antigen being tested. Positive controls should also be used routinely in each antibody staining run, remembering that it is more appropriate to employ neoplastic tissues known to express equivalent amounts of the antigen tested rather than non-lesional tissues that may express much higher levels of antigen. A negative control of tissues known not to express the antigen should also be employed. In addition, a nonspecific negative reagent control should be employed in place of the primary antibody to evaluate nonspecific staining. Ideally, a negative reagent control contains the same isotype as the primary antibody but exhibits no specific reactivity with human tissues in the same matrix or solutions as the primary antibody. All control tissues should be fixed, processed, and embedded in a manner identical to the test sample.

In addition to these technical aspects, consideration should also be given to the nature of the diagnostic specificity of the antibodies used and the properties of the target antigen. Much of this information is theoretical and beyond the control of the diagnostic laboratory. Nonetheless, you should have some familiarity with this aspect of the reagents. The information is often available in the literature, and it may be found in the product profiles provided by the manufacturer.

It is clear from the foregoing that immunohistology is not a simple matter of a positive or negative stain. While it is a powerful diagnostic tool, immunostaining is only an adjunct to histologic examination and requires careful optimization if it is to produce the highest diagnostic yield.

This book contains antibodies and antisera which we consider to be of diagnostic relevance. With the exception of a small number, such as the cytokeratins and the pituitary and pancreatic hormones, the antibodies are discussed separately and listed in alphabetical order for easy reference. The antibodies are listed under their main and alternate names, but specific clone numbers are not indexed.

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