Natasha Rekhtman
Marina K Baine
Justin A. Bishop

Quick Reference Handbook for Surgical Pathologists

Second Edition

Springer

The Art of Writing a Pathology Report:
What we Say … and What we Mean ☺

by Natasha Rekhtman, Diana Molavi, and Justin Bishop

What we Say:	What we Mean:
This is a difficult case	I have no idea what this is
This lesion is difficult to classify	I am not familiar with the new WHO classification
The differential diagnosis includes …	Your guess is as good as mine
Dr. X concurs with the diagnosis	Sure am glad somebody else here knows what this thing is
Case was shown at the quality assurance conference	We're all going down together
Invasion cannot be excluded	Probably invasive but don't feel like searching too hard … and it's time for my coffee break!
Recommend clinical correlation	Not my problem anymore!
Lesion is best seen on permanent sections	We missed it on frozen
Defer to permanents (as for thyroid frozen)	Maybe if I keep saying this … they will stop sending these?
Stains are suboptimal	Did not work at all
Stains are non-contributory	Stained the wrong block … or ordered the wrong antibody
Stains are non-evaluable	Forgot to order
Tissue with cautery artifact	Puh-leeze! Turn down that bovie!
Evaluation limited by processing artifact	Regular histotech is on vacation
Tumor approaches the margin	Positive margin but am going to dinner with the surgeon, so gotta be nice…
Tumor approaches the margin (#2)	Positive margin but am afraid of the surgeon
Focal acute appendicitis	Found a poly for you! You're welcome
An AFB stain is negative	But please don't hold me to it
Multiple step levels were examined	No, still not there. I am a pathologist, not a magician.
Representative sections submitted	One
Innumerable (as in polyps or mitotic figures)	More than 10
Rare (as in mitoses)	I didn't see any, but if I say zero there will be three on the first field when I show this case
Specimen did not survive processing	Was dropped on the floor and stepped on
Specimen was entirely submitted	Can't send me back to the bucket!
Possible lymph nodes (grossly)	Hunks of fat that I did not bother to dissect
Conservative re-excision is recommended	I forgot to ink the margins

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To Bob, Iris, Mark, Galina, and Katya.

Natasha Rekhtman

To Ian, Sophie, and Lily.

Marina K Baine

To Ashley, Riley, Avery, and Rory.

Justin A. Bishop

Preface to Second Edition

Welcome to the second edition of QRHSP! It has been over 7 years since the publication of the first edition in 2011. One of the great challenges in a book of this nature is that some of the content (e.g., IHC, molecular, grading) evolves rapidly, and some information inevitably goes out of date as soon as the book is published. Nevertheless, many general concepts and approaches covered here are fundamental and lasting, and provide a foundation on which future markers and applications can be built.

It has been immensely gratifying to know that the first edition of this book was useful to many of you, and that it has become a part of how many residents learn pathology. Although much of my clinical and academic work in the last decade has been focused on thoracic pathology and cytopathology, it still brings me great joy to work on this book. I love the process of distilling complex information into simple summaries and diagrams – which is the foundation of this book, and it has been a rewarding experience to go through this process with many talented contributors that worked with me on the current edition. I hope that this updated edition will continue to be your friend that you can count on when first learning a subject, preparing for the boards, or trying to remember that pesky IHC marker, fusion, or relevant syndrome while looking at a case.

The new edition follows largely the same framework as the first one, but now with thoroughly updated content. We have included many new markers that have entered practice in recent years (like p40, GATA3, SOX10, ERG, SF1, BAP1, and many others), added various newly described entities, like Ewing-like sarcomas with novel translocations and SDH-deficient neoplasms, and updated and expanded many IHC differentials, particularly in thoracic and ENT sections, inevitably reflecting the current focus of the main authors. Many other sections were significantly revised and updated. In particular the section on tumor genetics and cytogenetics was thoroughly updated and reorganized, such that the summaries are now organ-based, but include handy summaries and diagrams to help you remember which alterations are shared among different tumors. We also added quick summaries for a number of brand-new topics, such as the uses of IHC to detect hereditary tumor syndromes and quick reference for predictive markers and targeted therapies. Last, but not least, this edition contains significantly updated and expanded hematopathology sections. There are many new cartoon drawings which I have always found to help me remember complex information. Please check out the new cartoon for markers for carcinomas of unknown primary, many new cartoons in the glossary section, and a great new diagram for neck lymph node metastases created by Justin – it will help you think of the differential diagnosis based on which node is involved (it is a complex subject when you read it as dense paragraphs of text, but – as we hope is true for the rest of this book – it becomes very simple and easy to remember when summarized as a logical diagram).

As before, our approach was to capture the material that is difficult to keep in active memory, and for which well-organized quick references may be helpful. This book almost exclusively deals with neoplastic pathology, and is unique in that we cover not only pathology but also useful clinical information that can help you in making the diagnosis (like serologic tumor markers, patterns of metastases, syndromes). Most of the content in this book is at the level of a senior resident, but it is also useful as a quick reminder for a general pathologist. For the beginning resident, some of the differentials in IHC sections will make sense only after you have learned the entities; instead, start with the introductory material – IHC primers, potpourri of morphologic references and the glossary, and advance to other sections later in your training. As with the first edition, we did our best to vet out the content that may be too esoteric, marginally useful, or investigational. Many of these judgments are highly subjective and individual practice dependent (and I learned, increasingly difficult to apply to your favorite subspecialty area). As before, we apologize for any omissions, as some are unavoidable by the nature of this book.

I feel beyond fortunate to have had many extremely talented contributors participate in this edition, who made it possible for the update to come together. I particularly want to thank my main co-authors and co-editors Marina Baine and Justin Bishop, and other lead contributors – Jason Chang, Youran Zou, Xiaojun Wu, and Zenggang Pan. Special thanks to Marina Baine, who was the tireless lead contributor for multiple chapters. Also, many thanks to Diana Molavi, Shien Micchelli, and Laura Favazza for reviewing various portions of this edition and providing helpful feedback, and to my colleagues and trainees at MSKCC from whom I learn and get inspiration daily.

Last but not least, thanks to all of the readers for the feedback over the years. I greatly appreciate everyone who contacted me with specific suggestions and corrections, which we did our best to address in the current edition. Your feedback for this edition will be appreciated (rekhtman@mskcc.org).

Natasha Rekhtman

Acknowledgments

In addition to thanking all contributors to the current edition, many thanks to everyone who contributed to the first edition: Jennifer Broussard, Terina S. Chen, Amy S. Duffield, Tara Nikole Miller, Ross Allen Miller, and Janis M. Taube.

We would also like to thank the Springer team - Richard Hruska (Executive Editor), Barbara Lopez-Lucio (Developmental Editor), and Project Managers Rachel Taenzler, Kelita Katylin, and Prakash Jagannathan - for all their efforts with this project. Natasha also thanks Francis Bodd at Memorial Sloan Kettering Cancer Center for excellent editorial assistance.

Natasha Rekhtman, Marina Baine, Justin Bishop

Preface to First Edition

About This Book

This book is a compilation of high-yield at-a-glance summaries for various topics frequently needed in a quick reference format at the microscope (or when cramming for the boards). As recently minted pathologists, we compiled this book from the perspective of pathologists-in-training and we gathered topics which we wanted to have in quick summary format during our recent residency and fellowships. Although written with the trainees in mind, the book may also be of interest to practicing pathologists as a practical quick reference by the microscope.

The book has a unique layout in that most of the information is presented in tables and diagrams accompanied by minimal explanatory text. Our motto for this book was to boil the information down to the essentials and key elements but with just enough commentary to be accessible to a newcomer to pathology. This book is not intended as a substitute for original resources or authoritative texts, but rather its purpose is to bring under one roof compact summaries for various types of information that trainees and practicing pathologists now search for in many different sources, and give the conceptual "lay of the land" with emphasis on "must know" facts. Certainly decisions about what constitutes "must know" and "high-yield" are highly subjective, and we apologize for any omissions which are inevitable by the nature of this book.

Our other main objective was to make the format of the book as user-friendly and easy to navigate as possible, such that one can quickly find the needed information. We thank our Springer editors for agreeing to publish ths book in a non-standard format to help achieve this goal.

Content

The focus is not organ-based morphologic criteria for which there are many excellent quick-summary resources, but rather the focus is everything else that helps a pathologist make a diagnosis (and pass the boards) with emphasis on the vast and fast-growing fields of immunohistochemistry (IHC) and molecular markers.

The book starts with unique introductory "primers" – at-a-glance 1-page summaries with diagrams on the main types of marker applications and high-yield facts (such as peculiar principles of cytokeratin designation). We highlighted the rules and biological principles behind various immunostains and special stains to help residents reason through a problem rather than having to resort to memorized panels. The other part of the IHC section contains a large compilation of general and organ-based applications of IHC with numerous immunopanels. This includes the classics (such as lung adenocarcinoma versus mesothelioma) and more recent applications (such as the work-up for mismatch repair proteins).

Other sections of the book contain various quick references that are often needed at the microscope but require frequent reminders. This includes a compilation of grading systems, common prognostic systems, and other criteria that are difficult to keep committed to memory (such as size cut-points for various micro-entities like thyroid papillary microcarcinoma). Also included are summaries for tumor syndromes with a particularly practical "slide-to-syndrome" summary where we highlighted which diagnoses or features should trigger consideration of a syndrome. In tumor genetics and cytogenetics we highlighted which tumors have unique molecular characteristics that can aid in the diagnosis or are used in prognostic/predictive testing.

Another high-yield section that is not usually covered in most pathology books is a compilation of quick clinical references geared for pathologists. This section contains resources that help pathologists interpret clinical information that may be highly informative in the differential diagnosis of tumors, including a primer on metastasis (what metastatic patterns are classic vs. exceptional for certain tumors) and serologic tumor markers. We also included a brief summary of targeted therapies for which pathologists may be asked to perform predictive marker testing.

Even though the focus of the book is not organ-based morphologic criteria, we included several sections with differentials that cut across all organs. For example, this section includes at-a-glance differentials for small round blue cell tumors, and classic differentials for certain morphologic features (such as which tumors are classically associated with granulomas or have staghorn vessels). We also included an illustrated guide to microorganisms. Finally, we compiled an illustrated glossary of histopathologic descriptors with illustrations of common objects these terms are said to resemble (such as storiform or palisaded, and what Orphan Annie's eyes actually look like!). Keep this by your side as you begin to tackle the large pathology books! We are also very excited to include a handy guide for pathology web resources by Terina Chen and a user-friendly CPT coding summary by Diana Molavi.

Sources

We used a variety of sources, including standard books and mountains of primary literature. However, most importantly our "world view" of pathology this early in our careers comes primarily from our outstanding teachers at The Johns Hopkins Hospital and Memorial Sloan-Kettering Cancer Center. From them we learned the approaches and principles that come only after years of

experience but cannot be learned by reading books and papers. We were fortunate to learn pathology from these brilliant diagnosticians and generous educators, who shared their knowledge with us through sign outs, lectures and weekly unknowns during our residency at Johns Hopkins. We therefore can only take credit for organizing and presenting this stream of knowledge in a format easily accessible to a newcomer to pathology, and we give all credit for the many useful pearls and principles in this book to our teachers. On the other hand, we take full responsibility for any inaccuracies that may have inadvertently escaped our attention.

In Conclusion

It is our hope that this book will be your best friend both at the microscope and in the late night hours of studying for the boards. Because the type of information covered in this book is rapidly evolving, please be sure to check the most current sources.

Natasha Rekhtman and Justin Bishop

How This Book Came About – Part 1

I started working on this book in my second year of residency at The Johns Hopkins Hospital, although at that time I did not yet know that this was what I was doing. Like many pathologists, I am a very visual learner, and I firmly believe that a good table or diagram is worth many pages of text. Therefore I was desperately looking for resources that succinctly summarized the mountains of information I was trying to absorb, particularly in a format that was tabular or diagrammatic and was amenable to quick learning of the essentials. While there were many great resources for histologic criteria, what I felt was missing were quick references for the new and fast growing fields of immunostains and molecular markers, as well as other types of material frequently needed in pathologists' daily work but not available in a single source. I therefore started compiling these summaries and diagrams for my own use, and later started sharing them with my co-residents. After getting feedback that others were findings these summaries useful, and after I realized that creating them was an incredible motivator to learn and digest the information, I put together a small handbook which was generously printed by the Department of Pathology at Johns Hopkins as a Resident Manual in 2004 and 2007. Now in collaboration with Justin Bishop as my coeditor and main coauthor and with contributions from many former and current Hopkins residents and fellows and my current colleagues at Memorial Sloan-Kettering Cancer Center, this book has morphed into what it is today. Justin joined forces with me in the last two years, and I could not have dreamt of a more dedicated and talented collaborator, who made it possible to get this project completed.

Natasha Rekhtman

How This Book Came About – Part 2

My first interaction with this book (universally known as the "Green Book" at Hopkins) was in 2006. The more senior residents had copies of a magical book that had all the answers I was seeking as a pathology intern. Desperate for something to boil down the massive amounts of information into one resource, my fellow first-year residents and I assembled crude bootleg copies of it. At the end of that year as she left Hopkins, Natasha distributed a new edition which remains a fixture at my microscope to this day. However, as the years passed and new waves of residents entered our program, original copies of the Green Book became increasingly scarce, and the quality of copies became increasingly poor as they became 2nd and 3rd generation. My chief resident year, I was frequently confronted with a question from the junior residents: "Where can I get a copy of that Green Book?" We had heard rumors about the possibility of it being published, but no one at Hopkins knew the status of the now-mythical Green Book. Intent on getting an answer, I contacted Natasha. As luck would have it, she needed a collaborator to push the project past the finish line, and that collaborator became me. Initially a great way to study for my boards, working on the book then became a means to stay on top of the newest information as I started signing out surgical pathology. Although perhaps it was a bigger commitment than I initially realized, it was well worth the effort, and I am extremely grateful to Natasha for allowing me to be a part of this very special project.

Justin Bishop

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Common Abbreviations and Designations

**See IHC index for alternative designations of antibodies/antigens

AdenoCA – Adenocarcinoma
AFIP – Armed Forces Institute of Pathology
AJCC – American Joint Committee on Cancer
ALCL – Anaplastic large cell lymphoma
ALL – Acute lymphoblastic leukemia/lymphoma
AML – Acute myeloid leukemia
AT/RT – Atypical teratoid rhabdoid tumor
Bx – Biopsy
CA – Carcinoma
cc or CC – Clear cell
CD – Cluster of differentiation (as in CD3, CD20, etc.)
CHR – Chromogranin
CIS – Carcinoma in situ
CK – Cytokeratin(s)
CLL/SLL – Chronic lymphocytic leukemia/small lymphocytic lymphoma
CMV – Cytomegalovirus
CNS – Central nervous system
CRC – Colorectal carcinoma
CT – Computed tomography
DCIS – Ductal carcinoma in situ
DDx – Differential diagnosis
DFSP – Dermatofibrosarcoma protuberans
Diff – Differentiation
DLBCL – Diffuse large B cell lymphoma
DNA – Deoxyribonucleic acid
Dx – Diagnosis
EBV – Epstein-Barr virus
EM – Electron microscopy
ER – Estrogen receptor
ESS – Endometrial stromal sarcoma
FL – Follicular lymphoma
GBM – Glioblastoma multiforme
GCT – Germ cell tumor
GI – Gastrointestinal
GIST – Gastrointestinal stromal tumor
GU – Genitourinary
GYN – Gynecologic
HCC – Hepatocellular carcinoma
H&E – Hematoxylin and eosin
Heme – Hematopathology
HHV8 – Human herpesvirus 8
HMWCK – High molecular weight cytokeratins
HPF – High-power field (40X)
HPC – Hemangiopericytoma
HPV – Human papillomavirus
HSV – Herpes simplex virus
HTLV – Human T-lymphotropic virus
ID – Identification or identify
IHC – Immunohistochemistry
IMT – Inflammatory myofibroblastic tumor
IPMN – Intraductal papillary mucinous neoplasm
ISH – In situ hybridization
LMWCK – Low molecular weight cytokeratins
LN – Lymph node
LSIL – Low-grade squamous intraepithelial lesion
MALT – Mucosa-associated lymphoid tissue
MCL – Mantle cell lymphoma
MCN – Mucinous cystic neoplasm
MD – Moderately differentiated
ME – Myoepithelial
Met – Metastasis
MPNST – Malignant peripheral nerve sheath tumor
MRT – Malignant rhabdoid tumor
MZL – Marginal zone lymphoma
NE – Neuroendocrine
NET – Neuroendocrine tumor
NK – Natural killer
NLP-HL – Nodular lymphocyte predominant Hodgkin lymphoma
NOS – Not otherwise specified
PanNET – Pancreatic neuroendocrine tumor
PCR – Polymerase chain reaction
PD – Poorly differentiated
PEComa – Perivascular epithelioid cell tumor
PET – Positron emission tomography
PNET – Primitive neuroectodermal tumor
PR – Progesterone receptor
PTC – Papillary thyroid carcinoma
RBC – Red blood cell
RCC – Renal cell carcinoma
R-S cell – Reed-Sternberg cell
Rx – Therapy, treatment
SCCOHT – Small cell carcinoma of the ovary, hypercalcemic type
SFT – Solitary fibrous tumor
SCLC – Small cell lung carcinoma
SmCC – Small cell carcinoma
SqCC – Squamous cell carcinoma
SRBCT – Small round blue cell tumor
SYN – Synaptophysin
TB – Tuberculosis
UC – Urothelial carcinoma
undif. – Undifferentiated
vs. – Versus
WD – Well differentiated
WHO – World Health Organization

Immunohistochemistry reactivity code

+++	Overexpressed or consistently diffuse
+	Positive
+/–	Usually positive
–/+	Usually negative
–	Negative

(F+ = focally positive)

Chapter 1. Immunostains: Introduction

By Natasha Rekhtman, Marina K Baine, Youran Zou, Justin A. Bishop

Applications of Immunohistochemistry (IHC) in Anatomic Pathology

(select examples)

1. **Diagnosis of Tumors:**
   - **(a) Classification of poorly differentiated neoplasms:**
     - Carcinoma (cytokeratin+)
     - Lymphoma (CD45+)
     - Melanoma (SOX10/S100/Melan-A/HMB45+)
     - Others
   - **(b) Diagnosis of carcinoma of unknown primary:**
     - Lung (TTF-1/Napsin-A+)
     - Thyroid (PAX8/TTF-1+)
     - Prostate (PSA/NKX3.1+)
     - Colon (CDX2+)
     - Many others
   - **(c) Diagnosis of invasion:**
     - Loss of myoepithelial cells (breast cancer)
     - Loss of basal cells (prostate cancer)
     - Loss of basement membrane/collagen type IV (various carcinomas, rarely used)

2. **Assessment of Markers Reflecting Prognosis ("Prognostic" Markers):**
   - Ki67/MIB1 (general proliferation marker)
   - HER2 (adverse prognosis in breast and gastric cancer) – also predictive marker
   - CD38 (adverse prognosis in chronic lymphocytic leukemia)
   - Others

3. **Assessment of Markers Reflecting a Therapeutic Response ("Predictive" or "Theranostic" Markers)[1]:**
   - ER/PR (tamoxifen for breast cancer)
   - HER2 (Herceptin for breast cancer and gastric cancer)
   - ALK (crizotinib, recently ceritinib for lung carcinomas with ALK fusions)
   - PD-L1 (PD-1/PD-L1 inhibitory monoclonal antibodies for non-small cell lung carcinomas and other tumors)
   - Others

4. **Detection of Micrometastases:**
   - Melanoma (melanocytic markers)
   - Breast cancer (cytokeratins)

5. **Identification of Infectious Organisms[2]:**
   - Viruses (HSV, CMV, adenovirus, SV40 for *Polyomavirus*/JC virus)
   - Bacteria (*H. pylori*, anti-treponemal antibody for syphilis)
   - Other organisms (*Toxoplasma*)

1. Many predictive markers are assessed by molecular methods. Examples listed here are for markers assessed by IHC, more on this in Chapter 4.
2. IHC has recently become more widely used in identification of organisms in tissue, particularly when assessing for viral infection in transplanted organs (e.g., CMV and adenovirus in kidney transplant) or otherwise immunocompromised patients. However, in situ hybridization for some viruses is still the preferred method (EBV, HPV). For the vast majority of bacteria and fungi, the use of special stains remains to be the preferred method (GMS, AFB, etc.).

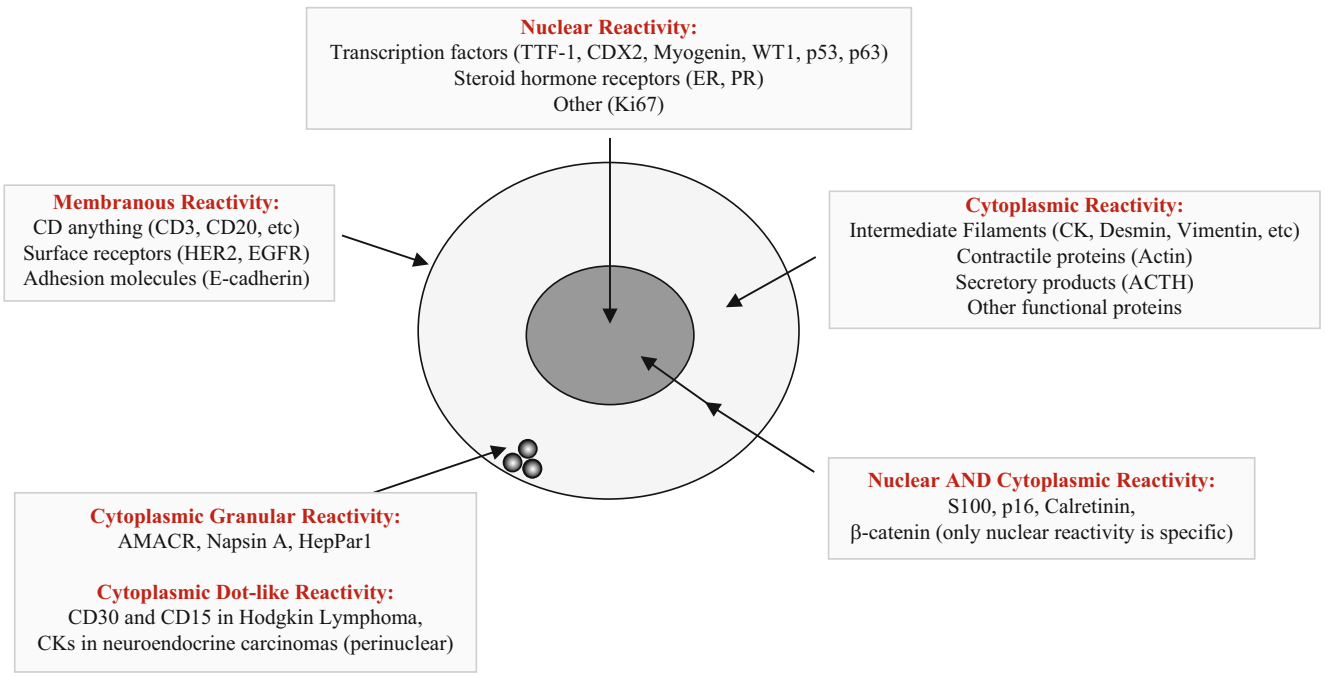
N. Rekhtman et al., *Quick Reference Handbook for Surgical Pathologists*, https://doi.org/10.1007/978-3-319-97508-5_1

Markers of Differentiation at a Glance

Differentiation	*Markers*
Mesenchymal	Vimentin (generally not useful for diagnosis)
Epithelial	Cytokeratins, EMA *[see epithelial primer]*
Smooth muscle	Desmin, muscle-specific actin, smooth muscle actin, calponin, h-caldesmon, smooth muscle myosin heavy chain *[see muscle primer]*
Skeletal muscle	Desmin, muscle-specific actin, myogenin, MyoD *[see muscle primer]*
Myofibroblastic	Partial smooth muscle phenotype: actins (MSA, SMA) in "tram-track" distribution and calponin but not h-caldesmon *[see muscle primer]*
Myoepithelial	Polyphenotypic markers: smooth muscle (complete phenotype – smooth muscle actin, calponin, others), neural (S100), glial (GFAP), epithelial (CK), and basal/stem cell factor (p40/p63) *[see muscle primer]*
Endothelial	CD34, CD31, ERG, Fli-1, D2-40 (lymphatic), Factor VIII (outdated) *[see vascular primer]*
Lipomatous	S100 (IHC generally not used)
Melanocytic	SOX10, S100, HMB45, Melan-A/MART-1, MITF, tyrosinase *[see melanocytic primer]*
Neuroendocrine	SYN, CHR, CD56, INSM1 *[see neuroendocrine primer]*
Glial	GFAP, OLIG2 *[see neuroglial primer]*
Neuronal	Neurofilament, NeuN, SYN *[see neuroglial primer]*
Nerve sheath (Schwannian)	SOX10, S100 *[see neuroglial primer]*
Serous acinar cells	PAS (general); BCL10, trypsin, chymotrypsin, and lipase (pancreas); SOX10 and DOG-1 (salivary)
Hematopoietic	Pan-hematopoietic: CD45/LCA Pan-B cell: CD20, CD19, CD79a, PAX5 Pan-T cell: CD3, CD43 Plasma cell: CD138, κ/λ light chains (normal κ:λ ratio is 2–3:1) Myeloid: CD43, CD117/c-kit, CD34, MPO MANY others *[see hemepath section]*
Histiocytic	CD68, CD163, enzymes (lysozyme/muramidase, α1-antitrypsin) *[see hemepath section]*

Location, Location, Location! Primer on Location of Antigens

- In order to properly interpret immunoreactivity, it is important to know the expected location of the antigen of interest. Knowing the biological function of a molecule of interest can be very helpful in intuitively anticipating the site of reactivity.
- Transcription factors (TTF-1, CDX2, myogenin, PAX8, WT1, p53, p63/p40) and steroid hormone receptors (ER, PR, AR) function in the nucleus, and therefore the expected IHC signal is **nuclear**. Proliferation marker Ki67 (MIB1) is also nuclear (except for peculiar membranous/cytoplasmic reactivity in hyalinizing trabecular tumor/adenoma of thyroid).
- In contrast, cytoskeletal, contractile, and other functional proteins are **cytoplasmic**. In fact, the majority of antigens in current use are cytoplasmic. This category includes all intermediate filaments (CK, desmin, vimentin, GFAP, neurofilament), contractile proteins (actin), melanosome-associated proteins (HMB45, Melan-A), secretory products (ACTH, trypsin), and various other functional molecules.
- **Membranous** reactivity is expected for receptors (EGFR), adhesion molecules (E-cadherin), and other surface molecules. This category includes virtually all CD (cluster of differentiation) antigens, such as CD3 (T-cell marker) and CD20 (B-cell marker). Occasionally, membranous reactivity may be difficult to distinguish from cytoplasmic signal; this distinction is important for several molecules where only membranous but not cytoplasmic reactivity counts as specific (HER2, EGFR).
- Although rare, several antigens have a characteristic **combined nuclear AND cytoplasmic** reactivity. This category most notably includes **S100**, **p16**, and **calretinin** (calretinin must be BOTH cytoplasmic and nuclear to be interpreted a positive staining in mesothelioma). **β-catenin** is membranous in most normal epithelia and cytoplasmic in stromal cells. However, it is the shift to nuclear reactivity that is a characteristic feature of several tumor types associated with mutations in adenomatosis polyposis coli (*APC*) or *CTNNB1* (encodes β-catenin) genes in the WNT pathway, such as desmoid-type fibromatosis and some colon CAs.
- **Granular** reactivity usually indicates localization to cytoplasmic organelles (mitochondria, Golgi, secretory vesicles, etc.). Distinctive granular cytoplasmic reactivity is typical of AMACR/racemase (mitochondrial/peroxisomal), HepPar1 (mitochondrial), and Napsin A (lysosomal). Dot-like CD30 in ALCL and classical HL and CD15 in classical HL are attributed to Golgi staining and are seen in conjunction with typical membranous staining generating the so-called "targetoid" or "ball and chain" appearance.
- Finally, **punctate** (aka perinuclear dot-like) reactivity is typical of some antigens that aggregate in the cytoplasm, most notably CK pattern in neuroendocrine CAs, including SmCC (pan-CK) and Merkel cell CA (CK20). This occurs due to the formation of CK tangles.
- ALK stain may show different patterns of staining in different tumors depending on the translocation partner!
- Note that there are some instances in which the **lack of immunoreactivity** is what is significant. One example is the loss of E-cadherin in lobular CA of the breast. Another is the loss of SMARCB1 (INI1) and rarely SMARCA4 (BRG1) in malignant rhabdoid tumors, as well as many other recently identified INI1/BRG1-deficient tumors.
- Beware of classic **false positives** (tissue edge effect, mast cell positivity, non-specific staining of hepatocytes due to high albumin content, positive staining in entrapped benign cells like TTF-1 staining of entrapped pneumocytes in tumors metastatic to the lung) and classic **false negative** as a result of failed IHC (always check controls, particularly normal structures serving as internal positive controls). Also beware of non-specific cytoplasmic reactivity for antigens with expected nuclear localization (such as TTF-1 or ER) – this should not be accepted as positive!

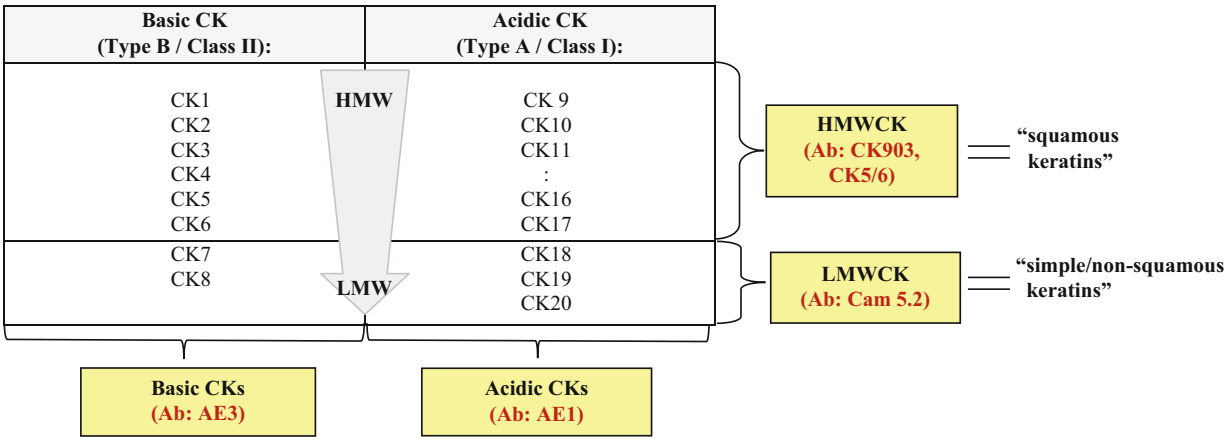


Abbreviations: *ALCL* anaplastic large cell lymphoma, *HL* Hodgkin lymphoma, *CK* cytokeratins

Primer on Cytokeratins

(*currently preferred designation for cytokeratins is “keratins”)

- Cytokeratins (CK) are cytoskeletal proteins that belong to a family of intermediate filaments (IFs). CKs are present in epithelial cells and are regarded as the most fundamental markers of epithelial differentiation. Other members of IF family are also used as markers of differentiation, including vimentin (for mesenchyme), GFAP (for glia), desmin (for muscle), and neurofilament (for neurons).
- There are 20 distinct types of CKs (plus hair- and nail-specific CKs).
- CKs were characterized by Moll et al., and the currently used CK designation system is known as the “Moll’s catalogue” [1].
- CKs are designated in a somewhat non-intuitive fashion based on their migration pattern in a two-dimensional (2D) gel electrophoresis, which separates proteins based on size and charge.
- Based on the 2D gel migration, CKs fall into two categories: basic (CK1 through 8) and acidic (CK9 through 20). Within each group, CKs are numbered in order of decreasing size, from high molecular weight (HMW) to low molecular weight (LMW), as diagramed below.



- In a cell, CKs exist as heterodimers, composed of acidic + basic subunits of a similar size. Therefore certain pairs of CKs (such as CK8/18, CK1/10) are expressed jointly.
- For diagnostic purposes, CKs are divided into LMWCK and HMWCK, as indicated on the diagram. This division corresponds to a distinct distribution of these two groups of CKs in normal tissues:
  - **HMWCK**s are expressed predominantly in squamous epithelia (and in basal cells), and they are known as “squamous keratins.” HMWCKs are large, and they are able to form a dense cytoplasmic network of filaments, accounting for resistance to mechanical stress of the surface epithelia. Large bundles of HMWCKs are known ultrastructurally (by electron microscopy) as “tonofilaments,” and these structures are the hallmark of squamous epithelia and SqCC.
  - In contrast, **LMWCKs** are loosely distributed in the cytoplasm and are unable to bundle. They are therefore characteristics of visceral organs, which experience little mechanical stress (such as the liver, kidney, and various glandular epithelia). LMWCKs are known as “non-squamous or simple keratins.” LMWCKs are expressed in all epithelial tissues with the exception of keratinizing squamous epithelium. Note that some glandular epithelia (such as breast) do co-express HMWCK in addition to LMWCK, and HMWCK can be induced in non-squamous epithelia as a result of reactive conditions (such as inflammation). So the rule of thumb “squamous epithelium = HMWCK” vs. “non-squamous epithelium = LMWCK” is not 100%.
  - A designation of “intermediate molecular weight CKs” is occasionally applied, which refers to the lighter CKs within the HMWCK group (CK 5, 6, 17). These are also known as “basal keratins” because they are expressed preferentially in basal cells.
- The above patterns of CKs are generally retained in corresponding CAs and can serve as useful diagnostic tools. As a word of caution – some CAs deviate from CK patterns of their parent epithelia. For example, high-grade SqCCs frequently co-express LMWCKs and HMWCKs. In addition, some adenoCAs are well known to co-express HMWCKs (such as CAs of the pancreas, endometrium, lung, and a subset of breast).
- To increase the yield of diagnostic IHC, expression of CKs is usually analyzed by mixtures of various CK antibodies (Abs), known as “Ab cocktails.” The commonly used Ab cocktails include:

Antibody cocktail	What it detects
AE1	All **acidic** CKs except CK 9, 12, 17, and 18
AE3	All **basic** CKs (CK1-8)
AE1/AE3 (pan-CK)	All types of CKs (except those missing in AE1)
OSCAR, PANK (MNF-116)	Broad-spectrum CK cocktail (similar to AE1/AE3)
Cam5.2	**LMWCK**s (CK 7, 8)
CK903 (K903; 34βE12)	**HMWCK**s (CK 1, 5, 10, 14)
CK5/6	**HMWCK**s (detects primarily CK5)