Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Special Studies

7

The pathologist's H&E is like the clinician's H&P (history and physical) - basic exams to be performed on every patient or specimen forming the cornerstone of diagnosis. However, the pathologist is no longer limited to the H&E; there are a wide variety of special studies available to evaluate pathologic processes, from simple histochemical stains to global gene expression patterns. Pathologists are now clinical cell biologists. Familiarity with the types of special studies available is important as the initial processing of the gross specimen may limit the types of studies that can be performed.

HISTOCHEMISTRY

Almost all histochemical stains are suitable for formalin-fixed tissues. Common stains and their uses are listed in Table 7-1. However, numerous other types of stains and modifications are used and pathologists must be aware of individual laboratory practices.

The WebPath section of the University of Utah site (http://library.med.utah.edu/webpath) has useful descriptions of special stains and illustrative photographs.

TABLE 7–1. HISTOCHEMICAL STAINS

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
AFOG (acid fuschin orange G; modified Masson's trichrome)	Nuclei – brown Connective tissue – blue Basement membrane – blue Proteins, fibrin, readsorption droplets in cells, immune complexes – red/orange/yellow RBCs – yellow	Evaluation of renal biopsies
Alcian blue	Acid mucins – blue (e.g., normal intestinal glands) Nuclei – red Cytoplasm – pink	Sometimes used to identify mucosubstances in mesotheliomas or intestinal metaplasia. Affected by pH. Hyaluronidase digestion can be used to identify hyaluronic acid.
Alcian blue/PAS	Intestinal metaplasia – dark purple Normal stomach – pink	Demonstrates both acid and neutral mucins
Alcian yellow	Free mucus – yellow Bacteria – dark blue	Identification of *H. pylori* in gastric biopsies
Acid-fast bacilli stains (Fite-Faraco, Ziehl-Neelson, Kinyoun)	TB – red and beaded MAI – red *Nocardia* – pink Tissue – blue	Identification of mycobacteria. Modifications are used to demonstrate *M. leprae* or *Nocardia*. Carnoy's fixed tissues cannot be used and B-5 is suboptimal. Slides must be examined under oil.
Alizarin red S	Calcium – orange red, polarizes	Identifies calcium in tissues
Bile	Bile – dark green on a yellow background	Identification of bile
Bodian's	Nerve fibers and neurofibrils – black Nuclei – black Tissue – blue	Neural tumors, identification of axons

Continued

TABLE 7–1. HISTOCHEMICAL STAINS—cont'd

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
Chloroacetate esterase (Leder; CAE)	Mature myeloid cells, mast cells – red granules Nuclei – blue	Evaluation of leukemias Identification of mast cells Cannot be used for tissue fixed in Zenker's or B-5.
Congo red	Amyloid – orange red with apple green birefringence after polarization Nuclei – blue	Detection of amyloid. Immunoperoxidase studies can be used to identify specific types. Overstaining can result in false positives.
Dieterle	Spirochetes, *Legionella*, other bacteria – brown to black Tissue – pale yellow or tan	Infectious lesions Melanin, chromatin, formalin pigment, and foreign material may also stain
Diff Quik (a modified Giemsa stain)	*H. pylori* – dark blue Other bacteria – blue Nuclei – dark blue Cytoplasm – pink	Evaluation of chronic gastritis
Elastic stains (Verhoeff–van Gieson)	Elastic fibers – blue black to black Nuclei – blue to black Collagen – red Other tissue – yellow	Identification of arteries and veins, vasculitis, invasion of lung tumors into visceral pleura, abnormal elastic fibers in elastofibromas
Fibrin (see Phosphotungstic acid hematoxylin or Mallory PTAH)		To demonstrate fibrin in renal biopsies
Fontana-Masson	Melanin, argentaffin granules, chromaffin granules, some lipofuschin – black Nuclei – red	Identification of melanin in melanomas and secretory granules in neuroendocrine tumors This stain has largely been replaced by IHC
Giemsa (May-Grunwald)	Bacteria (e.g., *H. pylori*) – blue Parasites (*Leishmania*, *Plasmodium*) – blue Mast cells – red to purple granules Nuclei – blue Cytoplasm of leukocytes – pink to blue depending on cell type and differentiation	Lymphoproliferative disorders (good nuclear and cytoplasmic detail) Identification of bacteria, rickettsias, and *Toxoplasma gondii*
Gram (Brown-Hopps, Brown-Brenn)	Gram-positive bacteria – blue Gram-negative bacteria – red Nuclei – red Tissue – variable	Identification of bacteria, some cases of actinomycetes, *Nocardia*, coccidioidomycosis, blastomycosis, cryptococcosis, aspergillosis, rhinosporidiosis, and amebiasis
Grimelius	Argentaffin and argyrophil granules – dark brown to black Nuclei – red Background – pale yellow-brown	Evaluation of neuroendocrine tumors (largely replaced by the use of immunohistochemistry for chromogranin)
Hematoxylin and eosin (H&E)	Nuclei – dark blue or purple Cytoplasm – pink to red	Standard stain for the routine evaluation of tissues
Iron (colloidal iron)	Ferric iron (e.g., hemosiderin) – blue Nuclei – red Background – pink	Bone marrow (iron stores, myelodysplasias), liver (hemochromatosis) Chromophobe renal cell carcinomas are positive
Jone's silver methenamine	Basement membrane – dark maroon to black	Evaluation of renal biopsies
Melanin bleach		Removes melanin from tissue, usually for IHC Melanin can be difficult to distinguish from IHC positivity

TABLE 7–1. HISTOCHEMICAL STAINS—cont'd

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
Methyl green-pyronin Y	DNA (nuclei) – green to blue-green RNA - red Goblet cells - mint green Plasma cell and immunoblast cytoplasm - pink to red Mast cells - orange Background - pale pink to colorless	Plasma cell lesions (largely replaced by IHC) Does not work well on tissues decalcified with formic acid.
Mucicarmine (Mayer)	Mucin - deep rose to red Capsule of *Cryptococcus* - deep rose to red Nuclei - black Tissue - blue or yellow	Identification of adenocarcinomas, identification of *Cryptococcus*
Oil red O	Fat - red Nuclei - blue	Requires frozen sections (lipids are dissolved by most fixatives or during processing). Tissue fixed in formalin can be used if tissue is frozen.
Periodic acid – Schiff (PAS)	Glycogen - red Basement membranes (BM) - red Mucins - red Colloid - red Fungi - red	Classification of tumors with glycogen (e.g., Ewing's/PNET, rhabdomyosarcoma, renal cell carcinoma), glomerular diseases (BM), identification of adenocarcinomas (mucin), fungal diseases (especially in argentophic areas – neutrophils and debris), spironolactone bodies in adrenal adenomas treated with this drug
Periodic acid – Schiff with diastase digestion (PAS-D)	As above except glycogen that has been digested will not be stained	Identification of glycogen in tumors Identification of fungus in glycogen-rich tissue (e.g., skin) PAS-D resistant deposits in liver are present in alpha-1-antitrypsin deficiency
Phosphotungstic acid hematoxylin (Mallory PTAH)	Glial fibers - blue Nuclei - blue Neurons - salmon Myelin - blue Skeletal muscle cross striations - blue Fibrin - blue Collagen - red brown	Identification of neural lesions Skeletal muscle differentiation (Zenker's fixative is preferred). This stain has been replaced by IHC for muscle markers.
Reticular fibers (Gomor's reticulin, Gordon and Sweets, Snook)	Reticulin - black Mature collagen, type 1 – brown Immature collagen, types 3 and 4 - black	Bone marrow (myelophthisis), liver (fibrosis, veno-occlusive disease), carcinoma vs. sarcoma (reticular network) (but largely replaced by IHC)
Silver stain (Grocott methenamine–silver nitrate – GMS)	Fungi - black *Pneumocystis* - black Mucin - taupe to gray Tissue - green	Evaluation of infectious diseases Bacteria will also stain black.
Steiner	Spirochetes, *H. pylori*, *Legionella*, other bacteria - dark brown to black Tissue - light yellow	Evaluation of infectious diseases
Sulfated Alcian blue	Myocytes – yellow Connective tissue – red-purple Amyloid – sea-foam green	Identification of amyloid in cardiac biopsies
Toluidine blue	Mast cells - deep violet Background - blue	Mast cell diseases, chronic cystitis

Continued

TABLE 7–1. HISTOCHEMICAL STAINS—cont'd

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
Trichrome (Gomori, Masson)	Mature collagen, type 1 – dark blue Immature collagen, types 3 and 4 - light blue Mucin - green or blue Nuclei - black Cytoplasm, keratin, muscle fibers - red	Liver (fibrosis)
Trichrome - modified	Viable myocardium - magenta to brick red Nonviable myocardium -dusky gray to mauve	Evaluation of myocardial biopsies
Von Kossa calcium	Calcium - black Tissue - red	Demonstration of phosphate and carbonate radicals with calcium in tissues, identification of malakoplakia (Michaelis-Gutmann bodies)
Warthin Starry	Spirochetes - black Other bacteria - black (including *Bartonella* sp.) Tissue - pale yellow to light brown	Infectious lesions (including syphilis, cat scratch fever, and bacillary angiomatosis)
Wright's	Eosinophilic granules - pink Neutrophilic granules - purple Lymphocytic cytoplasm - blue Nuclei -blue to purple	Blood smears

IMMUNOPEROXIDASE STUDIES

The development of methods to detect antigens on tissue sections with antibodies was a major advance in surgical pathology. Immunohistochemical (IHC) studies are most frequently used for the following purposes:

- Classification of tumors (e.g., carcinoma vs. lymphoma, B cell vs. T cell lymphoma).
- Identification of in situ lesions vs. invasive carcinomas (e.g., myoepithelial markers in breast cancers, basal cell markers in prostate).
- Prognostic factors (e.g., Ki-67 in glioblastomas).
- Predictive factors to guide specific therapy (e.g., c-KIT, estrogen and progesterone receptors, HER2/neu).
- Identification of extracellular material (e.g., β-2 microglobulin amyloid).
- Identification of infectious agents (e.g., CMV or HSV).

Use of Immunohistochemistry. A differential diagnosis is generated after examination of the H&E-stained slides. IHC is then used to gain evidence for or against diagnostic possibilities. "Trolling" cases through an immunohistochemistry laboratory by ordering numerous antibody studies without a clear reason in mind is more likely to lead to misguided diagnosis due to aberrant immunoreactivity than to provide an unexpected correct diagnosis.

Panels. There are no absolute rules for immunoreactivity in cells and tissues. Aberrant immunoreactivity or loss of immunoreactivity is occasionally observed for all antibodies, either due to biologic variability (e.g., occasional keratin-positive melanomas) or technical factors (e.g., impure antibodies, cross-reaction with other antigens, failure to preserve antigenicity). Thus, immunohistochemical markers are used most effectively as panels of markers with interpretation based on an immunohistochemical profile.

Slides for Immunohistochemistry. Tissue is often dislodged from normal glass slides during the treatments required for IHC. Thus, slides must be coated (e.g., with glue, poly-L-lysine, gelatin, albumin) or special commercial slides must be used. If slides are being prepared by another laboratory, the type of glass slide to be used must be specified.

Factors Affecting Immunogenicity. There are numerous variables that can affect antigenicity. The most common are listed below. Each laboratory must optimize its procedures for each antibody used. Studies on tissues or slides not prepared in the routine fashion for a laboratory must be interpreted with caution.

- **Type of fixative.** Some fixatives destroy some antigens (e.g., Bouin's diminishes ER immunoreactivity, keratins are not well preserved in B5).[1] Most studies are based on formalin-fixed tissue. Results cannot be assumed to be equivalent for other fixatives.
- **Length of time of fixation** in formalin causes protein cross-linking, and antigenicity generally decreases with fixation times over 24 hours. To some extent, this effect can be reversed using antigen retrieval methods. Antigenicity can also be reduced if the tissue is fixed for too short a period (e.g., less than 6 hours).
- **Prior decalcification in hydrochloric acid.** Decreases antigenicity of some epitopes (predominantly nuclear)

but not others (predominantly cytoplasmic).[2] Decalcifying agents using EDTA did not alter immunogenicity.
  - Decreased: estrogen receptor (ER), progesterone receptor (PR), Ki-67, p53, BerEp4 (tumor cells), H blood group.
  - Not affected: calcitonin, chromogranin, GCDFP-15, HMB 45, thyroglobulin, S100, prostate-specific antigen (PSA), keratins (CK 20, CAM5.2, AE1/AE3), A and B blood groups, others.
- **Temperature** of baking the slide.
- **Length of time since the glass slide was cut.** The immunoreactivity of the majority of antigens declines over days to weeks with potential complete loss at one month.[3,4] The loss may be due to exposure of tissue to air with oxidation of amino acids, as the immunogenicity of tissue deeper in the block can be preserved for many years. Antigen retrieval methods do not completely restore the antigenicity of old slides. Coating slides with paraffin, storing the slides in a nitrogen desiccator, and/or storing at lower temperatures can partially preserve antigenicity. However, studies should be performed on newly cut slides, if possible.
- **Antigen retrieval procedures** (e.g., proteolysis, heating [microwave, steam], special incubation fluids). To some extent these methods reverse the effects of formalin fixation. Variable effects are observed for different antibodies.
- **Type of antibody** (polyclonal vs. monoclonal vs. mixture of different monoclonals, epitope detected, mouse vs. rabbit). Very different results can be obtained with different antibodies to the same protein or different commercial sources of the same antibody.
- **Incubation time, incubation temperature, dilution of antibody.**
- **Methods of signal amplification.**

Controls. Controls are essential for the appropriate interpretation of immunohistochemical studies and ensure that all steps of this complicated procedure have been performed adequately.

Positive controls consisting of tissues known to be immunoreactive should be included each time an antibody is used for a test case. Internal positive controls should always be evaluated when present, as they control not only for the technique used but also for the antigenicity of the tissue under investigation. The immunoperoxidase table lists normal cells that are generally immunoreactive for each antibody. Some laboratories have used vimentin as a control for immunogenicity as almost all tissue should demonstrate positivity.[5] Given the wide and non-specific distribution of vimentin, smooth muscle alpha actin may be more useful in this context as pericytes, vascular smooth muscle, and myoepithelial cells present in most tissues are immunoreactive.

Examples of internal controls:

- S100: Normal nerves, melanocytes, and Langerhans cells in epidermis, cartilage, some myoepithelial cells, skin adnexa
- Estrogen and progesterone receptors: Normal luminal cells in ducts and lobules of the breast
- CD31, FVIII: Vascular endothelium
- c-KIT (CD117): Mast cells
- Smooth muscle alpha actin: Blood vessel walls, myoepithelial cells in the breast
- Vimentin: Blood vessels, stromal cells
- High molecular weight (MW) keratin: Squamous epithelium
- Low MW keratin: Glandular epithelium
- CD15: Polymorphonuclear leukocytes

Negative controls usually consist of replacing the primary antibody with non-immune animal serum diluted to the same concentration as the primary antibody. No positive reaction should be present. If multiple primary antibodies are used reactive with different target antigens, then they may serve as negative controls for each other. Although the best negative control would be to use antibody preabsorbed against the target antigen, this is rarely practical in a diagnostic laboratory. Diagnostic slides should also be evaluated for internal negative controls. Aberrant immunoreactivity of tissues that should not be positive is indicative that the immunoreactivity is nonspecific and the study should not be used for interpretation.

Evaluation of Studies

The following features must be taken into consideration when evaluating studies:

Location of Immunoreactivity. Antigens are present in specific sites. Some antigens may be present in more than one location or be extracellular.

Nonspecific positivity should be suspected when immunoreactivity is present in atypical locations:

- **Background:** Suspect nonspecific positivity if normal cells or noncellular components are positive. This can occur with suboptimal performance of the assay or suboptimal antibodies.
- **Edge artifact:** Antibodies can pool at edges or holes in tissue. True positivity should also be present in the center of the tissue.
- **Necrosis or crushing of cells:** Nonspecific positivity can be seen in disrupted cells. Although keratin is generally reliable in necrotic tumors, other markers generally should not be interpreted.
- **Inappropriate location** (e.g., cytoplasm instead of nucleus): Occasionally ER or PR are present in the cytoplasm instead of the nucleus. This is not interpreted as a positive result. Plasma cells have large amounts of cytoplasmic immunoglobulins that can crossreact with many antibodies.
- In rare cases, immunoreactivity in an unusual location is of diagnostic importance:
  - **TTF-1:** Cytoplasmic (instead of nuclear) positivity in hepatocellular carcinomas.
  - **Ki-67 (MIB1):** Cytoplasmic and membrane (instead of nuclear) positivity in trabecular hyalinizing adenomas of the thyroid and sclerosing hemangiomas of the lung.

- **Beta-catenin:** Nuclear (instead of cytoplasmic) positivity in solid pseudopapillary tumors of the pancreas and pancreatoblastomas. Both nuclear and cytoplasmic positivity is seen in the majority of colon carcinomas. Nuclear positivity is present in about 20% of endometrioid endometrial carcinomas and 70% of cases of desmoid-type fibromatosis.
- **ALK:** The pattern of immunoreactivity correlates with the different types of chromosomal translocations in anaplastic large cell lymphomas.
- **NPM** (nucleophosmin) shuttles between the cytoplasm and nucleus. NPM1 mutations occur in about 30% of adult AML and cause aberrant cytoplasmic expression of NPM ("NPMc+ AML"). These cases have a specific gene expression profile and distinctive clinical and prognostic features.

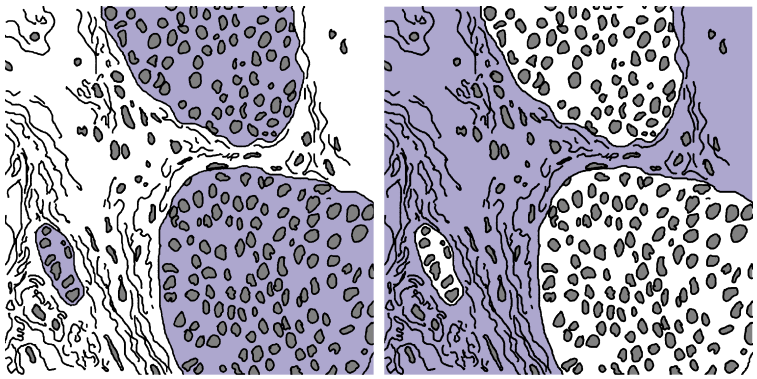
Examples of the normal location of antigens are shown in Figure 7-1.

Identification of Immunoreactive Cells. Immunoreactivity of tumor cells must be distinguished from immunoreactivity of normal entrapped cells (e.g., desmin [+] skeletal muscle cells infiltrated by tumor, S100 [+] Langerhans cells in tumors, smooth muscle alpha actin [+] blood vessels, etc.). Plasma cells have large amounts of cytoplasmic immunoglobulin and can react nonspecifically with many antibodies.

Intensity of Immunoreactivity. Some weak immunoreactivity may be present as a nonspecific finding. It is important to compare positive cells with control slides and with normally nonimmunoreactive cells to determine whether the immunoreactivity is significant.

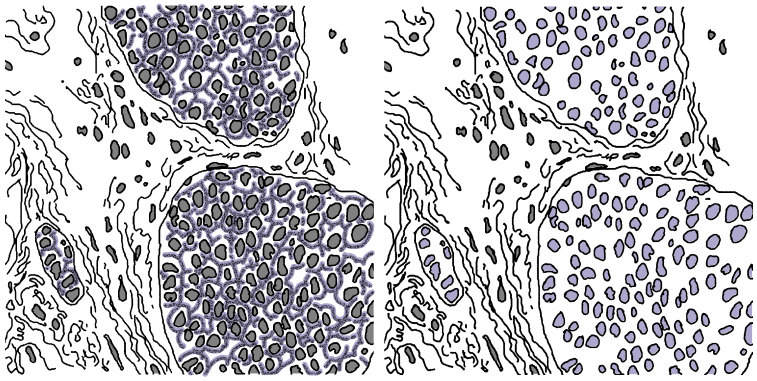
Number of Immunoreactive Cells. In some cases, the number of positive cells may be important as a criterion for positivity or as a prognostic marker (e.g., markers of proliferation such as Ki-67, HER2/neu). In other cases, rare weakly positive cells must be distinguished from intermingled normal cells or just nonspecific immunoreactivity.

Criteria for a "Positive" Result. Specific criteria for evaluating IHC have been developed for a few antibodies (see Tables 7-12 to 7-16). However, criteria do not exist for most antibodies or are not universally used by all



Nuclear: ER, TTF-1, P63, Myf4 (myogenin), Ki-67 (MIB1)

Membrane: EMA, HER2/neu, e-cadherin, EGFR



Cytoplasm:
Granular: organelles, secretory vesicles (chromogranin, HMB-45)

Fibrillary: intermediate filaments (actin, keratin)

Diffuse, patchy: cytosol, large vesicles (myoglobin, thyroglobulin)

Extracellular: Amyloid (β2-microglobulin, lambda chain, calcitonin)

Figure 7–1. Location of immunoreactivity (indicated in blue).

pathologists. The significance of immunoreactivity varies with the type of lesion, the antibody, and the specific assay. Strong positivity in the majority of cells is easily interpreted as positive. As the number of positive cells decreases, and the intensity of immunoreactivity weakens, the lower threshold of a "positive" result becomes more difficult to determine.

Time. Alkaline phosphatase chromogens (red color) fade over time. DAB (a brown color) is more permanent. This is not a problem in evaluating current pathology specimens. However, if slides are reviewed after a period of time, some chromogens may fade and once positive results may appear to be negative.

Common Panels for Immunohistochemical Studies

The following tables include information from the literature as well as the personal experiences of the staff at Brigham and Women's Hospital. Because of the many differences in specific antibodies, laboratory assays, and criteria for considering a result "positive," results may vary for different institutions. The results have been divided into five categories for general markers and four categories for hematopathology markers. Note that "%" refers to the number of tumors reported to be positive, not the number of cells positive within a tumor (Table 7-2).

The actual markers used to evaluate a case will depend upon the differential diagnosis based on the H&E appearance. In some cases, an initial panel, which is often used for typical cases, has been suggested. Not all markers listed would be used for all cases and some markers are included to indicate when they would not be useful for distinguishing the tumors listed in the table.

POSITIVE is defined as the presence of immunoreactive cells and NEGATIVE as the absence of immunoreactive cells. Unfortunately, "positive" has also been used in some studies to mean "absence of immunoreactivity" when this finding supports a diagnosis. For example, the absence of SMAD4 (DPC4) has been reported as a "positive" result for pancreatic carcinoma, as this marker is absent in the majority of these tumors. This method of reporting results becomes confusing as "positive" and "negative" are dependent on the expected diagnosis. It is preferable to report the findings (positive = immunoreactivity present; negative = immunoreactivity absent) and then interpret them as supporting, or not supporting, the diagnoses in the differential diagnosis.

Cytokeratin 7 and cytokeratin 20 tables

The combination of these two cytokeratins have been found to be useful to divide carcinomas into four main groups (Ck7+/Ck20+, Ck7+/Ck20-, Ck7-/Ck20+, Ck7-/Ck20-).

In Tables 7-3 to 7-7, other commonly used antibodies have been included to show differences within each group. The most useful additional antibodies will depend on the specific differential diagnosis.[6-8]

Small blue cell tumors

See Table 7-8.

Spindle cell lesions, soft tissue lesions, and sarcomas

See Table 7-9.

TABLE 7–2. EVALUATION OF POSITIVITY OF IMMUNOHISTOCHEMICAL STUDIES

GENERAL MARKERS			HEMATOPATHOLOGY MARKERS		
Category	**% of Tumors**	**Interpretation**	**Category**	**% of Tumors**	**Interpretation**
Positive (POS)	>90%	Almost always positive; a negative result would be unusual	+	>90%	Almost always positive
High	60-90%	Most tumors are positive	+/–	>50%	Majority positive
Moderate (Mod)	40-60%	May or may not be positive – usually the least useful type of marker	–/+	<50%	Minority positive
Low	10-40%	Most tumors are negative	–	<10%	Rarely positive
Negative (neg)	<10%	Almost all tumors are negative; a positive result would be unusual	Blank		Results unknown or too few cases to quantify
Blank		Results unknown or too few cases to quantify ?=Results based on very few cases (e.g., <10)			

TABLE 7–3. PREDOMINANTLY CK7+/CK20+

TUMOR	CK7+/ CK20+	CK7+/ CK20 –	CK7–/ CK20+	CK7–/ CK20–	34B E12	CAM-5.2	CK5/6	EMA	BER-EP4	CEA m	CEA p	TTF 1	p63	WT1	S100	CHRO	HEP	OTHER
Cholangio-carcinoma	High	Low	Low	neg	High	POS	Low	POS	POS	High	POS	neg	Low				rare	
Transitional cell carcinoma	POS	Low	neg	neg	Mod	POS	High	POS		Mod	Mod	neg	High	neg	neg	neg	neg	
Pancreas	High	Low	Low	neg		POS	Low	POS	POS	High	POS	neg	Mod	neg	neg	neg	Low	DPC4 lost in 55%
Ovarian mucinous	POS	Low	neg	neg		POS	neg	POS		Mod	Low		neg?	neg	High		Low	
Esophageal adenocar-cinoma	POS	neg	neg	neg								neg	Low		neg		Mod	

TABLE 7–4. NO DOMINANT CK7/CK20 PATTERN OR PATTERN UNKNOWN

	CK7+/ CK20+	CK7+/ CK20–	CK7–/ CK20+	CK7–/ CK20–	34b E12	CAM-5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	p63	WT1	S100	CHRO	HEP	OTHER
Gastric adeno-carci-noma	Low	Low	Low	Low	neg	POS	neg	High	POS	High	High	neg	Low	neg?	Low	neg	Low	
Amelo-blas-toma/ ada-manti-noma[a]					POS	neg		neg							neg?	neg?		
Lymphoepi-thelialcar-cinoma[b]					POS			High		Mod?	Mod?				neg[c]			p63 POS

[a]About 15% of ameloblastomas are positive for Ck7.
[b]About 50% of nasopharyngeal carcinomas are positive for Ck7. Many cases in Asian and North African patients (less commonly in US patients) are associated with EBV. EBV can be demonstrated by in situ hybridization, PCR, or occasionally by immunohistochemistry. These carcinomas are also positive for broad-spectrum keratins (AE1/AE3 and PANK).
[c]S100-positive dendritic cells are present.

TABLE 7–5. PREDOMINANTLY CK7+/CK20–

TUMOR	CK7+/ CK20+	CK7+/ CK20–	CK7–/ CK20+	CK7–/ CK20–	34b E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	p63	WT1	S100	CHRO	HEP	OTHER
Acinic cell carcinoma	neg	POS	neg	neg	POS	POS		Mod			Low				POS	Low		
Adenoid cystic carcinoma	neg	POS	neg	neg	POS	High	POS	Mod		POS	Low		POS		Mod	neg		GFAP Low
Breast ductal carcinoma	Low	High	neg	neg	neg[a]	POS	Low	POS	High	High	Mod	neg	Low[a]	Low	Mod	Low	neg	ER/PR[b] GCDFP Mod
Breast lobular carcinoma	Low	POS	neg	neg		POS	neg	POS	Mod	Mod	Mod	neg	Low			Low	neg	ER/PR[c] GCDFP Mod E-cad neg
Brenner tumor	neg	POS	neg	neg				POS			High			Low	neg?	POS		Calret low NSE POS
Cervical squamous cell carcinoma	neg	High	neg	Low	POS	neg	POS	POS		POS	Low	neg	POS			neg	neg	HPV POS p16 High
Choroid plexus	neg	High	neg	Low		POS		Low	neg						Mod			GFAP High
Chordoma	neg	POS	neg	neg	Mod	POS		POS	neg	neg	neg				POS	neg		GFAP neg
Craniopharyngioma	neg	POS	neg	neg	POS		POS											
Embryonal carcinoma	neg	POS	neg	neg	neg	POS		Low		Low	Low	neg?	neg		neg	neg	neg	PLAP High CD30 High
Endometrial carcinoma	Low	High	neg	neg		POS	Low	POS	POS	Low	Low	neg		neg?	High	neg	neg	Vim POS ER High
Lung – adenocarcinoma	Low	High	neg	Low	Mod	POS	neg	POS	POS	High	High	High	Mod	Low	Low	neg	Low	
Lung – BAL[c] non-mucinous	neg	POS	neg	neg	POS	POS				High	High	High	Mod	neg	Mod	neg		

Continued

TABLE 7–5. PREDOMINANTLY CK7+/CK20–—cont'd

TUMOR	CK7+/ CK20+	CK7+/ CK20–	CK7–/ CK20+	CK7–/ CK20–	34b E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	p63	WT1	S100	CHRO	HEP	OTHER
Meningioma – secretory type[d]	neg	POS	neg	neg		neg	High	POS		POS	POS				Low	neg		PR Mod ER neg
Mesothelioma	neg	High	neg	Low	High	POS	High	High	neg	neg	neg	neg	neg	High	neg	Low	neg	Calret High
Mixed tumor[e]	neg	POS	neg	neg		POS	POS	Low		Low	neg?		POS		POS		neg	GFAP High SMA POS Calp POS
Ovarian - endometrioid	neg	POS	neg	neg		POS	Low	POS	POS	Low	Low	neg?	Low	High	Low		neg	ER Mod
Ovarian – serous carcinoma	neg	POS	neg	neg		POS	Low	POS	POS	neg	neg	neg?	Low	POS	High		neg	ER High Calret Low
Renal cell – papillary & chromophobe	neg	POS	neg	neg	POS			POS						Mod[f]				
Thyroid - papillary	neg	POS	neg	neg	POS	POS	Mod	High		neg	Mod	POS	High		High	neg	neg	Thy POS Calci neg
Thyroid - follicular	neg	POS	neg	neg	neg		neg	Mod		neg	Low	POS			Mod	neg	neg	Thy POS Calci neg
Thyroid - medullary	neg	POS	neg	neg	neg		neg	neg		POS	Mod	POS				POS		Thy rare Calci POS

[a]P63 may be positive in breast "basal-like" carcinomas, some spindle cell metaplastic carcinomas, squamous cell carcinomas, and some papillary carcinomas. These subtypes may also have less typical keratin subsets such as Ck14 (detected by 34bE12), Ck17 (detected by MNF116), or Ck5/6.
[b]Most well- and moderately differentiated ductal carcinomas, and carcinomas of special type (except for medullary) will be positive for hormone receptors. Poorly differentiated carcinomas, metaplastic carcinomas, and medullary carcinomas are usually negative. Well- and moderately differentiated lobular carcinomas are almost always positive for ER, and usually positive for PR. Poorly differentiated lobular carcinomas may be negative for these markers.
[c]Non-mucinous bronchioloalveolar carcinomas have an immunophenotype similar to lung adenocarcinomas. Mucinous BALs are more likely to be CK20 positive (about 70% positive), CDX2 positive, MUC2 positive, and less likely to be TTF-1 positive (about 30% positive).
[d]Secretory meningiomas are frequently positive for CK7 and CEA, whereas other subtypes are usually negative for Ck7 and CEA. The majority of all types of meningiomas are positive for PR (incuding meningiomas in males).
[e]Mixed tumors (pleomorphic adenomas) occur most frequently in the salivary glands, but can also arise in soft tissues (myoepithelial tumors of soft tissue). These tumors have a similar immunophenotype with keratin (AE1/AE3 77%) or PANK (68%) or EMA (63%) present in the majority of tumors and frequent expression of markers associated with myoepithelial cells (e.g., calponin, GFAP, SMA, S100, p63). However, p63 is seen less frequently (23%) as compared to salivary tumors (100%).
[f]Chromophobe renal cell carcinomas may be positive for WT-1. Other types are negative.

TABLE 7–6. CK7–/CK20+

TUMOR	CK7+/ CK20+	CK7+/ CK20–	CK7–/ CK20+	CK7–/ CK20–	34b E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA M	CEA p	TTF-1	p63	WT1	S100	CHRO	HEP	OTHER
Merkel cell carcinoma	rare	neg	High	Low		High	neg	High	POS		POS	neg			Low	High	neg?	NSE High
Colon adenocarcinoma	Low	neg	High	Low	neg	POS	neg	High	POS	POS	POS	neg	Low	neg	Low	neg	neg	CDX2 POS

Rare colon carcinomas are either CK7 positive or CK20 negative, but they are rarely CK7+ CK20-. Although the majority of colon carcinomas are positive for CK20, almost one third of colon carcinomas with microsatellite instability (MSI-H positive) are CK20 negative. (see Table 19-24).

TABLE 7–7. PREDOMINANTLY CK7–/CK20–

TUMOR	CK7+/ CK20+	CK7+/ CK20–	CK7–/ CK20+	CK7–/ CK20-	34b E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	p63	WT1	S100	CHRO	HEP	OTHER
Adrenal cortical adenoma	neg	neg	neg	POS		neg	neg	neg		neg	Low	neg			neg	neg	Low	MelanA103 POS Inhibin POS
Carcinoid	neg	Low	Low	High	neg	POS	Low	Low		Mod	Mod	**VAR**[a]	neg		**VAR**[b]	POS	Low	
Epithelioid sarcoma	neg	Low	neg	POS	Mod	High	Low (foc)	POS (foc)					Low (foc)		neg		neg	
Esophageal squamous cell carcinoma	neg	Low	neg	High	POS	High?	POS	POS	High?	Low?	Low	neg	POS		neg	neg	neg?	
Seminoma	neg	Low	neg	High	neg	Low	Low	neg		neg	neg				neg	neg	neg	PLAP POS CD117 POS
Head and neck squamous cell carcinoma	neg	Low	Low	High	POS	neg	POS	POS			neg	neg	POS			neg	neg	
Hepatocellular carcinoma	Low	Low	neg	High	Low	POS	neg	Low	Low	neg	High[c]	High[d] (cyt)	Low		neg	neg	High	AFP Mod

Continued

TABLE 7–7. PREDOMINANTLY CK7–/CK20–—cont'd

TUMOR	CK7+/ CK20+	CK7+/ CK20–	CK7–/ CK20+	CK7–/ CK20-	34b E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	p63	WT1	S100	CHRO	HEP	OTHER
Lung – squamous cell carcinoma	neg	Low	Low	High	POS	High	POS			Mod	Low	neg	POS			neg	Low	
Lung – small cell carcinoma	neg	Low	neg	High	neg	High	neg	POS	POS	Mod	High	POS	rare		neg?	Mod	neg	
Pheo/paraganglioma	rare	rare	rare	POS	neg	neg	neg	neg				neg			High	POS		Inhibin neg MelanA103 rare
Prostatic carcinoma	neg	neg	Low	High	neg	POS	neg	Low	POS	neg	Mod	neg	neg	neg	neg	Low	neg	PSA POS
Renal cell carcinoma –clear cell	neg	Low	neg	High	neg	High	neg	POS	Low	Low	neg	neg	Low	neg?	Low	neg	neg	Vim POS
Squamous cell carcinoma[e]	neg	Low		High	POS	Low	POS	POS	neg	Mod	Low	Low	POS		neg	neg	neg	
Thymic carcinoma					POS	POS	POS	Mod	High	Low	neg?	neg	POS	neg	neg	Low		CD5 Mod
Thymoma	neg	Low	neg	High		High	High	Mod		Low	neg?	neg	POS	neg	neg	neg?	neg?	CD5 neg

[a]Non-pulmonary carcinoid tumors are negative for TTF-1. Some pulmonary carcinoids may be positive.
[b]Sustentactular cells may be positive for S100 and positivity can vary with site.
[c]CEA has a canalicular pattern in hepatocellular carcinoma, a diffuse cytoplasmic pattern in other carcinomas.
[d]TTF-1 immunoreactivity in HCC is cytoplasmic (not nuclear as in lung and thyroid carcinomas). Positivity can vary with the antibody used to detect TTF-1.
[e]Cervical carcinomas and basaloid squamous cell carcinomas of the tonsil are usually HPV positive. Nasopharyngeal carcinomas are usually EBV positive. Thymic squamous cell carcinomas are often CD5 positive.

TABLE 7–8. SMALL BLUE CELL TUMORS

TUMOR	PANK	CAM 5.2	CK20	EMA	S100	HMB 45	NSE	SYN	CHRO	CD99	SMA	HHF 35	DES MIN	MYF-4	LCA	NFP	WT1[a]	PAS[b]
Melanoma	rare	rare	neg	neg	POS	High[c]	High	Low	neg	Low	Low	neg	neg		neg	neg		
Esthesioneuroblastoma	Low	Mod		Low	POS		POS	High	Mod	Low			neg	neg?	neg	Mod		
Neuroblastoma	neg	neg	neg	Low	Mod	neg	POS	High	High	neg	neg	neg	neg		neg	High	Low	neg
Small cell carcinoma[d]	POS	Mod	neg	POS	neg	neg	High	Mod	Mod	Low		neg			neg	neg		neg
Merkel cell carcinoma[e]	POS	POS	POS	POS	neg	neg	High	Mod	High	Low			neg?		neg	Mod		neg
Desmoplastic small round cell tumor	POS	POS	neg	POS	Low	neg	High	Low	Low	Mod	Low	Low	POS	neg		neg	POS	POS
Ewing's (PNET)	Low	Low		Low	Low	neg	Mod	Low	neg	POS[f]	neg	Low	neg		neg	Low	neg	POS
Medulloblastoma	neg			neg?	Low		POS	POS		Low			Low			neg		
Rhabdomyosarcoma	neg	Mod		neg	Low	neg	Mod	neg	neg	Low	Low	POS	POS	POS	neg	Low	Mod	POS
AML	neg	neg	neg	neg	Low	neg	POS?			Mod					High			
Lymphoma	neg	neg	neg	neg	neg	neg	neg	neg	neg	Var	neg	neg	neg	neg	POS			neg[g]

[a]Polyclonal WT1 – nuclear immunoreactivity.
[b]PAS is a histochemical stain for glycogen. A PAS-D stain confirms the presence of glycogen by treatment of the tissue with diastase, which digests the glycogen and eliminates the positivity. Although used for these tumors in the past, these studies are currently not usually performed.
[c]MART-1 is also frequently positive in melanomas.
[d]Small cell carcinomas of the lung are positive for TTF-1.
[e]Merkel cell carcinomas demonstrate a dot like perinuclear pattern for most markers.
[f]Significant immunoreactivity is a membrane pattern in the majority of the cells.
[g]Some plasma cell lymphomas may be positive.
Ewing's (PNET), desmoplastic small round cell tumor, rhabdomyosarcoma, neuroblastoma, and medulloblastoma have characteristic cytogenetic changes (see Table 7-47).
EM has some advantages over immunohistochemistry in the evaluation of childhood small round blue cell tumors.[9]
Initial panel: Keratin, S100, LCA. Additional studies may be helpful depending on the histologic appearance and the results of the initial studies.

TABLE 7–9. SPINDLE CELL/SOFT TISSUE LESIONS/SARCOMAS

TUMOR	AE1/ AE3	CAM 5.2	EMA	S100	HMB 45	HHF-35	SMA	DES-min	H-CALDESMON	CD34	CD31	FVIII	C-kit CD117	CD99	OTHER
NEURAL															
Perineurioma	neg	neg	POS	Low	neg	Mod	Low	neg		Mod	neg	neg	neg	Mod	CLAUD-1 Low[a]
Neurofibroma	neg	neg	POS[b]	POS	neg	neg	neg	neg		High	neg			neg	
MPNST	Low	Low	Low	Mod	neg	Low	Low	neg	neg	Low			neg		GFAP Mod
Schwannoma	Low	neg	neg[c]	POS	neg	neg	neg	neg		Mod	neg	neg	neg		CD68 POS
Granular cell tumor[n]	neg	neg	neg	POS	neg	neg	neg	neg		neg					CD68 POS Calret POS Inhibin POS
MELANOMA	rare	rare	neg	POS	High[e]		neg	neg	neg	neg	neg	neg	Mod	Low	MelanA[a] High FLI-1 neg
CLEAR CELL SARCOMA	neg	neg	neg	High	POS	Low	neg	neg		neg	neg	neg	Low	Low	MelanA Mod
PECOMA[F]	neg	neg	neg	Low	POS	POS	POS	High	Mod	Low	neg	neg	VAR[h]		MelanA POS
GIST	neg	neg	neg	Low		Mod	Low	neg	High	High	neg		POS	POS	DOG1 POS
MUSCLE															
Rhabdomyosar-coma	Low	Low	Low	neg	neg	High	Mod	High	neg	Low	neg	neg	neg	Low	Myf4 POS WT1 Mod FLI-1 neg
Glomus tumor	neg	neg	neg	neg	neg	POS	POS	Low	High	Low	neg	neg	neg		
Leiomyoma or leiomyosar-coma	Low	Low	Mod	neg	neg	POS	POS	High	POS	Low	neg	neg	neg	Low	ER/PR High CD10 Low
ENDOMETRIAL STROMAL SARCOMA	Mod (foc)	Low (foc)			neg		High	Mod	neg	neg				neg	ER/PR High CD10 High

VASCULAR															
Angiosarcoma	Low[h]	Low[h]	rare	neg	neg	Low	Low	neg		High	High	High	Low		FLI-1 POS
Kaposi's sarcoma	neg	neg	neg	neg		neg	POS	neg		POS	High	Mod	neg		FLI-1 POS HHV 8 POS
Epithelioid hemangioendothelioma	High	neg	neg	neg	neg	neg	Low	neg		High	High	POS	neg		FLI-1 POS
"FIBROUS"															
Fibrosarcoma	neg	Low	neg	neg		neg	neg	neg		neg				Low	
Solitary fibrous tumor	neg	neg	Low	neg		neg	Low	neg	neg	POS	neg	neg	neg	High	
DFSP	neg	neg	neg	neg	neg	High	Low	neg	neg	POS	neg	neg	neg		
Dermatofibroma	neg	neg		neg	neg	High	High	neg[i]		Low[q]	neg			neg	
Fibromatosis	neg	neg	neg	Mod		High	High	Mod	neg	neg	neg		neg		ER rare
Post-op spindle cell nodule	Mod	Mod	Low	neg		High	High	Mod	neg	neg			neg		
Myofibroblastic tumors		neg	neg	neg		POS	High	Mod	neg	Mod		neg			ER High PR POS
Atypical fibroxanthoma	neg	neg	neg	neg	neg		Low	neg		Low					CD68 Mod
LIPOMATOUS[r]															
Well- or dedifferentiated liposarcoma															MDM2 POS CDK4 POS
Benign lipomatous tumors															MDM2 rare CDK2 rare

Continued

TABLE 7–9. SPINDLE CELL/SOFT TISSUE LESIONS/SARCOMAS—cont'd

	AE1/ AE3	CAM 5.2	EMA	S100	HMB45	HHF-35	SMA	DES-min	H-CALDESMON	CD34	CD31	FVIII	C-kit CD117	CD99	OTHER
OTHER															
Osteosarcoma	neg	neg	Low	Low		Mod	High	neg	neg					Low	
Chondrosarcoma	neg	neg	Low	POS	neg	neg	neg	neg		neg				Low	
Chrondroblastoma	neg	neg	neg	POS		Mod	Low	neg	neg?					POS	
Mesenchymal chondrosarcoma	neg	neg	neg	POS	neg		rare	Low						POS	My4 neg
Extraskeletal myxoid chondrosarcoma	neg	neg	Low	Low		neg	neg	neg		Low			Low	neg	
Alveolar soft part sarcoma	neg	neg		Low	neg	Low	Low	Low		Low	neg		neg	Low	MyoD1 neg Myogenin neg TFE3 POS[j]
Epithelioid sarcoma	POS	POS	POS	neg	neg	Low	Low	neg		Mod	neg	neg	neg	Low	FLI-1 neg
Synovial sarcoma[k]	High	High	High	Mod	neg	neg	Low	neg	neg	neg	neg	neg	neg	High	WT1 neg Claudin-1 POS[l] Calret Mod bcl[2] High
ADENOMATOID TUMOR	POS	POS	POS							neg	neg			neg	Ber-EP4 High Calret POS WT1 POS

MESOTHELIOMA – SARCOMATOID TYPE[m]	High	POS	Low			POS	High	Low		neg				Low	WT1[n] D2-40 High Calret Low
MENINGIOMA	neg[o]	neg[o]	High	Low	neg	Low	Low	neg		Low	neg	neg		POS	ER neg PR POS PANK Low
CARCINOMA – SPINDLE CELL[p]	VAR	VAR	VAR	VAR	neg	rare	rare	neg		neg	neg	neg			

[a]Some claudin-1 positive perineurial cells can be present in neurofibromas and schwannomas. About 30% of perineuriomas are positive.
[b]Perineural cells are positive for EMA in neurofibromas.
[c]EMA may be positive in capsule and perineural cells of schwannomas.
[d]Congenital granular cell tumors are positive for CD68 but negative for S100 and NSE.
[e]HMB-45 is less frequently present in spindle cell melanomas and usually negative in classic desmoplastic melanomas. Other markers for melanoma are also less frequently positive in these subsets.
[f]PEComa (perivascular epithelioid cell tumors) includes angiomyolipoma, lymphangioleiomyomatosis, clear cell sugar tumor of the lung, clear cell myomelanocytic tumor of ligamentum teres/falciform ligament, and abdominopelvic sarcoma of perivascular epithelioid cells.
[g]Results in the literature are conflicting. Angiomyolipomas are likely not positive for CD117.
[h]Keratin positivity may be present in ~25% of epithelioid angiosarcomas.
[i]Cellular dermatofbroma may show focal desmin immunoreactivity and a few will be CD34 positive.
[j]Alveolar soft part sarcomas are characterized by a translocation that fuses the TFE3 transcription factor gene at Xp11 to a novel gene at 17q25 called ASPL. These sarcomas demonstrate nuclear immunoreactivity for TFE3 (as do rare pediatric renal tumors with the same translocation) and this immunoreactivity is not present in other tumors or normal tissues. The characteristic cytoplasmic crystals are composed of monocarboxylate transporter 1 (MCT1) and its chaperone CD147. However, these proteins are found in many other cell types and are not specific for this tumor.
[k]Keratin and EMA positivity are usually only focal in monophasic synovial sarcomas.
[l]Claudin-1 is positive in glandular areas of synovial sarcoma but less so in spindle cell areas.
[m]The immunohistochemical pattern for epithelioid mesotheliomas is given in a separate table.
[n]WT-1 may be positive in a minor epithelioid component of sarcomatoid mesotheliomas, but is generally negative in the spindle cells.
[o]Secretory meningiomas are typically cytokeratin 7 positive (Ck20 negative) and also positive for CEA. Other subtypes are generally negative for keratin. However, malignant meningiomas may be positive for keratin.
[p]Squamous cell carcinomas with a spindle cell morphology are generally strongly positive for AE1/AE3 (less commonly for CAM5.2), EMA, and p63. Spindle cell carcinomas of the breast often express markers expressed by myoepithelial cells such as "basal keratins" (including cytokeratin 14, which is included in the group detected by PANK or MNF-116), smooth muscle alpha actin, and p63. Poorly differentiated carcinomas with a spindle cell morphology may only show focal positivity for keratins and EMA.
[q]Dermatofibromas may have weak peripheral positivity for CD34 which is distinguished from strong diffuse positivity in DFSP.
[r]Other sarcomas (e.g., ~60% of MPNST) can be positive for MDM2 or CDK4. These markers are helpful to distinguish between benign and malignant lipomatous tumors.

Metastatic tumors of unknown origin

Pathologists frequently receive specimens with metastatic tumors. Often, the site of origin is known to the clinician, but this information is not provided to the pathologist. A good clinical history is frequently more successful for correct classification than a battery of studies.

The Ck7/Ck20 pattern is generally helpful to narrow down the potential site of origin of carcinomas (see Tables 7-3 to 7-7). Additional studies can then be used to identify specific types of carcinoma.

The most important tumors to identify are those with specific therapeutic treatments for cure or palliation (Table 7-10).[10]

TABLE 7–10. METASTATIC TUMORS OF UNKNOWN ORIGIN

TYPE OF TUMOR	IHC	COMMENTS	POTENTIAL TREATMENT
Breast	ER/PR HER2/neu GCDFP-15	Gyn carcinomas can also be positive. Other carcinomas are rarely positive. It is unusual for other carcinomas to be strongly positive for HER2/neu. GCDFP-15 is not very sensitive, as many breast carcinomas are negative. The most common type of breast carcinoma to present as an occult primary is invasive lobular carcinoma. Rare women present with positive axillary nodes and no known primary. Most of these women will have breast cancer. The prognosis is the same, whether or not the primary is detected.	Palliated with hormonal treatment. HER2/neu-positive carcinomas can be treated with Herceptin.[a]
Carcinoid tumor	Chromogranin	Chromogranin positivity should be strong and diffuse. Focal and/or weak positivity can be seen in many carcinomas. Metastatic breast cancer and prostate cancer can closely resemble carcinoid tumor and both can be positive for chromogranin.	Palliation with tumor-directed pharmaceuticals.
Germ cell tumors	PLAP OCT-4	PLAP is not specific, but a germ cell tumor is unlikely if it is negative. Inhibin is more likely to be positive in choriocarcinomas. OCT-4 is highly specific for undifferentiated germ cell tumors (embryonal carcinoma and seminoma) among epithelioid and round cell malignant neoplasms. Other types of germ cell tumors (i.e., yolk sac tumor, teratoma, and choriocarcinoma) are negative. FISH can confirm an isochromosome 12p, even if the metastasis has the appearance of adenocarcinoma, sarcoma, or neuroendocrine carcinoma	Chemotherapy for possible cure.
GIST	c-kit (CD117)	Specific mutations are correlated with treatment response.	Treatment with Gleevec.[b]
Lung adenocarcinoma	TTF-1	Thyroid carcinoma should be excluded if TTF-1 is positive.	Up to 20% of patients will have specific activating mutations in EGFR (detected by PCR) and these patients may respond well to treatment with gefitinib.[c]
Lymphoma	LCA, B and T cell markers		Treatment for cure or long-term palliation.
Prostate	PSA or PrAP		Hormonal therapy effective for palliation.

TABLE 7–10. METASTATIC TUMORS OF UNKNOWN ORIGIN—cont'd

TYPE OF TUMOR	IHC	COMMENTS	POTENTIAL TREATMENT
Small cell carcinoma	TTF-1 (if of lung origin) Neuroendocrine markers	Diagnosis made by H&E appearance. P63 is usually negative and can be useful to exclude squamous cell carcinoma. Not necessary for diagnosis, but can exclude other diagnoses.	Chemotherapy for palliation.
Squamous cell carcinomas	Ck5/6, p63 p16 or HPV CD5	Not specific, but characteristic. H&E appearance usually sufficient to reveal keratin production or intercellular bridges. HPV or p16 are most commonly present in carcinoma of the cervix, but may be seen in carcinomas at other sites. About 26-38% of patients with a cervical LN metastasis of unknown primary will have an occult tonsillar carcinoma (usually basaloid type and p16 or HPV positive). Complete sampling of the tonsil may be necessary to identify these small carcinomas. May be positive in thymic carcinomas.	Radiation therapy often effective. Vaccine trials are being conducted.
Thyroid – papillary or follicular carcinoma	Thyroglobulin and TTF-1	Lung carcinomas are also TTF-1 positive, but will be thyroglobulin negative.	Highly effective treatment for cure with radioactive iodine.
Thyroid – medullary carcinoma	Calcitonin	If familial, important for counseling other family members.	Palliative treatment with tumor-directed radionucleotides.
Trophoblastic tumors	Inhibin	Inhibin is not specific, but a trophoblastic tumor is unlikely if it is negative.	Chemotherapy for possible cure.

[a]Trastuzumab (Herceptin) = a monoclonal antibody directed against the HER2/neu receptor.
[b]Imatinib mesylate (STI571, Gleevec™, Glivec™) is a small molecule tyrosine kinase inhibitor used for CML, ALL (Ph+), and GIST.
The KIT protein is encoded by the c-kit proto-oncogene and is a transmembrane receptor protein with tyrosine kinase activity. Mutated proteins may or may not respond to therapy with Imatinib. Mutations that render KIT independent of its ligand, SCF (stem cell factor), have been found in GIST, AML, germ cell tumors and systemic mastocytosis. Wild-type KIT and KIT with mutations in the juxtamembrane domain (the intracellular segment between the transmembrane and tyrosine kinase domains) are found in GISTs and are sensitive to imatinib. Other tumor types are associated with mutations in the enzymatic domain and the altered protein is generally not sensitive to imatinib.
[c]Gefitinib (Iressa) = a tyrosine kinase inhibitor effective against a small subset of lung adenocarcinomas with specific activating mutations.

Poorly differentiated tumors

See Table 7-11.

TABLE 7–11. POORLY DIFFERENTIATED TUMORS

TYPE OF TUMOR	IMMUNOHISTOCHEMICAL MARKER	COMMENTS
Carcinoma	Broad spectrum keratins AE1/AE3 or PANK (MNF-116)	Some carcinomas may express unusual keratin sub-types. If negative, try other keratin types (e.g., CAM5.2). The Ck7/Ck20 pattern may be helpful in determining the likely site of origin. Some non-carcinomas can have an epithelioid appearance and strongly express keratins (e.g., epithelioid angiosarcoma, epithelioid sarcoma, mesothelioma).
Melanoma	S100 protein	S100 is strongly positive in the vast majority of melanomas. Some carcinomas (especially breast) and sarcomas are also positive for S100 and additional markers may be required. HMB-45 and MART-1 are expressed by most epithelioid melanomas but may be focal or absent in non-epithelioid melanomas (e.g., spindle cell or desmoplastic melanomas).
Lymphoma	Leukocyte common antigen (LCA)	Present in almost all non-Hodgkin's lymphomas. May be absent in 30% of anaplastic (Ki-1) large cell lymphomas. These lymphomas are keratin negative but may express EMA. These tumors will be positive for CD30 (Ki-1) and ALK.

Estrogen and progesterone receptor evaluation

Hormone receptors are routinely determined on all invasive breast carcinomas and DCIS. ER and PR are weak prognostic markers and are more useful to predict the likelihood of response to hormonal therapies.

Many different methods are currently used to report the results of IHC studies for ER and PR. One method that has been used in multiple studies is the Allred score method (Table 7-12).

Patients with carcinomas that scored 3 (<1% of cells with intermediate intensity or 1% to 10% of cells with weak intensity) or above had improved disease-free survival when treated with endocrine therapy.[11] Patients with carcinomas with a total score of 2 (<1% weakly positive cells) had a survival rate similar to ER-negative carcinomas (total score of 0).

About 80% of DCIS cases are positive for ER using the same method of scoring. Women with ER-positive DCIS were shown to experience fewer local recurrences, contralateral recurrences, and distant recurrences when treated with tamoxifen (NSABP B24 study).

With optimization of IHC using newer antigen retrieval methods, 99.2% of carcinomas will have scores of 0, 7, or 8.[12] Therefore, many laboratories report results as positive or negative. The value of further subdividing cases by percent positive cells, H-score, or image analysis for either prognosis or to predict response to tamoxifen has not been demonstrated. Intensity of immunoreactivity is difficult to evaluate as most cases show strong reactivity with optimal assay methods and most carcinomas show considerable variability in intensity.

A possible method for reporting results is shown in Table 7-13. The same system can be used for reporting progesterone receptor results. The use of both ER and PR may be helpful for determining the likelihood of response to tamoxifen, as has been shown with data using the biochemical assay (Table 7-14). Presumably, the presence of the ER-regulated gene product PR is more predictive of an intact ER regulatory pathway.

Recent national guidelines for reporting ER and PR have been released and should be consulted for additional information about performing and interpreting these studies (Hammond ME, et al, American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer, J Clin Oncol 2010 Apr 19).

TABLE 7–12. ER AND PR—ALLRED SCORE

PROPORTION SCORE (PS)	% POSITIVE CELLS	INTENSITY SCORE (IS)	INTENSITY OF POSITIVITY
0	0	0	None
1	<1%	1	Weak
2	1% to 10%	2	Intermediate
3	10% to 33%	3	Strong
4	33% to 66%		
5	>66%		

The PS and IS Are Added Together for a Total Score:

Total Score (TS) = PS + IS	Interpretation
0, 2	Negative
3, 4, 5, 6, 7, 8	Positive

TABLE 7–13. REPORTING RESULTS OF ER AND PR EVALUATION

RESULT	CRITERIA	% OF CASES	COMMENTS
Positive	>10% of cells	70% to 80%	This group corresponds to PS scores of 3 and above. The majority of these carcinomas will have scores of 7 or 8.
Borderline or low positive	>0% to 10%	<5% to 10%	The clinical significance of this group is unclear. This group may be interpreted as "negative" or "positive" by some laboratories depending on the cut-off point chosen. This group could include cases with Allred scores of 2, 3, 4, or 5.
Negative	0	20% to 30%	This group corresponds to a TS score of 0.

TABLE 7–14. RESPONSE TO TAMOXIFEN

STATUS OF CARCINOMA	% OF CARCINOMAS	% OF PATIENTS RESPONDING TO TAMOXIFEN
ER+ PR+	63%	75% to 80%
ER+ PR–	15%	25% to 30%
ER– PR+	5%	40% to 45%
ER– PR–	17%	<10%

False negative results, and to a lesser extent, false positive results, can also be problems. **False negative results** may be due to a large number of causes including:

- Low sensitivity of the assay
- Errors in performing the assay
- Delayed fixation of tissue
- Over- or underfixation of tissue
- Overheating of tissue (e.g., with cautery during surgery)
- Decalcification of tissue

Most cases of false negativity can be suspected, as the normal breast tissue will also be negative. In such cases, the assay should be repeated on the same block, a different block from the same case, or blocks from another case, if available. If the normal tissue remains negative, the possibility of loss of antigenicity in the tissue can be mentioned in the report.

False positive results are quite unusual, as the antibody should not crossreact with other antigens.

- Entrapped normal ducts or lobules misinterpreted as carcinoma — this can be a difficult issue for DCIS as some ducts or lobules may be only partially involved by DCIS.
- Control placed on same slide misinterpreted as the carcinoma
- Sclerosing adenosis or myofibroblastoma (or other benign lesions) misinterpreted as invasive carcinoma

TABLE 7–15. HER2-IHC SCORING

SCORE	CRITERIA	% OF CASES	% OF CASES THAT SHOW AMPLIFICATION BY FISH
0 (Negative)	No immunoreactivity or immunoreactivity in <10% of tumor cells.	~ 60%	0% to 3%
1+ (Negative)	Faint weak immunoreactivity in >10% of tumor cells but only a portion of the membrane is positive.	~ 10%	0% to 7%
2+ (Equivocal)	Weak to moderate complete membrane immunoreactivity in >10% of tumor cells.	~ 5% to 10%	25% to 35%
3+ (Positive)	More than 30% of the tumor cells must show circumferential intense and uniform membrane staining. A homogeneous (chicken wire) pattern should be present.	~15% to 20%	75% to 90%

HER2/neu score

The HER2/neu immunoreactivity scoring system in Table 7-15 was recommended by an expert panel.[13] Other panel suggestions:

- If cytoplasmic positivity obscures the membrane pattern, repeat the assay or perform FISH.
- If normal ducts and lobules show definitive positivity, repeat the assay.
- In cases of invasive carcinoma, only the areas of invasion should be scored. In some cases the associated DCIS can show stronger immunoreactivity.
- Fixation must be in neutral buffered formalin and should, ideally, be between 6 and 48 hours for excisions, and at least 1 hour for needle biopsies. However, any effect from longer fixation has not been shown.
- Unstained slides should not be used if prepared >6 weeks earlier. Loss of antigenicity has been shown for other antigens, but not specifically for HER2.

Only membrane immunoreactivity is scored. Marked cytoplasmic immunoreactivity may make interpretation difficult. FISH studies may be preferred for such cases (Table 7-16).

TABLE 7–16. FISH RESULTS

RESULT	CRITERIA	COMMENT
Positive for amplification	>6.0 gene copies or >2.2 ratio	
Equivocal for amplification	4.0 to 6.0 genes or 1.8 to 2.2 ratio	Guidelines suggest counting additional cells for FISH, retesting, or performing IHC
Negative for amplification	<4.0 genes or <1.8 ratio	

Patients with a ratio of 2.0 or greater have been eligible for Herceptin trials.

In >90% of carcinomas with protein overexpression, the HER2/neu gene has been amplified. In 3% to 5% of cases, protein overexpression can occur due to other mechanisms. In <5% of cases, there may be gene amplification without protein overexpression. In general, there is a 20% to 40% response to Herceptin alone in patients with cancers showing gene amplification by FISH, and <5% of patients respond if the gene is not amplified. Therefore, FISH studies may be helpful for cases with 2+ positivity or difficult to interpret cases (e.g., with variable positivity or cytoplasmic positivity).

Well- and moderately differentiated lobular carcinomas are rarely positive (<5%). However, in some cases there may be edge enhancement of individual tumor cells that may be difficult to interpret. FISH studies may be helpful.

In rare cases, DCIS overexpresses HER2/neu but the accompanying invasive carcinoma does not. This is a source of potential false positive results for IHC or FISH.

Myoepithelial markers in breast carcinoma

Myoepithelial markers can be useful for the evaluation of breast lesions (Table 7-17):

- Invasive carcinoma vs. sclerosing adenosis (frequently involved by DCIS, LCIS, or apocrine metaplasia).
- DCIS vs. DCIS with microinvasion – Double immunolabeling with p63 (brown nuclear) and cytokeratin (AE1/AE3 – red cytoplasm) can be useful to highlight small nests of tumor cells lacking myoepithelial cells. A double stain with SMMHC and cytokeratin AE1/AE3 can also be helpful.
- DCIS vs. carcinoma invading as circumscribed tumor nests vs. lymphovascular invasion.
- Microglandular adenosis is the only "benign" breast lesion that lacks myoepithelial cells. However, this lesion may be a form of well-differentiated non-metastasizing invasive carcinoma. The cells are negative for ER and PR and strongly positive for S100.

Epidermal lesions of the nipple

See Table 7-18.

Breast carcinoma in males versus metastatic prostate carcinoma

See Table 7-19.

TABLE 7–17. MYOEPITHELIAL MARKERS IN BREAST CARCINOMA

MARKER	LOCATION	NORMAL LUMINAL CELLS	MYOEPITHELIAL CELLS	BLOOD VESSELS	MYOFIBROBLASTS	CARCINOMAS[a]	COMMENT
p63	Nucleus	neg	POS	neg	neg	rare	Only nuclear marker Clean background
SMA	Cytoplasm	neg	POS	POS	POS	rare	Most sensitive-marker
CD10	Membrane	neg	POS	neg	POS	rare	
SMM-HC	Cytoplasm	neg	POS	POS	High	rare	
Calponin	Cytoplasm	neg	POS	POS	Mod	rare	

[a]Rare carcinomas with myoepithelial features (adenoid cystic carcinomas, some spindle cell carcinomas, some basal-like carcinomas, some carcinomas associated with BRCA1 mutations) can show focal to diffuse positivity for myoepithelial markers.
S100 protein and cytokeratins (e.g., 34βE12) are not recommended for identifying myoepithelial cells, as fewer myoepithelial cells are positive and luminal cells can also be positive.
p63 is a good general marker for myoepithelial cells and is particularly helpful in cases with prominent myofibroblasts (e.g., sclerosing lesions) or with blood vessels closely apposed to tumor cells (e.g., papillary fronds in papillary DCIS). In some cases, SMA may be positive in more myoepithelial cells than p63.

TABLE 7–18. EPIDERMAL LESIONS OF THE NIPPLE AND PAGET DISEASE AT OTHER SITES

TUMOR	AE1/AE3	CAM 5.2 OR CK7	CK20	EMA	S100	HMB45	GCDFP-15	CEA-P	CEA-M	HER2	ER OR PR	MUC1	MUC2
Paget disease of the nipple	POS	POS	neg	POS	Mod	neg	Mod	Mod	Low	POS	Low	POS	neg
Toker cells	POS	POS	neg	Mod	neg	neg				neg	High	POS	neg
Squamous cell carcinoma	POS	Low	Low	POS	Low	neg	neg	Low	Mod	Low	neg	neg	
Melanoma	Low	Low	neg	Low	POS	POS	neg	Mod	neg	neg	neg	neg	
Vulvar Paget disease	POS	POS	High				POS			? Low		POS	neg
Perianal Paget disease	POS	Mod	POS									Low	POS

Most cases of Paget disease of the nipple are associated with DCIS deeper in the breast that involves the lactiferous sinuses, and about half will also have areas of invasion. Rare cases may be difficult to interpret due to the absence of associated disease in the breast or if the initial biopsy is shallow. In some cases, Paget cells may take up melanin and may be difficult to distinguish from melanoma. Toker cells are present in nipple epidermis in 40% to 80% of nipples and are Cam5.2 and Ck 7 positive but are negative for HER2/neu.
Paget disease of the vulva and perianal region has a similar distribution (i.e., tumor cells are present between an intact basement membrane and an overlying normal epidermal layer) but the tumor cells have different origins.
Initial panel: Cam5.2 (or Ck7), HER2, and S100 with additional antibody studies based on these findings, if necessary.

TABLE 7–19. BREAST CARCINOMA IN MALES VERSUS METASTATIC PROSTATE CARCINOMA

	CK7	ER	PSA	PAP
Breast carcinoma	**POS**	**High**	Mod	
Prostate carcinoma	neg		**POS**	**POS**

Carcinomas in the breast of males with prostate carcinoma can be difficult to classify, as these males are at increased risk for breast cancer, prostate carcinomas can mimic a well- or moderately differentiated breast cancer, and DCIS is often scant or absent. In addition, some breast carcinomas can express prostate markers. A panel of markers should distinguish these two types of carcinoma in most cases.

Signet ring cell carcinomas of the stomach and breast (lobular carcinoma)

See Table 7-20 and Fig. 7-2.

TABLE 7–20. SIGNET RING CELL CARCINOMAS OF THE STOMACH AND BREAST (LOBULAR CARCINOMA)

CARCINOMA	ER	PR	GDCFP	MUC1	MUC2	FCK7	CK20	E-CAD	CDX2	HEP PAR
Stomach	neg	neg	neg	Low	Mod	Mod	Mod	Mod	High	High
Breast	High	Low	High	POS	Low	POS	rare	Low	neg	neg

See Figure 7-2

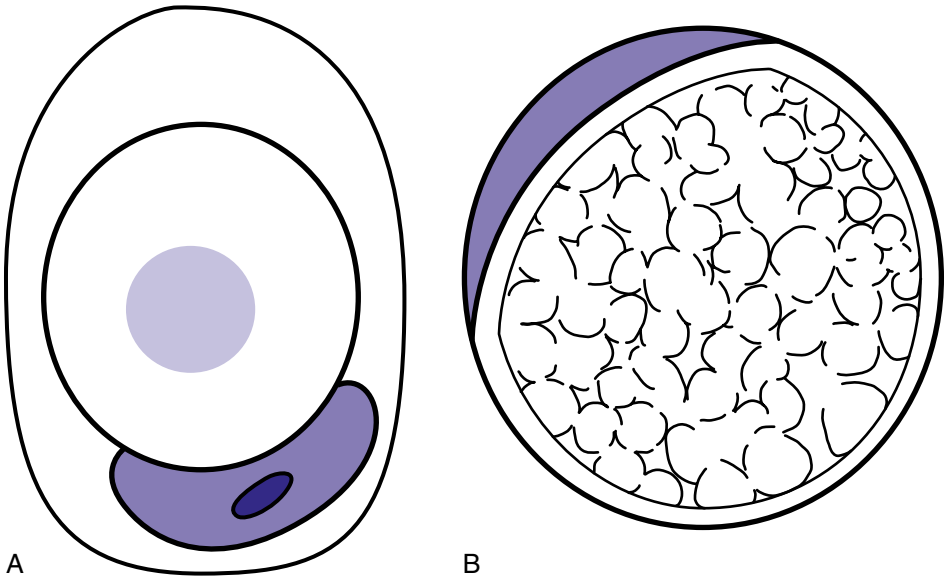


Figure 7–2. Metastatic lobular carcinoma of the breast can morphologically resemble primary signet ring cell gastric carcinomas. Both typically lack e-cadherin expression. In addition, lobular breast carcinomas can be clinically occult or can present as distant metastases many years after the initial presentation. Signet ring cells associated with breast carcinoma more commonly have a central mucin vacuole with a targetoid appearance (cell **A**). Gastric signet ring cells usually have many small vacuoles giving the cytoplasm a foamy appearance (cell **B**). These criteria are not reliable in distinguishing these two carcinomas. However, the presence of the first type of signet ring cell in a biopsy from the gastrointestinal tract should raise the possibility of metastatic breast carcinoma.[14] The majority of lobular breast carcinomas will be ER positive, and this is a reliable marker to exclude gastric carcinoma. In the minority of ER-negative cases, PR, GCDFP, MUC1, CDX2, and Hep Par may be helpful markers.[15]

Fibroblastic/myofibroblastic lesions of the breast

See Table 7-21.

TABLE 7–21. FIBROBLASTIC/MYOFIBROBLASTIC LESIONS OF THE BREAST

NAME	CD34	SMA	HHF-35	DES	KER*	p63	S100	ER	PR	COMMENTS
Normal stroma	100	–/+	–/+	rare	0	0	0	0	rare	
FA/phyllodes	100	66		12	0	0	12	0	–/+	
PASH/fibrous	100	+/–		+/–	0			0	+/–	
Myofibroblastoma	90	74	100	90	0	0	2	59	100	
Spindle cell lipoma	100	0	0	0			0			13q 16q rearrangements
Solitary fibrous tumor	95	13	7	7	0		9	0	17	
Fibromatosis	0	79	78	43	0		0	rare	rare	Some associated with FAP Nuclear beta-catenin
Nodular fasciitis	0	97	91	4			0			
To Be Distinguished From:										
Leiomyoma	18	90	90	70	0		6	90	90	Usually near nipple, long fascicles, more cytoplasm
Spindle cell carcinoma	0	55	30		55	40	30	rare	rare	May have epithelial areas, DCIS

*Spindle cell carcinomas may express keratins more typical of myoepithelial cells (e.g., CK 14 and CK 17). These keratins may be detected best with MNF-116 (= PANK; includes CK 17) or 34betaE12 (includes CK 14) or antibodies specific for these keratins.
Some epithelioid myofibroblastomas can closely resemble invasive lobular carcinoma. In these cases, the carcinoma will be strongly positive for typical keratins and also positive for ER and PR.

Ovarian carcinoma versus mesothelioma

See Table 7-22.

TABLE 7–22. OVARIAN CARCINOMA VERSUS MESOTHELIOMA

	CK7	CK20	CK5/6	CEA m	CEA p	CD15 (LEUM1)	ER	WT1	CALRET	BER-EP4
Peritoneal mesothelioma	High	neg	POS	neg	neg	rare	rare	High	High	neg
Ovarian serous carcinoma	POS	Low	neg	neg	neg	Mod	POS	POS	Low	POS
Ovarian endometrioid carcinoma	POS	neg	Low	Mod	Low		High	High	Low	POS
Ovarian mucinous carcinoma	POS	High	neg	Mod	Low		Low	neg	Low	

Primary ovarian carcinoma versus metastatic carcinomas

See Table 7-23.

TABLE 7–23. PRIMARY OVARIAN CARCINOMA VERSUS METASTATIC CARCINOMAS

	CK7	CK20	DPC4 (SMAD4)	CDX2	ER	CEA m	CEA p
Endometrioid ovarian carcinoma	POS	neg		Low	Mod	Mod	Low
Clear cell ovarian carcinoma	POS	neg		Mod ?	Mod		neg
Mucinous ovarian carcinoma	POS (diffuse)	High (patchy)	POS	Mod	Low	Mod	Low
Mucinous breast carcinoma	POS	Low	POS	neg[a]	POS	Mod	Low
Pancreatic carcinoma	POS	High	Mod	Mod	neg	High	POS
Appendiceal carcinoma	Low (patchy)	POS	POS			High	Low
Mucinous colon carcinoma	Low (patchy)	POS (diffuse)	High	POS	neg	POS	POS

[a]Breast cancers, in general, are negative for CDX2 and MUC2. The results for mucinous breast carcinomas have not been reported. 18% of mucinous ovarian carcinomas are positive for MUC2.

Endocervical carcinoma versus endometrial carcinoma

See Table 7-24.

TABLE 7–24. ENDOCERVICAL CARCINOMA VERSUS ENDOMETRIAL CARCINOMA

	CK7	CK20	VIM	CEA m	CEA p	p16	HPV(IN SITU)	ER	PR
Endocervical carcinoma	POS	rare	rare	POS	High	POS (diffuse, strong)	High	Low (focal)	Low
Endometrial carcinoma	POS	rare	POS	Low[a]	Mod	Low (patchy, weak)	neg	High (diffuse)	High

[a]27% of cases have some positivity but primarily in squamous areas and only focal in glandular areas.

Endometrial stromal sarcoma versus leiomyosarcoma

See Table 7-25.

TABLE 7–25. ENDOMETRIAL STROMAL SARCOMA VERSUS LEIOMYOSARCOMA

	CD10	DESMIN	H-CALDESMON	ER/PR
Endometrial stromal sarcoma	High	Mod	neg	High
Leiomyosarcoma	Low	High	POS	High

Trophoblastic lesions

See Table 7-26.

TABLE 7–26. TROPHOBLASTIC LESIONS

	KERATIN	ALPHA-INHIBIN	HPL[a]	HCG[a]	CD146[b] (MELCAM)	P63	KI67[c]	P57[d]	DNA PLOIDY[e]
Choriocarcinoma	POS	POS	weak (focal)	strong (diffuse)	POS	Mod (few cells +)	69%		
Placental site trophoblastic tumor	POS	POS	Mod (greater than hCG)	focal (less than HhPL)	POS	neg	>14%		
Epithelioid trophoblastic tumor	POS	POS	focal	focal	focal	POS	>14%		
Placental site nodule	POS	POS	weak (focal)	focal	focal	POS	<1%		
Exaggerated placental site			POS (diffuse)	focal	POS	neg	0%		
Partial mole	POS	POS	weak[b] (diffuse)	weak (diffuse)				POS	Triploid
Complete mole	POS	POS	weak[b] (focal)	strong (diffuse)				rare[f]	Diploid (paternal)
Hydropic fetus	POS	POS						POS	Diploid (60%) Triploid (40%)

[a]Evaluated in syncytiotrophoblast.
[b]Increases with advancing pregnancy.
[c]Implantation-site intermediate trophoblastic cells are evaluated for the number of Ki67 positive cells. CD146 can be used to help identify these cells using a double label technique. Lymphocytes can also be positive for Ki67 and should not be counted.
[d]p57 is a paternally imprinted gene, expressed from the maternal gene, which shows decreased expression in complete moles, whose DNA is completely derived from paternal DNA.
[e]Ploidy is usually determined by flow cytometry.
[f]In complete moles, p57 positivity is present in villous stromal cells and extravillous trophoblast but absent in intermediate trophoblast lining the villi.
Cytokeratin and alpha-inhibin (syncytiotrophoblastic cells and some intermediate trophoblastic cells) are not useful for the differential diagnosis of these lesions, but may be helpful if other types of tumors are in the differential diagnosis.

Metastatic adenocarcinomas in the abdomen

See Table 7-27.

TABLE 7–27. METASTATIC ADENOCARCINOMAS IN THE ABDOMEN

SITE OF ORIGIN	CK7	CK20	MUC2	MUC5AC	SMAD4*	CDX2	B-CAT	WT-1
Stomach	High (75)	Mod (45)	Mod (50)	Mod (55)	POS (100)	Mod (12-50)	High (63)	Mod (47)
Colon	neg (10)	POS (95)	High (60-100)	Low (25-40)	POS (95)	POS (90-100)	High (60-100)	High (63)
Appendix	Low (30)	POS (96)	POS (96)	High (85-100)	High (80-90)	POS (100)	Very low (9)	
Pancreas	POS (95)	High (75)	POS (100)	High (73-92)	Mod (45)	Mod (15-60)		Mod (54)
Uterus	POS (100)	Low (15)	neg (0)	Low (31)	POS (100)	Very low (7)	Mod (48)	Mod (50)
Ovary, serous	POS (100)	Low (15)	Low (12-38)	High	POS (95)	Mod (29-50)	Very low (0-10)	High
Ovary, mucinous	POS	High	Low (18)	POS	POS	Mod (34)		Low (12)
Breast, NST	POS (95)	neg (4)	Very low (9)	Low (37)	POS	neg (0)		Low
Breast, mucinous	POS	Low	POS (100)	Low (23)	POS			High (64)

*The lack of SMAD4 is found in about half of pancreatic carcinomas and is highly suggestive of this primary site.
In some published tables a "positive" result is the absence of positivity. In this table, "positive" signifies that the carcinoma shows immunoreactivity for SMAD4.

CNS neoplasms

See Table 7-28.

TABLE 7–28. CNS NEOPLASMS

	OLIG2[a]	GFAP	SYN[b]	NEUN	EMA	OTHER MARKERS
Astrocytoma	POS	POS	neg	neg	neg	
Oligodendroglioma	POS	Low (focal)	neg	neg	neg	
Ependymoma	Low	Low (focal)	neg	Low	Low	
Pilocytic astrocytoma	POS	POS	neg	neg	neg	
Ganglioglioma	POS	POS	POS	POS		
Central neurocytoma	Low	neg	POS	POS		
Medulloblastoma	Low	Low	POS	POS	neg	
Choroid plexus tumors		neg/rare	neg	neg	Low	CK POS
Meningioma	neg	neg	neg	neg	POS	HMB-45 POS in melanocytic variant CD34 neg
Hemangiopericytoma/ solitary fibrous tumor		neg			Low	CD34 POS
Atypical teratoid/rhabdoid tumor	Low	Low	Low		Low	INI1 neg[c] VIM POS
Schwannoma	neg				neg	
Lymphoma	neg	neg	neg		neg[d]	LCA POS
Melanoma	neg	neg	Low		neg	HMB-45 POS
Metastatic carcinoma	neg	neg	Low		POS	CK POS

[a]OLIG2: The majority of cells will be positive in diffuse gliomas. Other tumors can show smaller numbers of positive cells (typically much less than 50%).
[b]SYN: Any neural tumor can show focal positivity for synaptophysin.
[c]INI1/SMARCB1 protein: The absence of this protein is highly specific for atypical teratoid/rhabdoid tumor.
[d]EMA can be positive in myelomas

Hemangioblastoma versus metastatic renal cell carcinoma

See Table 7-29.

TABLE 7–29. HEMANGIOBLASTOMA VERSUS METASTATIC RENAL CELL CARCINOMA

	INHIBIN	RCC	CD10
Hemangioblastoma	POS	neg	neg
Metastatic renal cell carcinoma	neg	POS	POS

Tumors of germ cells and sex-cord stromal tumors

See Table 7-30.

TABLE 7–30. TUMORS OF GERM CELLS AND SEX-CORD STROMAL TUMORS

	AE1/AE3	CAM5.2	NSE	EMA	PLAP[a] (MEM)	OCT4	NANOG	AFP	CD30(Ki-1, BER-H2)	CD117 (CKIT)	SOX2[e]	VIM	HCG	HPL	INHIBIN	MELAN A103	OTHER
Seminoma	Mod	Low[b]	High	neg	POS	POS	POS	neg	Low	POS	neg	Mod	Low[c]	neg	neg	neg	
Intratubular germ cell neoplasia		neg			POS	POS	POS			POS		neg					
Embryonal carcinoma	POS	POS	High	Low	High	POS	POS	Low	High[d]	neg	POS	Low	Low	neg	neg	neg	
Yolk sac tumor	POS	POS	High	neg	Mod	neg	neg	High	Low	neg	neg	Low	neg	neg	neg	neg	
Choriocarcinoma	POS	POS	Mod	Mod	Mod	neg	neg	neg	neg		neg	neg	POS	POS	POS	neg	
Spermatocytic seminoma		Mod (focal)			neg	neg		neg	Mod?	Variable							
Leydig cell tumor	Mod	Mod		Low	Low	neg		neg				POS			POS	High	
Granulosa cell tumor	Low	Mod	Low	neg	neg	neg		neg		neg		POS			POS	High	WT1 High HHF35 High S100 Mod
Sertoli cell tumor	Mod	Mod		POS	neg	neg				neg		High			POS		

[a]PLAP is expressed in embryonic germ cells, but not in normal spermatogonia, spermatocytes, and spermatids.
[b]CAM5.2 is present as a strong dot-like paranuclear positivity. 80% of mediastinal seminomas are positive for CAM5.2 compared to 20% to 30% of testicular seminomas.
[c]HCG may be positive in trophoblasts in seminomas.
[d]Only 35% of metastatic embryonal carcinomas to lymph nodes after chemotherapy are positive for CD30.
[e]SOX2 is not specific for embryonal carcinoma, as many carcinomas can be positive for this marker.
FISH for 12p can be used to identify germ cell tumors and their metastases.
D2-40 (podoplanin) is strongly expressed in seminomas and ITGCN. It is also expressed in lymphatic endothelium, epithelioid mesotheliomas, and hemangioblastomas.

Adrenal and kidney tumors

See Table 7-31.

TABLE 7–31. ADRENAL AND KIDNEY TUMORS

	AE1/ AE3	CK7	CK20	PANK	CAM5.2	MUC-1 (EMA)	S100	CHROM	SYN	MART1 A103[c]	INHIBIN	NSE	NFP	AMACR	VIM	OTHER	IRON STAIN
Adrenal Tumors[d]																	
Cortical adenoma	neg	neg	neg	Low	Low	neg	neg	neg	POS	POS	High	High	neg	neg	High	TTF1 neg CD10 neg	
Cortical carcinoma					neg		neg	neg	High	POS	POS		neg				
Pheo/ paraganglioma		neg	neg	neg	neg	neg	High[a]	POS	POS	neg	neg	POS	POS		Mod	GFAP mod	
Kidney Tumors																	
RCC – clear cell	High	Low	neg	High	High	High (diff)	Low	neg	neg	neg	neg	Mod	neg		High	p63 neg TTF1 neg GFAP low RCC POS CD10 POS	Focal, coarse
RCC - papillary	POS	High	Low	POS		Mod (mem)								POS		RCC POS CD10 POS	Focal, coarse
RCC - chromo-phobe	High	High	neg	POS		POS (mem)								neg	neg	RCC Mod CD10 neg	Diff, strong
Oncocytoma[b]	Mod	High	neg	POS												RCC neg CD10 low	Focal, weak
Transitional cell carcinoma	Mod	POS	High	POS	POS	POS	neg	neg	neg	neg		Low		Low	Low	p63 POS CD10 mod	

[a]Positivity is present in sustentacular cells. These cells may be absent in malignant tumors.
[b]50% of oncocytomas have a punctate/dot-like pattern for CK 8 or 18 which is not seen in RCC. EM may be helpful to distinguish oncocytoma from chromophobe RCC (see Table 7-46).
[c]Antibody A103 is positive in adrenal cortical carcinomas. Another antibody to the same antigen, M2-7C10 is not positive in adrenal cortical carcinomas.
[d]Clear cell renal cell carcinoma metastatic to the adrenal can sometimes be confused with an adrenal cortical tumor (thus, the older term for clear cell carcinoma of "hypernephroma"). RCC has clear cytoplasm (compared to the bubbly cytoplasm of the adrenal cortex) and blood lakes are typically present. Glycogen is present in RCC and absent in adrenal lesions (demonstrated by PAS with and without diastase). Cytokeratin and EMA are useful IHC markers.
Diff = diffuse positivity; mem = positivity located on membrane.
Renal cell carcinoma subtypes have typical cytogenetic abnormalities (see Table 7-47).
CD117 (c-kit) has been reported to be positive in almost all papillary renal cell carcinomas (cytoplasmic) and chromophobe carcinomas (membrane) but is not present in clear cell carcinomas. Mutations in c-kit were only found in papillary carcinomas.

Tumors of bladder, prostatic, and renal origin

See Table 7-32.

TABLE 7–32. TUMORS OF BLADDER, PROSTATIC, AND RENAL ORIGIN

	CK7	CK20	KERATIN HMW	PSA	PAP	AMACR	CEA m	CEA p	P63	CA125	MUCI
Prostatic carcinoma	Low	Low	neg	High	POS	POS	neg	Mod	neg	neg	neg
Transitional cell carcinoma	POS	High	Mod	neg	neg	Low	Mod	Mod	High	neg	neg
Bladder adenocarcinoma	High	High	neg	neg	neg		Mod	High		Low	POS
Renal cell carcinoma – clear cell	Low	neg	neg	neg			Low	neg	Low	neg	neg
Rectal adenocarcinoma	Low	POS	neg	neg	neg		POS	POS		neg	POS
Seminal vesicle carcinoma	High	neg		neg	neg		VAR	POS		High	

Prostate carcinoma versus other lesions

See Table 7-33.

TABLE 7–33. PROSTATE CARCINOMA VERSUS OTHER LESIONS

	34βE12 (BASAL CELLS)	P63 (BASAL CELLS)	AMACR (504S) (GLANDULAR CELLS)	PSA
Benign glands	POS	POS	neg	POS
PIN	POS	POS	High	POS
Invasive carcinoma	neg	neg	POS	POS
Nephrogenic adenoma	Mod	neg	High	neg

Antibody cocktails: These antibodies can be combined to facilitate the evaluation of small lesions:
34βE12 + p63 = labels a greater number of basal cells than either marker alone.
AMACR + p63 and/or 34β12 = facilitates the identification of small foci of invasive carcinoma.

Hepatic tumors

See Table 7-34.

TABLE 7–34. HEPATIC TUMORS

	CK7	CK20	AE1/AE3	CAM5.2	KERATIN HMW	CEA m	CEA p	TTF-1	HEP	AFP	CD10	CHROM	MUC1D	BILE	CIRRHOSIS	HBV
Hepatocellular carcinoma	Low	neg	Low	POS	neg	neg	High[a]	High[b] (cyt)	High	Mod	High[a]	neg	neg	may be present	65-90%	50%
Hepatoblastoma			Low	POS		Low	High[a]		POS	High		Low			absent	rare
HCC – fibrolamellar	Mod?	neg					POS[a]		POS	neg?				may be present	absent	rare
Cholangiocarcinoma	POS	Mod	POS	POS	High	High	POS	neg	neg	neg	neg		High	negative	rare	rare
Metastatic carcinoid tumor	Low	Low	High	POS		Mod	Mod	Low[c] (nuc)	neg		Low	POS		negative	absent	absent

[a]Bile canalicular pattern. Other carcinomas have a membrane or cytoplasmic pattern.
[b]TTF-1 is seen in the cytoplasm (unlike the nuclear pattern seen in lung and thyroid carcinomas)
[c]Carcinoids arising at sites other than lung are very unlikely to be positive for TTF-1. Lung carcinoids may be positive and are more likely to express Ck7.
[d]Mucin histochemical stains can also be used. HCCs will be negative and 75% to 100% of cholangiocarcinomas will be positive.
Cyt = cytoplasmic immunoreactivity; nuc = nuclear immunoreactivity.
Sinusoids of HCC show diffuse CD34 positivity in 80% to 90% of cases, but this is not seen in normal liver. CD34 positivity can also be seen in focal nodular hyperplasia. Metastatic carcinomas can show diffuse positivity in 20% of cases, but the positive endothelial cells are present throughout the tumor and the cells do not surround nests of tumor cells, as is seen in HCC.
Reticulin stains can be helpful in the evaluation of fine needle aspirates or core needle biopsies of liver lesions. HCC has an abnormal pattern of absent, decreased, or expanded trabecula, whereas benign lesions will show a normal trabecular pattern.
Metastatic carcinomas can usually be distinguished from HCC by frequent expression of Ck7, only rare expression of HepPar1, the absence of a bile canicular pattern for CEAp and CD10, and the absence of cytoplasmic positivity for TTF-1.
Metastatic carcinomas to the liver often cannot be reliably distinguished from cholangiocarcinomas by histologic appearance or immunohistochemical pattern, with the exception of colorectal carcinomas. If the patient has a known primary carcinoma, it is most helpful to compare the two tumors.

See Table 7-35.

TABLE 7–35. THYROID AND PARATHYROID LESIONS

	KER-HMW	CK19	HBME[a]	GALECTIN-3	CALCI-TONIN	SYN	CHRO	RET	P27	PPAR GAMMA	THY	TTF-1	S100[b]	CEA m	CEA p	CD57	RBPRO-TEIN	VIM	OTHER
Thyroid Lesions:																			
Hyperplastic nodule		Low	Low	Low	neg				POS	neg	POS	POS				Low	POS		
Follicular adenoma	neg	Low	Low	Low	neg	Mod	neg	neg	POS	Low 10%	POS	POS	Low			Low	POS	POS	
Follicular carcinoma	neg	Low	Mod	Low	neg	High	neg	neg	POS	Low 30%	POS	POS	Mod	neg	Low	Mod	neg?	POS	
Papillary carcinoma — follicular variant		POS	POS	Mod	neg			Low	Mod	Low 10%	POS	POS				High	neg		
Papillary carcinoma	POS	POS	High	High	neg	High	neg	Low	POS	Low 10%	POS	POS	High	neg	Mod	POS	neg	POS	p63 POS
Medullary carcinoma	neg			Mod	POS	POS	POS			neg	Low	POS		POS	Mod	Mod	Mod	High[c]	Ck7 POS PANK POS
Anaplastic carcinoma[d]		Mod									rare	rare							P53, Cyclin D1, High MIB1 index, BCL2 neg
Parathyroid adenomas and carcinomas		**POS**			Low	Low	POS		High[e]	neg	neg	neg	Low				POS	neg/ weak	PTh POS RCC POS Cyclin D1 POS

[a]Tumors with Hurthle cell changes may be negative for HBME. [b]Hurthle cells (both benign and neoplastic) are positive for S100 (nuclear and cytoplasmic). [c]Spindle cells may be positive for vimentin.
[d]Anaplastic thyroid carcinomas are frequently negative for TTF-1, thyroglobulin, and Ck20 but positive for p53 and Cyclin D1. [e]p27 is low in parathyroid carcinomas.
Thyroid adenomas, follicular carcinomas, papillary carcinomas, and medullary carcinomas are Ck7+ and Ck20-. Variable immunoreactivity has been reported for Ck7 in anaplastic carcinomas.
Metastatic carcinomas to the thyroid will be negative for thyroglobulin, TTF-1 (except for lung carcinomas), and calcitonin.
DDIT3 and ARG2 are new markers that may prove helpful for distinguishing follicular carcinoma (~ 70-80% positive) from adenoma (90% negative)

Differential diagnosis of epithelial mesothelioma and lung adenocarcinoma

See Table 7-36.

Initial panel: AE1/AE3, calretinin, WT-1 (clone 6F-H2), CEA, Leu-M1, and TTF-1 with additional studies ordered in difficult cases.

Other antibodies generally reported as negative in epithelial mesotheliomas and positive in lung adenocarcinomas include the following: MOC-1, B72.3, Ber-EP4, and BG-8. Cytokeratins 5/6 are reported to be positive in mesotheliomas and negative in lung carcinomas. However, in our experience, these markers have proven less useful than the ones listed earlier. The use of EMA is controversial. Strong membrane positivity is characteristic of epithelial mesothelioma, whereas cytoplasmic positivity is characteristic of adenocarcinomas.

Less is known about the immunophenotype of pure sarcomatoid mesotheliomas. The spindle cells are positive for cytokeratin, but are less frequently positive for the other markers as compared to the epithelioid cells. Tumors that can, on occasion, resemble mesotheliomas are generally negative for cytokeratins, with the notable exceptions of some cases of angiosarcoma, epithelioid hemangioendothelioma, synovial sarcoma, epithelioid sarcoma, and leiomyosarcoma (see Table 7-9).[16]

TABLE 7–36. DIFFERENTIAL DIAGNOSIS OF EPITHELIAL MESOTHELIOMA AND LUNG ADENOCARCINOMA

	EPITHELIAL MESOTHELIOMA	LUNG ADENOCARCINOMA
Immunohistochemistry		
AE1/AE3 keratin	POS (perinuclear)[a]	POS (membrane)[b]
Calretinin	POS	NEG
WT-1 (clone 6F-H2)	POS (nuclear)[c]	NEG[d]
CEA (polyclonal)	NEG	HIGH[e]
Leu-M1 (CD15)	NEG	HIGH
TTF-1	NEG	HIGH
Mucins		
Mucicarmine	3-4%	60%
PAS-D	<3%	65%
Alcian blue	30%	Pos ?%
Alcian blue + hyaluronidase	Staining lost	Staining preserved
Ultrastructure (EM)		
Microvilli	Elongated, serpiginous, and branched	Short, blunt, rigid appearing
Length to diameter ratio	10 to 16:1	4 to 7:1
Cytogenetics	Deletions of 1p, 3p, 17p, loss of 9 and 22	Deletions of 3p, highly variable changes

[a]Keratin immunoreactivity is accentuated around the nucleus and is present in the cytoplasm, without a prominant membrane accentuation.
[b]Keratin immunoreactivity is diffusely present in the cytoplasm with membrane accentuation in some cells.
[c]WT-1 immunoreactivity is nuclear.
[d]Metastatic adenocarcinomas are generally negative for WT-1 except for ovarian serous carcinomas and some renal carcinomas (see Table 7-5).
[e]Most metastastic adenocarcinomas will be positive for CEA, but there are some exceptions (see Table 7-5).
Tissue should be obtained for EM and cytogenetics, if possible.

Lung carcinoma

See Tables 7-37 and 7-38.

TABLE 7–37. LUNG CARCINOMAS

	KERATIN 7	KERATIN 20	TTF-1	P63	CHROMO-GRANIN	SYNAPTO-PHYSIN	CDX2	ER/PR
Adenocarcinoma	POS	Low	HIGH	Low	neg	Low	neg	Low/mod
Bronchioloalveolar carcinoma — nonmucinous	POS	Low	HIGH	HIGH	neg	neg	neg	
Bronchioloalveolar carcinoma — mucinous	HIGH	HIGH	Low				neg[c]	
Squamous cell carcinoma	Low	neg	neg	POS	neg	neg	neg	
Large cell carcinoma ("non small cell")	High	Low	Mod	Mod	neg	Low	neg	
Small cell carcinoma[a]	Low	neg	POS	neg	Mod	Mod	neg	neg
Carcinoid tumor	Mod	neg	Low/ neg	neg	POS	POS	neg	
Metastatic colon carcinoma	POS	Low	neg	neg	neg	neg	POS	
Metastatic breast carcinoma[b]	POS	neg	neg	neg[b]	neg[b]	Low	neg	Variable

[a]Small cell carcinomas arising at other sites can also be TTF-1 positive.
[b]If metastatic breast cancer is suspected, the lung lesion should be compared with the breast primary. Most metastatic breast cancers will have the same pattern of ER, PR, and HER2/neu expression. Some breast carcinomas can be strongly chromogranin positive. Rare breast cancers can be p63 positive (squamous cell carcinomas, metaplastic carcinomas [including spindle cell carcinomas] or triple negative carcinomas).
[c]The mucinous type of bronchioloalveolar carcinoma (BAC) may be difficult to distinguish from metastatic colon carcinoma as some cases are CK7 negative, CK20 positive, TTF-1 negative, and can be focally positive for CDX2. However, colon carcinomas are usually diffusely positive for CDX2.

TABLE 7–38. DIFFERENTIAL DIAGNOSIS OF LUNG CARCINOMAS

DIFFERENTIAL DIAGNOSIS	MOST USEFUL MARKERS
Adenocarcinoma vs. squamous cell carcinoma	Keratin 7, keratin 20, TTF-1, p63
Small cell carcinoma vs. basaloid squamous cell carcinoma	P63, TTF-1
Small cell carcinoma vs. carcinoid tumor	Mitoses, necrosis, amount of cytoplasm
Large cell neuroendocrine carcinoma vs. carcinoid tumor	Mitoses, necrosis
Mucinous lung carcinoma vs. metastatic colon cancer	TTF-1, CDX2 (mucinous BAC can be focally positive for CDX2)
Lung carcinoma vs. metastatic breast carcinoma	TTF-1, compare ER/PR/HER2 pattern in primary breast carcinoma and lung tumor

B-cell neoplasms

See Table 7-39.

TABLE 7–39. B-CELL NEOPLASMS

	B-CELL MARKERS																
	CD45 LCA	CD19 B4	CD20 L26	CD22	CD79a	SIG	CIG	CD5 LEU1	CD10 CALLA	CD23	CD43 LEU22	CD34	BCL-2	BCL-6	CD138 SYNDECAN	CYCLIN D1	OTHER
Precursor lymphoblastic lymphoma/ leukemia	+/–	+	+/–	+/–	+ cyt	–	+M	–	+[a]	–	+/–	+/–	–	–	–	–	TdT + CD99 +
Small lymphocytic lymphoma/CLL	+	+	+ wk	+ wk	+	+ M/D wk	–/+	+	–	+	+/–	–	+	–	–	–	CD11c+ wk CD79b – FMC7 –
Mantle cell lymphoma	+	+	+	+	+	+M/D	–	+	–	–	+	–	+	–	–	+	CyclinD + FMC +
Marginal zone lymphoma (MALT)	+	+	+	+	+	+	+/–	–	–	–	+/–	–	+	–	–/+[b]	–	CD11c +/– CD21+ CD35+
Follicular lymphoma	+	+	+	+	+	+M	–	–	+	–/+	–/+	–	+/–	+	–	–	CDw75 +
Burkitt lymphoma and Burkitt-like lymphoma	+	+	+	+	+	+M	+/–	–	+	–	+/–	–	–	+		–	TdT- MIB-1 100% EBER in situ in 52% MYC[c]
Mediastinal large B-cell lymphoma	+	+	+	+	+/–	–	–	–	–			–		+	–	–	CD30+/– wk traf1 60%

Continued

TABLE 7–39. B-CELL NEOPLASMS—cont'd

	B-CELL MARKERS																
	CD45 LCA	CD19 B4	CD20 L26	CD22	CD79a	SIG	CIG	CD5 LEU1	CD10 CALLA	CD23	CD43 LEU22	CD34	BCL-2	BCL-6	CD138 SYNDECAN	CYCLIN D1	OTHER
Large B-cell lymphoma	+/–	+	+	+	+	+/–	+/–	–/+	–/+		–/+	–	–/+	+/–		–	CD30 +/– MIB-1 >40% traf1 <5%
Lymphoplasmacytic lymphoma	+/–	+	+	+	+	+M/D	+M/G st	–	–	–	+/–				–/+[b]	–	
Hairy cell leukemia	+	+	+	+	+	+		–	–	–	–	–	–	–	–	–/+	DBA.44+ CD79b – CD11c + CD103+ CD25+ st FMC7 +
Primary effusion lymphoma	+	–	–		–	–	–					–		–	+	–	CD30 (Ki-1)+ HHV8+ EBER +/–
Plasmacytoma/ myeloma	–/+	–	–/+	–	+	–	+G/A st	–	–/+	–	+/–		–		+	–/+	CD56+ CD38 + EMA +

[a]Lymphoblasts in t(4;11)(q21;q23) ALL are CD10 negative and frequently CD24 negative.
[b]Positive in plasma cell component.
[c]The myc gene (8q24) is translocated to Ig genes:
t(8;14) (heavy chains) 85% of cases.
t(2;8) (kappa light chain)
t(8;22) (lambda light chain)
Cyt = cytoplasmic immunoreactivity; st = strong immunoreactivity; M, D, G, A = type of heavy Ig chain present; wk = weak immunoreactivity.

T-cell neoplasms

See Table 7-40.

TABLE 7–40. T-CELL NEOPLASMS

	CD45 LCA	TCR	CD2 TE/ T11	CD3 T3	CD43 LEU22	CD5 LEU1	CD7 LEU9	CD4 T4	CD8 T8	CD25 IL2R	TIA-1	GRAN-ZYME b	CD56 NCA m	CD30 KI-1	TDT	ALK	OTHER
Precursor lymphoblastic lymphoma/ leukemia	+	–	+/–	+	+/–	+/–	+	+/–	+/–	+/–	–	–	–	–	+	–	CD34+ CD99+ CD1a +/–
T-cell prolympho-cytic leukemia	+	+	+	+wk	+	+	+	+/–	–/+	+/–	–	–	–		–	–	CD1a-
Adult T-cell lymphoma/ leukemia	+	+	+	+	+	+	–/+	+	–	+	–	–	–	+/–		–	
Mycosis fungoi-des and Sezary syndrome		TCRβ+	+	+	+	+	–	+	–/+	–/+	–/+	+/–	–	–	–	–	HECA+
Peripheral T-cell lymphoma, NOS	+	+	+/–	+/–	+	+/–	–/+	+/–	–/+		+	+/–	–/+	+(large cells)	–	–	
Hepatosplenic T-cell lymphoma		TCRδ1+ TCRαβ–	+	+	+	–	+/–	–	–	–	+	–	+/–	–	–	–	CD57- CD16–/+ LMP-1- Perforin –
Panniculitis-like T-cell lymphoma																	
CD56+		–	+	+	+			–	–	–	+	+	+	–	–	–	CD95+
CD56–		+	–	–	+			–	+	–	–/+	+	–	–	–	–	CD95–
Angioimmuno-blastic lymphoma	+	+	+	+	+	+	+	+	–/+	–	+	+	–	–	–	–	CD10+/– CD57+ bcl–6+/–
Enteropathy-type T-cell lymphoma	+			+	+	–	+	–	–/+	–	+/–	+/–	+(small cells)	+ (large cells)	–	–	CD103+

Continued

TABLE 7–40. T-CELL NEOPLASMS—cont'd

	CD45 LCA	TCR	CD2 TE/ T11	CD3 T3	CD43 LEU22	CD5 LEU1	CD7 LEU9	CD4 T4	CD8 T8	CD25 IL2R	TIA-1	GRAN-ZYME b	CD56 NCA m	CD30 KI-1	TDT	ALK	OTHER
Anaplastic large cell lymphoma (Ki-1 lymphoma)	+/–	+/–	+/–	–/+	+/–	–/+	–/+	+/–	–/+	+/–	+/–	+/–	–/+	+(mem, golgi)	–	+/–[b] (cyt, nuc)	Clusterin+[a] EMA+/– Perforin +/– EBER- BSAP–
Extranodal NK/T-cell lymphoma, nasal type	+	–	+	– CD3ε+ (cyt)	+	–	–/+	–	–	–	+	+	+	–/+	–	–	EBER+ CD16+ CD57–
Blastic NK-cell lymphoma		–	–/+	–	+/–		–/+	+/–		–			+	–	+/–	–	CD33- Myelo-

[a]Expressed in all cases of systemic ALCL but less commonly in primary cutaneous ALCL and very rarely in diffuse large B-cell lymphoma, peripheral T-cell lymphoma, and NS HD.
[b]Only positive in systemic ALCL (subset); negative in primary cutaneous ALCL.
Cyt = cytoplasmic; nuc = nuclear; wk = weak immunoreactivity.

Hodgkin lymphoma

See Table 7-41.

TABLE 7–41. HODGKIN LYMPHOMA

	CD45 LCA	CD20 L26	CD3 T3	CD15 LEUM1	CD30 Ki-1	EMA	SIG	CD79A	CDW75	OCT2	BOB.1	BSAP	LMP1	OTHER
Classical Hodgkin lymphoma (HL)	–	–/+	–	+/–	+	– rare	–	–/+	–	–	–/+	+	+/–	traf-1 + bcl2 +
Nodular sclerosis HL	–	–/+	–	+/–	+	– rare	–	–/+	–	–	–/+	+	–/+	
Lymphocyte-rich HL	–	–/+	–	+/–	+	– rare	–	–/+	–	–	+/–	+	+/–	
Mixed cellularity HL	–	–/+	–	+/–	+	– rare	–	–/+	–	–	–/+	+	++/–	
Lymphocyte-depleted HL	–	–/+	–	+/–	+	– rare	–	–/+	–	–	–/+	+	+ (if HIV +)	
Nodular lymphocyte-predominant HL	+	+	–	–	–/+	+/–	+	+ wk	+/–	+	+	+	–	bcl-6 + bcl 2 –

Wk = weak.

Amyloid

Amyloidosis (Greek for *amylon* = starch plus *eidos* = resemblance) is seen in many different clinical settings and is associated with many diseases. Pathologists can narrow down the differential diagnosis considerably to help guide clinical decision making. Finding an amyloid deposit in any tissue is similar to finding metastatic carcinoma in a lymph node – in both settings clinical information (e.g., history, physical examination, radiology studies, results of laboratory tests) is essential in arriving at the correct interpretation. A little immunohistochemistry and a lot of clinical judgment by the pathologist can help establish the cause with a greater degree of certainty.[17]

Finding and characterizing amyloid deposits:

1. Examine the H&E slide for noncellular material in the correct location for the suspected disease (Table 7-42).

TABLE 7–42. AMYLOID

TYPE OF AMYLOID-RELATED DISEASE	TYPE OF PROTEIN (AVAILABLE TESTS)	UNDERLYING DISEASE	ORGAN INVOLVEMENT	OTHER FEATURES
Primary AL	Kappa and lambda light chains (IF and IHC)	Multiple myeloma (15% have amyloid) Benign monoclonal gammopathy	Heart, bone marrow (only amyloid at this site), kidney, neuromuscular, joints, liver, spleen, tongue, larynx	May have Factor X deficiency (binds to light chains) Localized forms of amyloid are not associated with systemic disease
Secondary AA	Serum amyloid protein A (IF[b])	Chronic inflammation: infection, RA, Crohn's disease, sarcoid, familial Mediterranean fever, malignancy (RCC, HD)	Spleen (100% – sago [tapioca] or lardaceous), kidneys (75%), adrenals (40%), heart (symptoms rare), joints (rare)	
Dialysis-associated	Beta-2-microglobulin (IHC)	Long-term hemodialysis, rarely seen in peritoneal dialysis or with chronic renal failure	Joints (periarticular tissue), carpal tunnel, rarely systemic (GI, vessels), rarely involves fat, does not involve spleen	
Medullary carcinoma-associated	Calcitonin (IHC)	Medullary carcinoma of the thyroid	Associated with the tumor	Calcitonin can also be used to detect C cell hyperplasia
Other tumor-associated amyloid	Peptide hormones	Endocrine tumors	Associated with the tumor	
Alzheimer's	Beta amyloid (IHC)	Alzheimer's disease	Brain – senile plaque cores, neuritic plaques, neurofibrillary tangles	Also seen in Lewy body dementia, Down's syndrome, hereditary cerebral amyloidosis (Dutch type)
Other hereditary dieases	Transthyretin (IHC)	Familial amyloid polyneuropathy, senile/cardiac amyloidosis	Heart (usually without symptoms), joints, prostate	
Amyloid P component AP				Associated with all forms of amyloid. May be used to detect amyloid radiologically.

[a]Light chains are detected best by immunofluorescence (IF) on unfixed frozen tissue. IF and IHC can be performed on paraffin sections, but with less specificity. Only 50% of cases of light chain disease amyloid will be positive because the amyloid protein is often derived from the variable domain whereas antibodies detect the common domain.
[b]Serum amyloid protein A can only be detected by IF on unfixed frozen tissue.

2. Amyloid deposits will be orange-pink on Congo Red stains or sea-foam green on Sulfated Alcian blue stains. Amyloid may be more apparent on these stains. HOWEVER, beware of overcalling cases in which there is not a histologic correlate for amyloid in the stained tissue. If there is background positivity in normal tissue due to overstaining, the slide cannot be interpreted. Positive controls must show appropriate specific positivity.
3. Congo red-positive amyloid should become an apple green color when viewed under polarized light. This may require the high-quality polarizers that are built in to the microscope. Lower quality polarizers (i.e., the cut squares of polarizing material) may not be adequate. Collagen (silver when H&E is polarized) and fibrin (does not polarize) may mimic amyloid.
4. The amyloid deposits can be further characterized using immunohistochemistry or immunofluorescence (see Table 7-42) based on the clinical information, the organ or structures involved, and the distribution of amyloid deposits in the tissue. Amyloid can also be identified using EM (non-branching fibrils, 7.5 to 10 nm width and up to 1 micron in length).
5. A firm diagnosis is not always possible. The final diagnosis should be based on a combination of histologic, immunohistochemical, and clinical data.

Antibodies for immunohistochemistry

See Tables 7-43 and 7-44.

Results

The results of studies are incorporated into the surgical pathology report. The following information is included:

1. The type of tissue studied: formalin-fixed (or other fixatives) tissue, cryostat sections, cytology preparations, etc.
2. The type of immunoagents used, being as specific as possible. For example, do not just list keratin but specify the type of keratin (e.g., AE1/AE3).
3. The results of the studies in great enough detail to allow interpretation. For example the type of cell that is immunoreactive (e.g., tumor vs. nontumor), intensity of immunoreactivity (e.g., weak, strong) and/or the number of cells immunoreactive (e.g., focal vs. diffuse).
4. Integration of the results into the final diagnosis specifying whether they confirm or support a diagnosis, make one diagnosis more likely than others, or exclude one or more diagnoses.

Text continues on page 157.

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
Actin (alpha smooth muscle actin) *(SMA, SM-ACT)*	Smooth muscle isoform of actin *(Cytoplasm or membrane)*	Smooth muscle, myoepithelial cells, blood vessel walls, pericytes, some stromal cells of intestine, testis, and ovary, myofibroblasts in desmoplastic stroma. Not in striated muscle or myocardium.	Smooth muscle tumors, myofibroblastic tumors, PEComas, glomus tumors, KS, some spindle cell carcinomas (e.g., with features of myoepithelial cells)	ID of smooth muscle differentiation (muscle or myofibroblasts) in tumors. Noninvasive lesions of breast (myoepithelial cells present if benign or DCIS) vs. invasive carcinoma. Microglandular adenosis also lacks myoepithelial cells.	Good marker for myoepithelial cells of the breast but also positive in myofibroblasts in stroma. P63 is more specific, but less sensitive for myoepithelial cells.
Actin (muscle-specific actin) (HHF35, MSA, muscle common actin, EM ACT)	Alpha and gamma smooth muscle actins, recognizes a common epitope of alpha skeletal, cardiac, and smooth muscle *(Cytoplasm)*	Smooth, striated, and cardiac muscle, smooth muscle of blood vessels, pericytes, myoepithelial cells, myofibroblasts	Numerous tumors including tumors of muscle, glomus tumor, PEComa, GIST, DFSP, dermatofibroma, myofibroblastic tumors, spindle cell carcinomas, salivary gland tumors, mesothelioma, others	ID of muscle differentiation in tumors.	Sensitive but not specific. Present in tumors not of muscle origin.
Alpha fetoprotein (AFP, alpha 1-fetoprotein)	Glycoprotein present in fetal liver *(Cytoplasm, granular)*	Fetal liver, regenerating liver cells	HCC (but not the fibrolamellar variant), hepatoblastomas, yolk sac tumors, embryonal carcinoma (but less commonly)	HCC (+/–) vs. other cell types (however, AFP is rarely present in other carcinomas such as breast and ovary). Yolk sac tumors (+) vs. other germ cell tumors (–/+).	Correlates with extracellular hyaline eosinophilic globules in yolk sac tumors
Alpha 1-antitrypsin *(AAT, alpha-1-AT)*	Glycoprotein that inhibits proteolytic enzymes produced in the liver *(Cytoplasm)*	Histiocytes, reticulum cells, mast cells, Paneth cells, salivary gland	HCC, germ cell tumors, true histiocytic neoplasms, colon and lung carcinoma, others	Accumulates in liver cells in AAT deficiency	Not specific for tumor type. CD68 is somewhat more specific for macrophages.
AMACR *(P504S, alpha-methylacyl-CoA racemase)*	Mitochondrial and peroxisomal enzyme involved in the metabolism of branched-chain fatty acid and bile acid intermediates *(Cytoplasm)*	Not present in normal tissues	Colorectal carcinoma (92%), colonic adenomas (75%), prostate carcinoma (83%), PIN (64%), nephrogenic adenoma (58%), breast cancer (44%), ovarian carcinoma, TCC, lung carcinoma, RCC, lymphoma, melanoma	Can be combined with p63 to distinguish prostate carcinoma (AMACR +, p63 absent in basal cells) from benign mimics (AMACR -, p63 present in basal cells). However, ~20% of small cancers on core may be negative for AMACR.	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
Androgen receptor *(AR)*	Mediates the function of androgens *(Nucleus)*	Prostate, skin, oral mucosa	Osteosarcoma, prostatic carcinoma, breast carcinoma, ovarian carcinoma, others		
B72.3 *(Tumor-associated glycoprotein 72, TAG-72, CA 72-4)*	Oncofetal glycoprotein, may be a precursor of the MN blood group system, sialosyl-Tn antigen *(Cytoplasm, membrane)*	Not present in most benign adult epithelial cells (may be present in secretory endometrium), apocrine metaplasia, and fetal GI tract	Adenocarcinomas (especially ovary, colon, breast)	Adenocarcinoma (+ >90%) vs. mesothelioma (5%) or mesothelial cells (-)	Other markers are more useful for mesothelioma vs. adenocarcinoma.
bcl-2 *(B-cell lymphoma 2)*	Protein involved in inhibition of apoptosis *(Membrane, cytoplasm)*	Medullary lymphocytes and epithelial cells of the normal thymus, mantle and T zone small lymphocytes	Synovial sarcoma, solitary fibrous tumor, myofibroblastic tumors, schwannoma, neurofibroma, granular cell tumor, GIST, KS, melanoma Small lymphocytic lymphoma/CLL, mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma (MALT), some large B-cell lymphoma	Synovial sarcoma (+/-) vs. mesothelioma (-) Thymic carcinomas strongly express bcl-2 compared to thymomas. Small lymphocytic lymphoma, mantle cell lymphoma, and marginal zone lymphoma (MALT) (+) vs. reactive follicles (-).	The bcl-2 gene is involved in the t(14;18) found in follicular lymphomas.
Ber-EP4 *(Epithelial-specific antigen [ESA], Ep-CAM)*	Glycoprotein *(Membrane)*	All epithelial cells except superficial layers of epidermis	Most carcinomas	Adenocarcinoma (+; strong and diffuse in 60 to 100%) versus mesothelioma (- or focal in 26%)	Other markers are better for distinguishing adenocarcinoma vs. mesothelioma
Beta-amyloid *(6F/3D)*	Amyloid present in Alzheimer's disease (AD) and in cerebral amyloid angiopathy *(Extracellular)*	None	Senile plaque core in AD, amyloid cores, neuritic plaques, neurofibrillary tangles	Diagnosis of AD, other diseases	Found in AD, Lewy body dementia, Down's syndrome, hereditary cerebral amyloidosis (Dutch type)

Beta-catenin	Component of the adherens junction that binds to e-cadherin and functions in cell adhesion and anchoring the cytoskeleton; signaling molecule of the Wnt/wingless pathway *(Membrane, cytoplasm)*	Urothelium, breast epithelium, colon, esophagus, stomach, thyroid	TCC, colonic adenocarcinomas and adenomas, breast carcinoma, esophageal squamous cell carcinoma, head and neck squamous cell carcinomas, gastric carcinoma, ovarian carcinoma, thyroid carcinoma, prostate carcinoma, HCC, brain neoplasms Nuclear positivity in solitary fibrous tumor (40%), endometrial stromal sarcoma (40%), synovial sarcoma (28%)	Aberrant nuclear expression in solid-pseudopapillary tumors of the pancreas (95%) and pancreatoblastomas (78%) Aberrant nuclear expression in desmoid fibromatosis (80% deep, 56% superficial) vs. low grade myofibroblastic sarcoma (30%), solitary fibrous tumor (22%), infantile fibrosarcoma (20%), desmoplastic fibroblastomas (6%)	
Beta-2 microglobulin	Immunoglobulin-associated protein *(Extracellular deposits of amyloid)*	Plasma cells		Identification of amyloid in patients on dialysis	Amyloid tends to accumulate around joints and in the GI tract
BG8	Lewis blood group y antigen *(Cytoplasm)*	Red blood cells, endothelial cells	Adenocarcinomas (95%), rare mesotheliomas (about 5%)		Other markers are better for distinguishing adenocarcinoma vs. mesothelioma
Blood group antigens	A, B, and H antigens *(Membrane)*	Epithelial cells and red blood cells, endothelial cells	Lost or abnormally expressed in many carcinomas	Can be helpful to identify potentially misidentified specimens if patients' blood types are known.	H is diminished by decalcification but not A and B antigens.
CA 125 *(OC125)*	Mucin-like glycoprotein, antibody to ovarian carcinoma antigen *(Luminal surface)*	Epithelial cells, mesothelial cells	Adenocarcinomas of ovary, breast, lung (bronchioloalveolar), and others (rarely colon), TCC, adenomatoid tumor of the uterus, squamous cell carcinoma, seminal vesicle carcinoma, anaplastic lymphoma	Seminal vesicle carcinoma (+) vs. prostate carcinoma (-)	Used as a serum marker for monitoring ovarian cancer

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
CA19-9 *(Carbohydrate antigen 19-9)*	Antigen of sialyl Lewis[a]-containing glycoprotein; antibody to colon carcinoma *(Cytoplasm)*	Epithelial cells of breast, colon, kidney, liver, lung, pancreas, salivary gland, others	Adenocarcinomas of GI tract, pancreas, ovary, lung, and bladder, rare in mesotheliomas Chronic pancreatitis		Used as a serum marker for monitoring gastrointestinal and pancreatic carcinomas
Calcitonin	Peptide hormone produced by C cells *(Cytoplasm and extracellular amyloid)*	C cells of the thyroid	Medullary carcinoma of the thyroid (within tumor cells and in amyloid)	ID of C-cell hyperplasia ID of medullary thyroid carcinoma	Used as a serum marker for medullary carcinoma.
Caldesmon *(h-caldesmon)*	Actin and calmodulin binding protein in smooth muscle *(Cytoplasm)*	Vascular and visceral smooth muscle cells, some myoepithelial cells of the breast	Smooth muscle tumors, PEComa, GIST	Smooth muscle tumors (+) vs. myofibroblastic lesions (-) or endometrial stromal tumors (-)	
Calponin *(CALP)*	Protein that binds to calmodulin, F-actin, and tropomyosin to regulate smooth muscle contraction *(Cytoplasm)*	Vascular and visceral smooth muscle cells, myoepithelial cells of the breast, periacinar and periductal myoepithelial cells of the salivary gland	Myoepithelioma, some smooth muscle tumors, myofibroblastic lesions	Can be helpful to identify myoepithelial cells in breast lesions	
Calretinin	Intracellular calcium-binding protein of the troponin C superfamily with an EF-hand domain *(Cytoplasm, nucleus)*	Subsets of neurons, pineal cells, germinal epithelium of ovary, mesothelial cells, keratinocytes, breast, sweat glands, neuroendocrine cells, thymus	Epithelial mesotheliomas (less + in sarcomatoid type), adenomatoid tumor, some lung squamous cell carcinomas, rare adenocarcinomas, mesenchymal tumors (e.g., synovial sarcoma), granular cell tumor, Leydig cell tumor, granulosa cell tumor	Epithelial mesotheliomas (>90%) versus adenocarcinoma (<10%)	Useful positive marker for mesotheliomas.
Carcinoembryonic antigen *(CEA. CD66e)*	Glycoproteins with immunoglobulin-like regions found in fetal tissues *(Cytoplasm)*	Fetal tissues	Adenocarcinomas (liver, colon, pancreas, bile duct, and lung more than breast, ovary), TCC, medullary carcinoma of the thyroid Usually absent in RCC, prostate carcinoma, and papillary or follicular thyroid carcinomas	Adenocarcinoma (+) versus mesothelioma (–) HCC: polyclonal CEA has a canalicular pattern	Different reactivity patterns occur with different antibodies and with polyclonal versus monoclonal antibodies

CD5 *(Leu 1)*	Transmembrane glycoprotein *(Membrane)*	T cells and B cell subsets (mantle zone)	Thymic carcinoma, adenocarcinomas, mesothelioma (cytoplasmic) T-cell leukemias and lymphomas, aberrantly expressed in low-grade B-cell lymphomas (CLL or mantle cell lymphoma).	Thymic carcinoma (+/-) vs. thymoma (-). Thymic carcinoma (+/-) vs. metastatic squamous carcinoma (-) Classification of low grade B-cell lymphomas. Evaluation of T-cell lymphomas (this marker is frequently lost).	
CD10 *(CALLA [common acute leukemia antigen], J5)*	Cell surface metalloendopeptidase that inactivates peptides *(Membrane)*	Precursor B cells, granulocytes, rare cells in reactive follicles, myoepithelial cells of breast, bile canaliculi, fibroblasts, brush border of kidney and gut	Endometrial stromal sarcoma, RCC (clear cell and papillary types), HCC, TCC, rhabdomyosarcoma, pancreatic carcinoma, schwannoma, melanoma Precursor lymphoblastic lymphoma/leukemia, follicular lymphoma, Burkitt lymphoma, CML, angioimmunoblastic lymphoma	Myoepithelial cell marker in breast Endometrial stromal sarcoma (+) vs. leiomyosarcoma (-/+) (but caldesmon is preferred for this purpose) Evaluation of low-grade lymphomas. Evaluation of leukemias	Not specific for nonlymphoid neoplasms.
CD15 *(LeuM1)*	3-fucosyl-N-acetyl-lactos-amine, X-hapten - CHO moiety linked to cell membrane protein *(Membrane and cytoplasm)*	Granulocytes, monocytes	Adenocarcinomas CMV-infected cells RS cells (not LP HD) in a membranous and golgi pattern, some large T-cell lymphomas, MF, some leukemias	Adenocarcinomas (+) versus mesotheliomas (-) Evaluation of HD	
CD30 *(Ki-1)*	Single-chain transmembrane glycoprotein homologous to the nerve growth factor superfamily *(Cytoplasm, membrane, and golgi)*	Activated B and T cells, some plasma cells, immunoblasts, interdigitating cells, histiocytes, follicular center cells, decidualized endometrium, reactive mesothelial cells, most other tissues negative	Embryonal carcinoma, some vascular tumors (not KS), some mesotheliomas Anaplastic large cell (CD30+) lymphomas, mediastinal large B-cell lymphoma, primary effusion lymphoma, HD (but not LP HD), some other B- and T-cell lymphomas, EBV transformed B cells	ID of anaplastic large cell (CD30+) lymphomas. Evaluation of HD (RS cells are positive except in LP HD). ID of peripheral T-cell lymphoma (large cells may be positive).	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
CD31 *(PECAM-1, platelet-endothelial cell adhesion molecule)*	Transmembrane glycoprotein functioning in cell adhesion *(Cytoplasm, membrane)*	Endothelial cells, platelets, megakaryocytes, plasma cells, histiocytes, other hematopoietic cells	Vascular tumors (> 80% of angiosarcomas), KS, histiocytic neoplasms, PEComa, very rarely other tumors	ID of endothelial differentiation in tumors Evaluation of angiogenesis	Most sensitive and specific marker for endothelial cells
CD34 *(HPCA-1, hematopoietic progenitor cell, class 1, QBEnd10)*	Single-chain transmembrane glycoprotein, leukocyte differentiation antigen *(Cytoplasm, membrane)*	Hematopoietic progenitor cells (decreases with maturation), endothelial cells, fixed connective tissue cells (e.g., in skin), fibroblasts	Acute leukemia, sarcomas of vascular origin, KS, epithelioid sarcoma, GIST, DFSP, solitary fibrous tumor, neurofibroma, schwannoma, spindle cell lipoma, phyllodes tumor, fibroadenoma	ID of endothelial or fibroblastic differentiation in tumors. Evaluation of angiogenesis. Evaluation of the number of blasts in bone marrow in acute leukemia. Solitary fibrous tumor (+) vs. sarcomatoid mesothelioma (-) DFSP (+) vs. dermatofibroma (-)	Not specific but can be useful in context with other features
CD44v3 *(CD44 variant 3, H-CAM)*	Transmembrane glycoprotein that mediates cell adhesion *(Membrane)*	Many, including myometrium	Many, including endometrial carcinomas	Possibly helpful to distinguish cellular leiomyoma (+) from endometrial stromal sarcoma (-)	Many splice variants of CD44 are present in normal and malignant cells.
CD57 *(Leu 7, HNK-1)*	Lymphocyte antigen that cross reacts with a myelin-associated glycoprotein *(Membrane)*	T-cell subsets, NK cells, myelinized nerves, neuroendocrine cells, prostate, pancreatic islets, adrenal medulla	Nerve sheath tumors (occasional), leiomyosarcoma, synovial sarcoma, rhabdomyosarcoma, neuroblastoma, gliomas, neuroendocrine carcinomas, neurofibromas, some prostate carcinomas Angioimmunoblastic lymphoma, T gamma lymphoproliferative disorder (large granular cell lymphocytic leukemia)	ID of neuroendocrine differentiation in tumors ID of angioimmunoblastic T-cell lymphoma Evaluation of NK neoplasms.	Not very specific for solid tumors
CD63 *(NKI/C3, melanoma-associated antigen, ME491)*	Member of the tetraspanin or transmembrane 4 superfamily (TM4SF) found on lysosomes *(Cytoplasm or membrane)*	Melanocytes, mast cells, histiocytes, salivary gland cells, sweat gland cells, pancreatic cells, islets of Langerhans, prostatic cells, Paneth cells, peribronchial glands, pituitary	Nevi, melanomas, carcinoids, medullary carcinomas of the thyroid, some adenocarcinomas	Cellular neurothekoma (NKI/C3 + and S100 -) versus melanocytic lesions (NKI/C3 and S100 +) ID of melanocytic lesions	May be negative in desmoplastic melanomas

CD68 *(KP1, CD68-PG-M1, Mac-M)*	Intracellular glycoprotein associated with lysosomes *(Cytoplasm, membrane)*	Macrophages, monocytes, neutrophils, basophils, large lymphocytes, Kupffer cells, mast cells, osteoclasts	Neurofibroma, schwannoma, MPNST, granular cell tumors, PEComa, melanomas, atypical fibroxanthoma, RCC Some lymphomas, histiocytic sarcomas, APML, Langerhans proliferative disorders	Best general marker for macrophages, although not specific to this cell type.	The antibody PG-M1 does not react with granulocytes. Not very specific for solid tumors.
CD99 *(MIC-2, 12E7, Ewing's sarcoma marker, E2 antigen, HuLy-m6, FMC 29, O13 [different epitope])*	MIC2 gene product – glycoproteins (p30 and p32) involved in rosette formation with erythrocytes *(Membrane)* (*Membrane* [immunoreactivity is more specific than cytoplasmic])	Cortical thymocytes, T lymphocytes, granulosa cells of ovary, pancreatic islet cells, Sertoli cells, some endothelial cells, urothelium, ependymal cells, squamous cells	PNET/Ewing's sarcoma, chondroblastoma, mesenchymal chondrosarcoma, synovial sarcoma, solitary fibrous tumors, GIST, some alveolar rhabdomyocarcomas, desmoplastic small cell tumors, small cell carcinomas, granulosa cell tumors, yolk sac components of germ cell tumors, Sertoli-Leydig cell tumors, atypical fibroxanthoma, meningioma B- and T-cell precursor lymphoblastic lymphoma/leukemia	Thymic carcinomas (lymphocytes +) versus other carcinomas. ID of PNET/Ewing's sarcoma (immunoreactivity should be clearly membranous in the majority of the cells) Evaluation of lymphoblastic lymphoma/leukemia	O13 is the most commonly used antibody. Immunoreactivity is highly dependent upon the antigen retrieval system used
CD117 *(c-kit, stem cell factor receptor)*	Transmembrane tyrosine kinase receptor (ligand is stem cell factor) - apoptosis is inhibited when the ligand is bound *(Cytoplasm, membrane)*	Mast cells, interstitial cells of Cajal (ICC - pacemaker cells of the GI tract found through-out the muscle layers and in the myenteric plexus), epidermal melanocytes, mononuclear bone marrow cells (4%), Leydig cells, early spermatogenic cells, trophoblast, breast epithelium	GIST (>95%), seminomas (>70%), intratubular germ cell neoplasia, mature teratomas (>70%), papillary renal cell (cytoplasmic – associated with mutations), chromophobe renal cell (membrane - not associated with mutations), some melanomas (focal), mast cell tumors, some carcinomas (including adenoid cystic carcinoma), some brain tumors, some PNET/Ewing's sarcoma, some angiosarcomas AML (>50%), CML in myeloid blast crisis	ID of GIST (+) vs. leiomyomas (-) and schwannomas (-). ID of seminomas ID of mast cells (mastocytosis) – abnormal mast cells commonly have the imatinib resistant mutation D816V.	Mast cells are an excellent internal control. CD117 (+) does not correlate with mutations and/or oncoprotein activity in tumors not known to have activating mutations and is, in general, not of clinical or therapeutic significance in this setting (e.g., to detect tumors likely to respond to therapy directed against the protein, such as Gleevec.

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
CD123 *(interleukin-3 receptor alpha chain)*	Alpha chain of the IL-3 receptor *(Membrane)*	Myeloid precursors, macrophages, dendritic cells, mast cells, basophils, megakaryocytes	Plasmacytoid dendritic cell tumors		
CD141 *(thrombomodulin, TM)*	Transmembrane glycoprotein, receptor for thrombin *(Cytoplasm [epithelial cells], membrane [mesothelial cells])*	Endothelium, platelets, monocytes, synovial cells, syncytiotrophoblast, mesothelial cells, dermal keratinocytes, islet cells, peripheral nerves	Mesotheliomas, TCC, KS, squamous cell carcinomas, choriocarcinomas, rarely adenocarcinomas, benign and malignant vascular tumors	Mesothelioma (+ 80%) vs. adenocarcinoma (+ 10%) (but variable results have been reported in other studies)	Other markers are better for distinguishing adenocarcinoma vs. mesothelioma.
CD146 *(melanoma cell adherin molecule, MELCAM, MCAM, MN-4, MUC18, A32 antigen, S-Endo-1)*	Membrane cell adhesion glycoprotein of the Ig gene superfamily *(Membrane)*	Implantation site intermediate trophoblast, myofibroblasts, endothelium, pericytes, Schwann cells, ganglion cells, smooth muscle, cerebellar cortex, breast luminal and myoepithelial cells, external root sheath of hair follicle, subcapsular epithelium of thymus, follicular dendritic cells, basal cells of bronchus and parathyroid, subpopulations of activated T cells	Melanoma, angiosarcoma, KS, leiomyosarcoma, placental site trophoblastic tumor, choriocarcinoma May be focally positive in squamous cell carcinoma and small cell carcinoma of the lung, mucoepidermoid carcinoma, breast carcinoma, some leukemias, neuroblastoma	ID of placental site trophoblastic tumors	
CD163 *(M130)*	Endocytic receptor to scavenge haptoglobin and hemoglobin complexes *(Membrane, cytoplasm)*	Tissue macrophages (high expression), monocytes (low expression) including Kupffer cells, Hofbauer cells but not follicular dendritic cells or plasmacytoid monocytes	Neoplasms of histiocytic differentiation Leukemias of monocytic differentiation Synovial type giant cell tumors of the vertebral column Langerhans cell histiocytosis (~60%), benign fibrous histiocytoma (~67%) Littoral cell angioma of the spleen	ID of true histiocytic derivation of tumors	More specific for monocyte/histiocyte derivation than CD68

CDK4 *(cylin-dependent kinase 4)*	A kinase involved in cell cycle regulation *(Nuclear)*	None	Liposarcoma, glioblastoma, anaplastic astrocytoma, large B-cell lymphoma, osteosarcoma, breast carcinoma	Atypical lipomatous tumor/well-differentiated liposarcoma and dedifferentiated liposarcoma (>90% +) vs. benign adipose tumors (<5% +)	MDM2 can also be used for this differential diagnosis
CDX2 *(caudal-related homeobox transcription factor, CDX-88)*	Homeobox nuclear transcription factor specific for the intestinal tract that regulates MUC2 expression *(Nucleus)*	Small intestine, colon, and endocrine pancreas	Colon carcinomas (usually strong and diffuse), small intestine carcinomas, mucinous ovarian carcinomas, bladder adenocarcinomas, some gastric, esophageal, pancreatic, and bile duct carcinomas HCC, breast, lung, and head and neck carcinomas are usually negative	ID of colon carcinomas and other carcinomas of the gastrointestinal tract. However, other carcinomas (e.g., mucinous ovarian carcinoma or mucinous lung bronchioloalveolar carcinoma) can also be positive	
Chromogranin A	Acidic glycoprotein in neurosecretory granules *(Cytoplasm, granular)*	Islet cells of pancreas, bronchial Kulchitsky cells, parathyroid, adrenal medulla, anterior pituitary, C-cells of thyroid	Pheochromocytoma, carcinoids (not rectal), small cell carcinoma, neuroblastoma, some breast and prostatic carcinomas, Merkel cell tumors, islet cell tumors, medullary carcinoma of the thyroid, parathyroid lesions, Brenner tumor	ID of neuroendocrine differentiation in tumors. Not present in pituitary prolactinomas. Pheochromocytoma (+) versus adrenal cortical carcinoma (–). Parathyroid (+) vs. thyroid (–)	Most specific marker of neuroendocrine differentiation Also can be detected in serum Bouin's solution or B5 fixation may increase immunogenicity
Claudin-1 *(CLDN1)*	Protein component of the tight junction complex *(Membrane – not cytoplasmic)*	Epithelial cells, perineurial cells, some endothelial cells (venules)	Perineurioma (30%), synovial sarcoma (epithelioid areas, lower in spindle cell areas), carcinomas Some perineurial cells may be present in neurofibromas and schwannomas	Perineurioma (+ 30%) vs. DFSP (-), fibromatosis (-), low grade fibromyxoid sarcoma (–) Meningiomas – 50% +	
Collagen IV	Major constituent of basement membranes *(Basement membrane)*	Mesangial cells within glomeruli, basement membranes, basal lamina of capillaries	Tumors with external lamina (schwannomas, smooth muscle tumors)	Absence or loss may be associated with stromal invasion by carcinomas	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
D2-40 *(podoplanin, M2A)*	Oncofetal Membrane O-linked sialoglycoprotein *(Membrane)*	Lymphatic endothelium, germ cells of testis, interstitial cells of Cajal, follicular dendritic cells, myoepithelial cells of the breast	Lymphatic tumors, some angiosarcomas, some epithelioid hemangioendotheliomas, epithelioid mesotheliomas, seminomas, ITGCN, KS, GIST, ovarian serous carcinomas	Identification of LVI ID of seminoma (+, diffuse) vs. embryonal carcinoma (- or focal) Epithelioid meso (+) vs. adenocarcinoma (–) ID of follicular dendritic cell sarcoma	Myoepithelial cells of the breast can show cytoplasmic positivity — limiting usefulness in the breast for LVI
Desmin	Intermediate filament in muscle *(Cytoplasm)*	All striated muscle (Z bands) and many smooth muscle cells, myofibroblasts, smooth muscle of some BVs	Rhabdomyosarcoma (80% +), leiomyosarcoma (50-70% +), PEComa, desmoplastic small round cell tumors (usually dot-like), some myofibroblastic tumors, endometrial stromal sarcoma	ID of muscle differentiation in tumors	
DOG1 *(discovered On GIST-1)*	Protein of unknown function expressed in GIST *(Cytoplasm or membrane)*	Interstitial cells of Cajal	GIST (positivity in other tumor types is <10%)	ID of GIST (may be + in some CD117 neg GIST) Positive in 79% of GIST with PDGFRA mutations, whereas CD117 is positive in 9% of this group	
DPC4 *(homozygously deleted in pancreatic carcinoma, locus 4, Smad4)*	Transcriptional regulator interacting with the TGFbeta signaling pathway *(Nucleus)*	Normal tissues	Expressed in most carcinomas Lost in 31% of Pan IN-3, 55% of pancreatic carcinomas, and 22% of stage IV colon carcinomas	Mucinous ovarian carcinoma (+) vs. metastatic pancreatic carcinoma (negative in 55%)	Mutated in familial juvenile polyposis in 25% to 60% of cases
E-cadherin	Transmembrane cell adhesion molecule that binds to catenins for cell polarization, glandular differentiation, and stratification *(Membrane)*	Epithelial cells	Most carcinomas – may be lost in poorly differentiated carcinomas Not present in LCIS and invasive lobular carcinoma of breast or gastric signet ring cell carcinomas	Ductal (+) vs. lobular (–) lesions of the breast	Can be helpful to distinguish DCIS from LCIS.

EGFR *(epidermal growth factor receptor, HER1)*	Transmembrane protein receptor of the type 1 growth factor family with tyrosine kinase activity *(Membrane positivity scored, cytoplasmic positivity is not scored)*	Many types of epithelium, skin eccrine and sebaceous glands, mesenchymal cells, perineurium The strongest membrane positivity is present in hepatocytes, bile ducts, basilar squamous cells, pancreatic ducts, breast epithelium, lung alveolar lining cells, mesothelial cells, prostate epithelium, endometrial glands and stroma	Adenocarcinomas (esp. colon), squamous cell carcinomas, TCC, neural tumors, sarcomas	Expression is increased in tumors of higher grade and poorer prognosis Colon carcinomas (80-90% positive) – response to cetuximab is not related to IHC score Lung adenocarcinoma – specific mutations (but not IHC) predict response to TK inhibitors GBM – 40% show gene amplification and overexpression by IHC. TK domain mutations are rare	
Epithelial membrane antigen *(EMA, MUC1, HMFG, DF3, CA 15-3, CA 27.29, PEM, many others)*	Episialin, glycoprotein found in human milk fat globule membranes *(Cytoplasm [more common in malignant cells], membrane [more common in benign cells])*	Epithelial cells, perineurial cells, meningeal cells, plasma cells, usually negative in non-neoplastic mesothelial cells	Carcinomas, mesotheliomas (thick membrane pattern), some sarcomas (synovial sarcoma, epithelioid sarcoma, leiomyosarcoma, some osteosarcomas), adenomatoid tumor, chordoma, perineurioma, neurofibroma, meningioma, desmoplastic small round cell tumor, Sertoli cell tumor Some anaplastic large cell lymphomas (CD30 +), plasma cell neoplasms	ID of epithelial differentiation in tumors – however, keratin is more specific for this purpose Synovial sarcoma (typically focal positivity) vs. other sarcomas Demonstrates the "inside out" glands of invasive micropapillary breast carcinoma	There are over 50 monoclonal antibodies recognizing different glycosylation patterns in normal tissues and tumors[18]
Epstein-Barr virus					
EBV-encoded nonpolyadenylated early RNAs *(EBERS)*	RNA produced by EBV *(Nucleus)*	EBV-infected B cells	All EBV-related tumors	Most sensitive marker for EBV	Detected by in situ hybridization for RNA on paraffin sections
LMP-1	Latent membrane protein *(Membrane)*	EBV-infected B cells	Nasopharyngeal carcinomas, RS cells (not LP HD), transplant lymphomas, AIDS-related lymphomas, endemic Burkitt lymphoma (rare in sporadic cases)	Evaluation of EBV-related neoplasms	
EBNA 2 *(nuclear antigen 2)*	Nuclear protein *(Nucleus)*	EBV-infected B cells	Transplant-related lymphomas, AIDS-related lymphomas Not present in Burkitt lymphoma, nasopharyngeal carcinomas, or HD	Evaluation of transplant- and AIDS-related lymphomas	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
Estrogen receptor *(ER, 1D5, SP1, 6F11, H222, others)*	Steroid-binding protein *(Nucleus)*	Breast epithelial cells (not myoepithelial cells), epithelial and myometrial cells of the uterus	Breast carcinomas (>70%), myofibroblastoma of breast, gynecologic carcinomas, some skin appendage tumors, rare in other carcinomas, present in some meningiomas, smooth muscle tumors, some melanomas, some thyroid tumors, desmoid tumors, vulvovaginal stromal tumor	Prognosis and prediction of response to hormonal therapy of breast cancer. Only nuclear positivity is scored ID of metastatic breast cancer	ABs recognize different epitopes and have varying sensitivities in formalin fixed tissue. Antigenicity may be diminished after decalcification or exposure to heat during surgery.
Factor VIII-related antigen *(VWF, FVIII:RAg, von Willebrand factor)*	Glycoprotein involved in coagulation, part of FVIII complex *(Cytoplasm)*	Endothelial cells, megakaryocytes, platelets, and mast cells, endocardium	Vascular tumors (often absent in angiosarcomas) Not present in KS, PEComa Megakaryocytic AML (M7)	ID of endothelial differentiation in tumors (specific but not very sensitive) Evaluation of angiogenesis Evaluation of M7 (megakaryocytic) leukemias	May not detect smaller blood vessels (see CD 31 and 34). Present in Weibel-Palade bodies. Not a sensitive marker for vascular neoplasms.
Factor XIIIa *(Factor XIII subunit A)*	Transglutaminase involved in the coagulation pathway *(Cytoplasm)*	Fibroblasts, dendritic reticulum cells in reactive follicles, dermal dendrocytes, liver, placenta, platelets, megakaryocytes, monocytes, macrophages	Fibroblastic neoplasms, dermatofibroma		Not very specific
Fascin *(p55)*	Actin binding protein thought to be involved in the formation of microfilament bundles and cell motility *(Cytoplasm)*	Interdigitating reticulum cells in lymph nodes, dendritic cells of lymph node, thymus, spleen and peripheral blood, histiocytes, smooth muscle, endothelial cells, squamous mucosal cells, lining cells of splenic sinuses, neurons	RS cells and their variants (but not LP HD), rare non HD lymphomas Reticulum cell tumors Some sarcomas Many carcinomas, especially those of advanced stage Glial tumors (more common in high-grade tumors)		Not very specific
Fibronectin	Glycoproteins found in BMs and extracellular matrix, bind to integrins *(Extracellular)*		Stroma of many tumors		

FLI-1 *(Friend leukemia integrin-site 1)*	Transcription factor (ETS family) – translocation in Ewing's can result in an EWS-FLI-1 fusion protein *(Nucleus)*	Endothelial cells (hemangioblasts, angioblasts), small lymphocytes	Ewing's sarcoma/PNET, vascular tumors (including KS), Merkel cell carcinoma, melanoma Can also be weakly present in lymphomas, synovial sarcoma, some carcinomas	ID of vascular tumors (unlike other vascular markers, FLI-1 is nuclear). ID of Ewings/PNET – not specific but very sensitive (70%)	Reactivity can be variable with high background and may be difficult to interpret
Galectin-3 *(Gal-3)*	Lectin with anti-apoptosis function (galactoside-binding protein) *(Nucleus, cytoplasm, membrane, extracellular matrix)*	Many epithelial cells, lymphocytes, mesenchymal cells, macrophages, activated endothelial cells	Many carcinomas, adenomas, lymphomas, soft tissue tumors	Thyroid carcinomas (papillary and to a lesser extent follicular) show higher expression than benign lesions In some carcinomas, expression is diminished in higher grade lesions	
Glial fibrillary acidic protein *(GFAP)*	Intermediate filament *(Cytoplasm)*	Normal and reactive astrocytes, developing and reactive ependymal cells, developing oligodendrocytes, choroid plexus, Schwann cells, enteric glial cells, pituitary cells, chondrocytes	Tumors of astrocytes, ependymal cells, and oligodendrocytes, MPNST, myoepitheliomas (salivary glands and soft tissue), sweat gland tumors, Merkel cell carcinomas, chordomas	ID of neural differentiation in tumors (30% of MPNSTs are +). Neuroblastomas are negative, schwannomas may be focally +. Merkel cell carcinoma (+) versus small cell carcinoma (–) (but Ck20 is a better marker for this purpose). ID of myoepithelial neoplasms.	
GLUT-1 *(glucose transporter 1)*	Component of transmembrane glucose transport *(Membrane)*	Erythrocytes, perineurium, blood vessels, trophoblasts, renal tubules, germinal center cells	TCC, lung carcinoma, squamous cell carcinoma, adenocarcinomas of colon, lung, bile ducts, kidney, ovary, pancreas, stomach, and endometrium, germ cell tumors		Not very specific
Gross cystic disease fluid protein-15 *(GCDFP, CDP, BR-2, BRST-2)*	Protein found in breast fluid *(Cytoplasm)*	Apocrine sweat glands, apocrine metaplasia of the breast	Breast carcinomas (60%), sweat gland carcinomas, some salivary gland tumors, some prostate carcinomas	ID of apocrine differentiation in tumors ID of breast metastases (however, only positive in about 60%)	
HepPar-1 *(hepatocyte paraffin-1, HP1)*	Mitochondrial protein *(Cytoplasm, coarsely granular)*	Liver	HCC, some cases of gastric adenocarcinoma, esophageal adenocarcinoma, others negative or only rarely positive	HCC (80–95%) vs. metastatic carcinomas to the liver	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
HBME-1	Antigen to microvilli on mesothelioma cells *(Membrane and cytoplasm)*	Mesothelial cells, epithelial cells	Mesotheliomas (epithelial type - thick, membrane staining), adenocarcinomas, chordomas, chondrosarcomas	Positivity higher in thyroid carcinomas than in adenomas May be absent in thyroid carcinomas with Hurthle cell features	Not a specific marker for mesotheliomas
HER-2/neu *(c-erbB2, A0485, Sp3)*	Growth factor receptor (tyrosine kinase) homologous to epidermal growth factor receptor *(Membrane, some cytoplasm)*	Absent or rare in normal cells	Breast carcinomas (20 to 30%), Paget disease of nipple (>90%), less frequently in other carcinomas (ovary, uterus, GI, pancreas), some synovial sarcomas	Poor prognostic factor in breast cancer Membrane positivity used to select patients for treatment with Herceptin (scored from 0 to 3+) (see separate table)	Only membrane positivity is scored. Gene amplification (detected by FISH) correlates with strong complete membrane immunoreactivity in >90% of cases
HHV8	Latent nuclear antigen of human herpes virus type 8 *(Nucleus)*	Absent in normal tissue	KS (endothelial cells and some perivascular cells) Primary effusion lymphoma (PEL), AIDS-associated multicentric Castleman's disease	Evaluation of KS and PEL	
HMB-45 *(E-MEL, melanoma specific antigen)*	Oligosaccharide side-chain of a melanosomal antigen, gp100/pmel17 *(Cytoplasm)*	Fetal melanocytes and some normal adult superficial melanocytes, retinal pigment epithelium	Melanoma (epithelioid but not spindle cell or desmoplastic type), clear cell sarcoma, PEComa, tumors associated with tuberous sclerosis, melanotic schwannoma, others	ID of metastatic melanoma. Melanophages can also be positive. Melan-A may be more specific. ID of PEComa.	NKI-betab detects the same protein. Tissues fixed in B5 may have high background staining

hMLH1 *(human mutS homologue 2),* **hMSH2** *(human mutL homologue 1),* **MSH6, PMS2**	Proteins involved in mismatch repair of DNA (the first two genes account for 95% of HNPCC) *(Nucleus)*	Most normal tissues May be lost in areas of chronic inflammation	Expression (or non-expression) is not specific for tumor type	Absence is associated with germline mutations in HNPCC patients and with gene silencing by methylation in 15% of sporadic colon carcinomas – correlated with characteristic clinical, pathologic, and treatment response features IHC will not detect the 5% of patients with mutations in other genes or rare patients with mutated gene products that are immunoreactive	Other assays for microsatellite instability utilize PCR (90% sensitive for MSI)
Hormones (ER and PR are listed separately)	Insulin, gastrin, glucagon, somatostatin, calcitonin, ACTH, FSH, LH, PRL, TSH, others *(Cytoplasm)*	Hormone-producing cells	Hormone-producing tumors	ID of hormone products in tumors.	May not correlate well with serum levels of the same markers.
Human chorionic gonado tropin *(hCG, B-HCG)*	Beta chain of the hormone *(Cytoplasm)*	Syncytiotrophoblasts	Choriocarcinoma, giant cells in seminomas, placental site tumors (weak)	ID of trophoblastic differentiation in tumors	
Human placental lactogen *(HPL, hPL)*	Hormone *(Cytoplasm)*	Trophoblast	Choriocarcinoma (may be weak), complete moles (strong), partial moles (weak), some lung and stomach carcinomas	ID of trophoblastic differentiation in tumors.	
Inhibin— alpha subunit	Hormone produced by ovarian granulosa cells and prostate, inhibits FSH production *(Cytoplasm)*	Ovarian granulosa cells, Sertoli cells, pregnancy luteomas, ovarian follicles, syncytiotrophoblast, adrenal cortex, hepatocytes	Granulosa cell tumors, juvenile granulosa cell tumors, Sertoli and Leydig cell tumors, ovarian stromal cells around other tumors, hydatidiform moles, choriocarcinoma, thecofibroma, adrenal cortical tumor, granular cell tumor	ID of sex cord stromal differentiation in ovarian tumors. Distinguishes adrenal cortical tumors (>70% +) vs. HCC (<5% +) and RCC (<5% +)	
INI-1 *(BAF47/Snf5)*	Chromatin remodeling complex *(Nucleus)*	All normal cells	Deleted or mutated in pediatric rhabdoid tumors (tumors are negative) and CNS atypical teratoid/rhabdoid tumors	Lost in 90% of epithelioid sarcomas (conventional and proximal) and lost in 50% of epithelioid MPNST ID of rhabdoid tumors and atypical teratoid/rhabdoid tumors	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
Keratins	Intermediate filaments *(Cytoplasm)*	Epithelial cells	Carcinomas, mesotheliomas, desmoplastic small round cell tumors (dot-like pattern), thymomas, chordomas, synovial sarcomas, epithelioid sarcoma, leiomyosarcoma, trophoblastic tumors, some other sarcomas, rarely melanomas	ID of poorly differentiated carcinomas Cytokeratins 7 and 20 can be used to help identify the site of origin of carcinomas	
AE1/AE3	Two monoclonal antibodies. AE1 detects 10, 15, 16, and 19. AE3 detects 1 to 8. *(Cytoplasm)*	Epithelial cells, mesothelial cells	Most carcinomas. The only common carcinomas that are frequently negative are HCC (70% negative) and RCC, clear cell type (20% negative) Epithelioid hemangioendothelioma, epithelioid sarcoma, synovial sarcoma, mesothelioma, adenomatoid tumor, germ cell tumors	ID of epithelial differentiation in tumors HCC (–/+) versus cholangiocarcinoma and metastatic carcinomas (+)	Good broad spectrum keratin
CAM 5.2	8, 18 *(Cytoplasm)*	Simple and glandular epithelium	Most carcinomas including those usually negative for Ck7 and 20: HCC, prostatic carcinoma, thymic carcinoma, gastric carcinoma, renal cell carcinoma, small cell carcinoma Carcinoid tumor, thymoma, germ cell tumors, mesothelioma, dendritic cells Synovial sarcoma, epithelioid sarcoma Many squamous cell carcinomas are negative	ID of carcinomas that may be negative for Ck7 and Ck20 Paget disease (+) versus squamous cell carcinoma (-) Positivity for dendritic cells in lymph nodes and elsewhere may be confused for micrometastases	May be positive when other keratins are negative

Keratin 5/6	5/6 *(Cytoplasm)*	Basal cells, stratum spinosum of epidermis, mesothelial cells	Squamous cell carcinomas, TCC, epithelioid mesotheliomas, squamous metaplasia in adenocarcinomas, thymic carcinoma	Less frequently present in non-squamous cell carcinomas Epithelioid mesothelioma (+) vs. pulmonary adeno (–) Present in some "triple negative" breast cancers — may identify a poor prognostic group	Has limited use in routine practice
Keratin 7	7 *(Cytoplasm)*	Simple epithelia, respiratory epithelium, transitional epithelium, endothelial cells of small veins and lymphatics Not present in squamous epithelium	Most adenocarcinomas of glandular epithelial origin, TCC, mesothelioma, neuroendocrine neoplasms Not Merkel cell carcinoma or colon carcinoma Rare in clear cell RCC (but present in other variants), prostate carcinoma, HCC, lung small cell carcinoma, thymoma, carcinoid Not present in squamous cell carcinomas of the skin, but may be present in squamous cell carcinomas arising from non-keratinizing epithelium (e.g., cervical carcinoma)	The combination of Ck7 and Ck20 is used to distinguish carcinomas arising at different sites (see Tables 7-3 to 7-7)	
Keratin 14	14 *(Cytoplasm)*	Squamous cells, myoepithelial cells	Squamous cell carcinomas, thymoma, myoepithelial neoplasms, oncocytic neoplasms (Hurthle cell adenoma of the thyroid), some triple negative ("basaloid") breast cancers	ID of keratin in spindle cell breast carcinomas and other triple negative breast cancers	
Keratin 20	20 *(Cytoplasm)*	Gastric foveolar epithelium, intestinal villi and crypt epithelium, Merkel cells, taste buds, umbrella cells of urothelium, subsets of epithelial cells Not present in nonepithelial cells	Colon carcinoma, Merkel cell carcinoma, TCC, adenocarcinoma of the bladder, pancreatic carcinoma, cholangiocarcinoma, mucinous ovarian carcinoma, esophageal adenocarcinoma	Merkel cell carcinomas Ck20 positive, whereas most similar tumors are negative ID of metastatic colon carcinomas (the pattern of Ck7 negative, Ck20 positive, is most frequently seen in this carcinoma, but can rarely be seen in other types)	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
PAN-K *(MNF-116)*	Broad-spectrum detection of keratins including 5, 6, 8, 17, and 18 *(Cytoplasm)*	Epithelial cells including simple epithelium and squamous cells		Detection of keratin in all carcinomas, including poorly differentiated carcinomas (especially spindle cell squamous cell carcinomas) May be more sensitive than AE1/AE3 for carcinomas with myoepithelial ("basal") features due to inclusion of the "basal" keratin Ck17	
34βE12 *(903)*	HMW keratins including 1, 5, 10, 14 *(Cytoplasm)*	Complex epithelia, basal cells, myoepithelial cells	TCC, cholangiocarcinoma, squamous cell carcinoma, non-mucinous bronchioloalveolar lung carcinoma, RCC (papillary and chromophobe types), mesothelioma, papillary thyroid carcinoma, thymic carcinoma, lymphoepithelial carcinoma	TCC (+) versus prostate carcinoma (-) or RCC (-). Prostate carcinoma (no basal cells) versus benign lesions (with some + basal cells present). Can be combined with p63 for this use. ID of keratin 14 in triple negative ("basaloid") breast cancers (Ck14 is also available separately).	
Ki-67 (MIB-1)	Protein found during the entire cell cycle but not in G0 *(Nucleus)*	Any cycling cell	Any cycling tumor	Used as a prognostic marker for some tumors Detects number of cycling cells in Burkitt lymphoma and large B-cell lymphoma Aberrant membrane and cytoplasmic immunoreactivity is present in trabecular hyalinizing adenoma of the thyroid and sclerosing hemangioma of the lung	MIB-1 recognizes an epitope preserved in formalin-fixed tissue
Laminin	Component of basement membranes *(Basement membrane)*	Basement membranes	Nerve sheath tumors, smooth muscle tumors	Loss associated with stromal invasion by carcinomas Present in microglandular adenosis of the breast.	

Lysozyme *(Ly)*	Muramidase (mucolytic enzyme) *(Cytoplasm)*	Circulating monocytes, some tissue macrophages, granulocytes, salivary gland, lacrimal gland, stomach and colon epithelial cells (inflamed or regenerative), apocrine glands, Paneth cells, some other epithelial cells	Salivary gland tumors, stomach and colon carcinomas AML with monocytic differentiation	Marker for histiocytes but not specific. May mark activated phagocytic macrophages. Evaluation of myeloid leukemias.	Not specific for identification of solid tumors
MAC 387 *(L1 antigen, calprotectin, calgranulin, cystic fibrosis antigen)*	Three polypeptide chains released with activation or death of neutrophils *(Cytoplasm)*	Neutrophils, monocytes, some tissue macrophages, eosinophils, squamous mucosa, reactive skin, synovial lining cells	Lung carcinomas (not small cell or carcinoid), squamous cell carcinomas Histiocytic neoplasms (but not Langerhans cells)	Marker for macrophages (but not as specific as CD68)	Belongs to the S100 protein family Cells can passively take up antigen resulting in false positive results
Mammaglobin *(MGB1)*	Secretory glycoprotein *(Cytoplasm)*	Breast epithelium, sweat glands, endocervix, endometrium	Breast cancer (50%), endometrioid adenocarcinoma (~40%), salivary gland carcinoma (~20%), melanoma (~6%)	ID of metastatic breast cancer (+ in about 50%)	
MDM2 *(mouse double minute 2 homolog)*	A ubiquitin protein ligase that regulates p53 stability *(Nucleus)*	Not seen in normal cells	Liposarcomas (>90%) and MPNST (60%)	Atypical lipomatous tumor/well-differentiated liposarcoma and dedifferentiated liposarcoma (>90% +) vs. benign adipose tumors (<5% +)	CDK4 has a similar pattern
MELAN-A or MART-1 *(melanoma antigen recognized by T cells, A103, M2-7C10)*	Melanocyte differentiation antigen *(Cytoplasm)*	Antibody MC-7C10 is positive in melanocytes of skin, uvea, and retina Antibody A103 is also positive in adrenal cortex, granulosa and theca cells of the ovary, Leydig cells	Melanomas (but < 50% of spindle cell or desmoplastic melanomas), PEComas Antibody A103 is also positive in adrenocortical tumors, Leydig cell tumor, granulosa cell tumor	ID of melanomas. The antibodies are not positive in melanophages and may be more specific for the detection of micrometastases in lymph nodes. Antibody A103 distinguishes adrenocortical tumors (≥50% +) vs. HCC (-) and RCC (-).	More sensitive than HMB45 Peptides are used for melanoma immunotherapy A103 has a broader spectrum of immunoreactivity than MC-7C10

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
MITF *(microphthalmia transcription factor, Mit-f, D5, MiTF)*	Basic-helix-loop-helix-leucine zipper transcription factor that regulates tyrosinase and other melanogenic proteins *(Nucleus)*	Melanocytes, pigmented cells of the retina, mast cells, osteoclasts	Melanoma, PEComa, angiomyolipoma, clear cell sarcoma	Melanoma vs. other tumors – not as specific as other melanoma markers (also present in other tumors)	Mutations result in autosomal dominant Waardenburg syndrome type 2a (hereditary deafness and skin hypopigmentation)
MUC2 and **MUC1**	Mucins *(Cytoplasm)*	MUC1 is typical of pancreaticobiliary-type differentiation and MUC2 of intestinal differentiation	Many adenocarcinomas	Identification of colonic metastases (expression more conmmon than in lung or ovary) For pancreatic/ampullary tumors, MUC2 positive tumors may have better prognosis than MUC1 tumors Cholangiocarcinoma (MUC1 80%) vs. HCC (negative) IPMN is usually MUC2+/MUC1–, most PanIN are MUC2-/MUC1+, ductal adenoca is MUC1+ except colloid type which is MUC2+	MUC2 is a marker of intestinal cells – similar pattern as CDX2
Myf-4 *(MRF4, myogenin)*	Human homologue of myogenin - muscle regulatory protein *(Nucleus)*	Striated muscle	Rhabdomyosarcoma	ID of skeletal muscle differentiation in tumors	Better than MyoD1
MyoD1	Nuclear phosphoprotein, role in myogenic regulation *(Nucleus)*	Developing muscle tissues (myoblasts), adult muscle is negative	Rhabdomyosarcoma (especially poorly differentiated tumors), mixed Mullerian tumors	ID of skeletal muscle differentiation in tumors	Background positivity is often high, making interpretation difficult

Myoglobin	Oxygen-binding protein *(Cytoplasm)*	Striated muscle (including cardiac muscle), not smooth muscle	Tumors of striated muscle (rhabdomyosarcoma + 50%), but often negative in poorly differentiated tumors	ID of skeletal muscle differentiation in tumors	More specific but less sensitive than actin and desmin
Myosin – smooth muscle myosin heavy chain *(SM-MHC; SMMS-1, M3558)*	Contractile protein in smooth muscle that interacts with actin *(Cytoplasm)*	Visceral and vascular smooth muscle, myoepithelial cells of the breast	Tumors with myoepithelial cells	Marker for myoepithelial cells in the breast – may have less positivity in vascular smooth muscle cells and myofibroblasts	Antibodies to different isoforms will detect different types of muscle fibers
Myosin – fast myosin *(MY-32, M4276)*	Contractile protein in skeletal muscle that interacts with actin *(Cytoplasm)*	Striated muscle - Type 2 fibers (not present in cardiac or smooth muscle)	Rhabdomyosarcoma (some; especially pleomorphic variant)	ID of skeletal muscle differentiation in tumors	
NANOG	Embryonic stem cell transcription factor *(Nucleus)*	Embryonic cells	Embryonal carcinoma, seminoma, CNS germinoma	Seminoma and embryonal carcinoma vs. other germ cell tumors May detect "stem cells" in tumors	Stronger and more diffusely positive compared to OCT3/4. Named after Tir Na Nog, the mythologic Celtic land of eternal youth
Nestin	Intermediate filament *(Cytoplasm)*	Neural stem/progenitor cells, embryonic neural cells, neuronal and glial cells Retina, striated muscle, cardiac muscle, skin, liver, pancreas, kidneys, testes, adrenals	Neurocytomas, neuroblastomas, gliomas, glioblastomas, astrocytomas, ependymomas, medulloblastomas, Schwannomas Carcinomas, GIST, others		
NeuN *(NEUronal Nuclei, A60)*	DNA binding neuron-specific protein expressed at terminal differentiation *(Nucleus)*	Neuronal cells including cerebellum, cerebral cortex, peripheral ganglion cells Not glia, pineocytes, Schwann cells	Central neurocytomas May be focally positive in other CNS neoplasms	ID of neuronal differentiation	
Neurofilaments *(70 + 200kD, NFP)*	Intermediate filaments with three subunits *(Cytoplasm)*	Neuronal cells, adrenal medulla	Tumors of neuronal origin or with neuronal differentiation, neuroblastoma, medulloblastoma, retinoblastoma, Ewing's/PNET, esthesioneuroblastoma, Merkel cell carcinoma, some endocrine tumors (carcinoids, pheochromocytomas)	ID of neuronal differentiation in tumors ID of Merkel cell carcinomas	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
Neuron- specific enolase *(NSE – do not confuse with the enzyme non-specific esterase)*	Gamma-gamma enolase isoenzyme *(Cytoplasm)*	Neuroectodermal and neuroendocrine cells, more weakly striated and smooth muscle, megakaryocytes, T cells, some platelets, neurons, pituitary cells, hepatocytes	Neuroectodermal and neuroendocrine tumors, melanomas (including desmoplastic melanomas), many breast carcinomas, germ cell tumors, alveolar soft part sarcoma	ID of neuronal or neuroendocrine differentiation in tumors	Lacks specificity
OCT4 *(OCT3, OCT3/4, POU5F1)*	POU-domain transcription factor (POU5F1 gene) *(Nucleus)*	Embryonic stem cells and pluripotent germ cells	Seminoma, intratubular germ cell neoplasia, embryonal carcinoma	Identification of seminoma and embryonal carcinoma. Other epithelioid and round cell neoplasms are negative	More specific than PLAP for this purpose
OLIG2	Member of the basic helix-loop-helix transcription factor family *(Nucleus)*	Oligodendrocytes	Diffuse glioma (100%), may be positive in other CNS tumors. Sparse positivity in ependymomas T-ALL with OLIG2 translocation	Primary CNS tumor (+) vs. metastasis (-)	
p16 *(MTS1, CDKN2)*	Binds to and inhibits the cyclin-dependent kinases cdk4 and cdk6 *(Cytoplasm and nucleus)*	Absent	Cervical squamous cell carcinomas and adenocarcinomas (both in situ and invasive), endocervical carcinoma, endometrial carcinoma Some basaloid squamous cell carcinomas of the tonsil in young patients that are associated with HPV16.	Evaluation of cervical lesions Possible use predicting tonsillar site for metastatic squamous cell carcinoma of the head and neck	Overexpression is due to HPV-induced cell cycle dysregulation
p53 *(multiple antibodies to wild type and mutant forms)*	Tumor suppressor gene product – probably most frequently mutated gene in malignancy *(Nucleus)*	Overexpression uncommon or absent in normal cells or benign tumors	Many malignant tumors – but not specific for malignancy	Overexpression may be used as a prognostic factor	Different antibodies recognize different wild type and mutant forms of the protein and will give different results

p57 *(kip2, p57^{KIP2})*	Cyclin-dependent kinase inhibitor (CDKI) acting to inhibit cell proliferation, paternally imprinted *(Nucleus)*	Cytotrophoblast, intermediate trophoblast, villous stromal cells, decidual stromal cells, absent in syncytiotrophoblast		Diploid complete moles show absent or low expression in cytotrophoblast and villous stromal cells (may be present in villous intermediate trophoblast and decidual stromal cells), partial moles and hydropic abortions have normal expression	
p63	Protein with at least six major isotypes, including deltaNp63, member of the p53 family *(Nucleus)*	Proliferating basal cells of cervix, urothelium, prostate, and myoepithelial cells of breast, basal squamous cells, squamous metaplasia	Squamous cell carcinomas, TCC, adenomyoepithelioma, adenoid cystic carcinoma, nasopharyngeal carcinoma, "basal type" breast carcinomas, papillary carcinoma of the thyroid, others	ID of myoepithelial cells in breast lesions Diagnosis of prostatic carcinoma by showing absence of basal cells (more sensitive when combined with 34BE12) Basaloid squamous lung cancer (+) vs. small cell (-) ID of metastatic poorly differentiated squamous cell carcinomas	Easier to interpret than SMA in breast lesions as myofibroblasts are negative
Placental alkaline phosphatase *(PLAP)*	Alkaline phosphatase secreted by trophoblast *(Cytoplasm)*	Placenta (trophoblast)	Germ cell tumors (but not spermatocytic seminoma), intratubular germ cell neoplasia, partial moles, some carcinomas of breast, ovary, lung, stomach, and pancreas, some rhabdomyosarcomas (esp. alveolar type)	Absence of immunoreactivity makes a germ cell tumor unlikely. However, spermatocytic seminomas and immature teratomas are negative. ID of intratubular germ cell neoplasia.	
Progesterone receptor *(PR, PgR, PgR636)*	Steroid binding protein *(Nucleus)*	Normal breast epithelial cells, endometrial cells, many smooth muscle cells, breast lobular stroma	Breast carcinomas, gynecologic carcinomas, some skin adnexal tumors, secretory meningiomas, endometrial stromal sarcomas, some leiomyomas, myofibroblastic tumors, rarely other tumors	Prognosis and treatment of breast cancer ID of metastatic breast cancer	
Prealbumin *(Transthyretin, TTR)*	Plasma transport protein for retinol and thyroxine *(Cytoplasm)*	Pancreatic islet cells, choroid plexus, retinal pigment epithelium, liver	Pancreatic islet cell tumors, carcinoid tumors, choroid plexus papillomas, choroid plexus carcinomas (may be focal or absent)	ID of choroid plexus neoplasms Evaluation of some forms of amyloidosis	Major subunit protein in some forms of inherited systemic amyloidosis

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
Prostate specific antigen *(PSA)*	Member of kallikrein family of serine protease isolated from human seminal plasma *(Cytoplasm)*	Normal prostatic epithelium, urachal remnants, endometrium, transitional cells of bladder	Prostatic carcinomas, some breast carcinomas	ID of prostatic carcinomas (may be lost in some poorly differentiated carcinomas). Seminal vesicle carcinomas are negative	More specific than PAP Used as a serum screening test for prostate cancer
Prostate acid phosphatase *(PrAP, PAP)*	Isoenzyme of acid phosphatase *(Cytoplasm)*	Normal prostatic epithelium, periurethral glands, anal glands, macrophages	Prostatic carcinomas, TCC, rectal carcinoids	ID of prostatic carcinomas (may be lost in some poorly differentiated carcinomas)	
RCC *(Renal cell carcinoma marker, gp200)*	Glycoprotein on surface of renal tubules, breast epitethelial cells, epididymis *(Cytoplasm, membrane)*	Renal tubules, breast, epididymis	Clear cell and papillary RCC, breast carcinoma, embryonal carcinoma	Clear cell and papillary RCC (+) vs. chromophobe carcinoma (–/+) and oncocytoma (-)	
RET *(Rearranged during transfection)*	RET-proto-oncogene – surface glycoprotein of the receptor tyrosine kinase family *(Cytoplasm)*	Neurons, embyronic kidney	Papillary thyroid carcinomas (78%), follicular variant of papillary carcinoma (63%), Hurthle cell carcinoma (57%), insular carcinoma (50%), medullary carcinoma, not present in follicular carcinomas or benign lesions Neuroblastoma, pheochromocytoma	Evaluation of thyroid tumors	Germline mutations occur in MEN2A and 2B (10q11.2), familial medullary thyroid carcinoma, and some cases of Hirschsprung's disease

S-100 protein	Calcium-binding protein isolated from the CNS (member of the EF hand family) *(Nucleus and cytoplasm)*	Glial and Schwann cells, melanocytes, chondrocytes, adipocytes, myoepithelial cells, Langerhans cells, macrophages, reticulum cells of lymph nodes, eccrine glands, others	Melanoma (including spindle cell and desmoplastic types), clear cell sarcoma, schwannoma, chordoma, ependymoma, astroglioma, Langerhans proliferative disorders, some carcinomas (e.g., breast, ovary endometrial, thyroid), granular cell tumor, histiocytic sarcoma, myoepithelioma	ID of melanoma (if negative, melanoma is highly unlikely) ID of Langerhans proliferative disorders Sustentacular cells in pheochromocytomas (loss may be poor prognostic factor) ID of neural tumors ID of cellular schwannomas (more strongly and diffusely positive than in MPNST)	Langerhans cells and macrophages in tumors may be misinterpreted as positivity in the tumor itself S100 is very soluble and may be eluted from frozen tissues
Smoothelin	Smooth muscle specific cytoskeletal protein *(Cytoplasm)*	Fully differentiated smooth muscle cells (not present in noncontractile smooth muscle cells or myofibroblasts), weak in perivascular smooth muscle		May distinguish muscularis propria (+, strong, diffuse) vs. muscularis mucosaae (– or weak and focal) in urinary bladder biopsies	
SOX2	Embryonic stem cell transcription factor *(Nucleus)*		Embryonal carcinoma	Embryonal carcinoma (+) vs. seminoma (–)	
Synaptophysin	Transmembrane glycoprotein found in presynaptic vesicles *(Cytoplasm)*	Neuroectodermal and neuroendocrine cells, neurons	Medulloblastoma, neuroblastoma, pheochromocytoma, paragangliomas, carcinoids, small cell carcinoma, medullary carcinoma of the thyroid, neural neoplasms, pancreatic islet cell tumors	ID of neuroendocrine differentiation in tumors ID of neuronal differentiation in CNS tumors	
Synuclein-1	Neuron-specific protein associated with synaptosomes *(Lewy bodies)*	Brain	Present in Lewy bodies (Lewy body dementia and Parkinson's disease)		
Tau	Microtubule-associated protein *(Cytoplasm, extracellular)*	Normal neuronal cell bodies and dendrites, neuropil, glial cells	Abnormal amounts in Alzheimer's disease in neurofibrillary tangles and senile plaques	Evaluation of Alzheimer's disease, Pick's disease, supranuclear palsy corticobasal degeneration, others	
TFE3	Transcription factor *(Nucleus)*	Not reported	Alveolar soft part sarcoma, Xp11.2 or TFE3 translocation renal carcinomas in children and young adults	Other tumors and normal adult tissues are negative	These carcinomas have translocations involving the TFE3 gene resulting in its overexpression

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
General Markers					
Thyroglobulin	Glycoprotein produced by thyroid follicular cells *(Cytoplasm)*	Thyroid follicles	Thyroid carcinomas (papillary, follicular, and Hurthle cell types, rarely present in medullary carcinomas)	ID of metastatic thyroid carcinoma Loss may be a poor prognostic factor	Thyroglobulin can diffuse into metastatic tumors to the thyroid.
TTF-1 *(thyroid transcription factor 1)*	Transcription factor for thyroglobulin, thyroid peroxidase, Clara cell secretory protein, and surfactant proteins *(Nucleus; aberrant cytoplasm positivity in HCC)*	Thyroid, lung, and some brain tissues	Thyroid carcinomas (including medullary carcinoma; may be negative in anaplastic carcinoma), lung adenocarcinomas (75% – but lower in mucinous bronchioloalveolar carcinomas), small cell carcinoma of lung (>90%), HCC (cytoplasmic), absent or focal in most other adenocarcinomas	Mesothelioma (–) vs. adenocarcinoma (+/–) Lung adenocarcinoma (+/–) vs. metastatic breast carcinoma (–) Small cell carcinoma of lung (+) vs. metastasis from other sites (–), but some extrapulmonary small cell carcinomas can also be (+) HCC (cytoplasmic 71%), rare in other tumor types	The detection of cytoplasmic TTF-1 may depend on the specific antibody used and the antigen-retrieval method
Tyrosinase	Melanogenic protein *(Cytoplasm)*	Melanocytes		Melanoma vs. other tumors (sensitivity similar to MART-1 and HMB-45)	
Ulex *(Ulex Europaeus I lectin, UEA 1)*	Lectin, fucose residues on blood group H *(Cytoplasm)*	Endothelial cells	Vascular tumors, some carcinomas	Evaluation of angiogenesis	Not very specific
Vimentin	Intermediate filament *(Cytoplasm)*	Mesenchymal cells, fibroblasts, endothelial cells, chondrocytes, histiocytes, lymphocytes, many glial cells, myoepithelial cells, smooth muscle	All mesenchymal tumors, neural tumors, melanomas, meningiomas, chordoma, Leydig cell tumor, granulosa cell tumor, Sertoli cell tumor, adrenal cortical adenoma May be co-expressed with keratin in carcinomas of endometrium, thyroid, kidney (clear cell), adrenal cortex, lung, salivary gland, ovary, and liver	May be poor prognostic factor if co-expressed with keratin or GFAP	Can be used as an internal control for immunogenicity Not a specific marker for tumor type or line of differentation

WT1 *(Wilms tumor 1 protein)*	Zinc finger transcription factor *(Cytoplasm, nucleus)*	Sertoli cells, decidual cells of uterus, granulosa cells of ovary, blood vessels, myelocytic cells	Wilms tumors (epithelial and blastemal components), epithelial mesotheliomas (nuclei – 80% to 90%), acute leukemia (nuclei), adenocarcinomas (cytoplasmic; especially breast (mucinous breast cancers can have nuclear positivity (64%), ovary), desmoplastic small cell tumors (nuclear and cytoplasmic), rhabdomyosarcoma	Mesothelioma (+, nuclear) vs. adenocarcinoma (adenocarcinoma usually negative for nuclear positivity except for ovarian) – use mouse monoclonal antibody Desmoplastic small cell tumors	Gene located on 11p13 and is inactivated in 5 to 10% of sporadic Wilms tumors and nearly 100% of Denys-Drash syndrome patients ABs detect epitopes at different ends of the protein and may give different results. Not very specific.
Hematopathology Markers					
ALK protein *(Anaplastic lymphoma kinase, ALK-1, p80, CD246)*	The ALK gene (2p23) (a tyrosine kinase receptor) is translocated to part of the nucleophosmin (NPM) gene (5q35) to form the fusion protein p80 and is overexpressed *(Cytoplasm, nucleus)*	Nervous system, T cells	Anaplastic (CD30+) large cell lymphomas (about one third have t[2;5][p23; q35]). ALK negative anaplastic lymphomas may have trisomy 2. Some inflammatory myofibroblastic tumors	ID of anaplastic large cell lymphomas	The pattern of immunoreactivity varies with the translocation present
Alpha-1-antichymotrypsin *(ACH)*	Serine protease inhibitor *(Cytoplasm)*	Histiocytes, granulocytes, others	Histiocytic tumors, many adenocarcinomas, melanomas, many sarcomas	Marker for histiocytes but CD68 is more specific	Not specific for tumor type
Alpha-1-antitrypsin *(AAT, alpha-1-AT)*	Glycoprotein synthesized in the liver that inhibits proteolytic enzymes (especially elastase) *(Cytoplasm)*	Histiocytes, reticulum cells, mast cells, Paneth cells, salivary gland	HCC, germ cell tumors, histiocytic neoplasms, colon and lung carcinomas, others	Accumulates in liver cells in AAT deficiency	Not specific for tumor type CD68 is a more specific marker for macrophages

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
Bcl-2	Protein involved in inhibition of apoptosis *(Membrane, cytoplasm)*	Medullary lymphocytes and epithelial cells of the normal thymus, mantle and T zone small lymphocytes	CLL, mantle cell lymphoma, follicular center cell (FCC) lymphoma, marginal zone lymphoma Synovial sarcoma, other soft tissue tumors	FCC lymphomas (+) vs. reactive follicles (–). Hyperplastic marginal zones of the spleen, abdominal LNs, and ilial lymphoid tissue are (+) Malignant thymomas may have greater reactivity than other thymomas Synovial sarcoma is more frequently positive compared to mesothelioma	Involved in the t(14;18) found in 90% of FCC lymphomas Not specific for ID of solid tumors
Bcl-6	Proto-oncogene – Kruppel-type zinc finger protein with homology to transcription factors *(Nucleus)*	Normal germinal center B cells	Follicular lymphomas, diffuse large B-cell lymphomas, Burkitt lymphoma, mediastinal large B-cell lymphoma, LP HD Not present in B-CLL, hairy cell leukemia, mantle cell lymphoma, and marginal zone lymphomas	Evaluation of B-cell lymphomas	Involved in gene rearrangements at 3q27 in lymphomas
Blood group antigens	A, B, and H antigens *(Membrane)*	Epithelial cells, endothelial cells, erythrocytes	Abnormally expressed or lost in many carcinomas	Sometimes helpful in identifying specimens	H is diminished by decalcification but not A and B antigens
BOB.1 *(B-cell Oct-binding protein 1)*	Coactivator that interacts with Oct transcription factors in B cells *(Cytoplasm)*	B cells (including plasma cells)	B-cell lymphomas and leukemias Reed-Sternberg cells in LP HD, usually absent in other HD types	Evaluation of HD	BOB.1 and Oct2 are necessary (but not sufficient) for Ig expression
BSAP *(B-cell specific activator protein, Pax-5)*	Transcription factor encoded by the Pax-5 gene that regulates B-lineage specific genes *(? Nuclear)*	B cells	All B-cell neoplasms and HD	Merkel cell tumors and pulmonary small cell carcinomas have been reported to be positive	Not reliable in Zenker's fixed tissue

CD1a *(T6)*	Membrane glycoprotein *(Membrane)*	Cortical thymocytes (immature T cells), Langerhans cells, dendritic cells	Langerhans proliferative disorders, lymphoblastic lymphoma	Evaluation of Langerhans proliferative disorders Evaluation of lymphoblastic lymphoma	
CD2 *(TE, T11, rT3, Leu 5a + b, LFA-2)*	Glycoprotein mediating adhesion of activated T cells and thymocytes with antigen presenting cells and target cells, functions in E rosette formation *(Membrane)*	T cells, NK cells, cortical thymocytes	T-cell neoplasms, may be aberrantly lost in peripheral T-cell neoplasms	Pan T-cell marker	
CD3 *(T3)*	C3 antigen (five polypeptide chains) *(Membrane, cytoplasm)*	T cells, cortical thymocytes	T-cell neoplasms, may be aberrantly lost in peripheral T-cell neoplasms Anaplastic large cell lymphoma is often negative	Best pan T-cell marker	In paraffin sections, NK cells may also be positive
CD4 *(TH, T4, Leu 3)*	Transmembrane glycoprotein, HIV receptor *(Membrane)*	T helper/inducer cells, macrophages, Langerhans cells	MF, other T-cell neoplasms	Evaluation of MF Evaluation of T-cell neoplasms	
CD5 *(Leu 1)*	Transmembrane glycoprotein *(Membrane)*	T cells and B cell subsets (mantle zone)	T-cell leukemias and lymphomas, aberrantly expressed in low-grade B-cell lymphomas (CLL or mantle cell lymphoma) Thymic carcinoma, adenocarcinomas, mesothelioma (cytoplasmic)	Classification of low-grade B-cell lymphomas Evaluation of T-cell lymphomas (this marker is frequently lost) Thymic carcinoma (~40%) vs. thymoma (<10%) vs. pulmonary squamous cell carcinoma (<5%)	
CD7 *(Leu 9)*	Membrane-bound glycoprotein *(Membrane)*	Precursor T cells, T cell subsets, NK cells, thymocytes	T-cell lymphomas and leukemias	Frequently (50%) lost in T-cell lymphomas versus reactive T cells (+) Evaluation of T-cell leukemias	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
CD8 *(T8, Leu 2)*	Two glycoprotein chains *(Membrane)*	T cell subsets, NK cells, T cytotoxic/suppressor cells	T-cell lymphomas and leukemias	Evaluation of MF and T-cell lymphomas (this marker is frequently lost)	
CD10 *(CALLA [common acute leukemia antigen], J5, neprilysin)*	Cell surface metalloendopeptidase that inactivates peptides *(Membrane)*	Precursor B cells, granulocytes, rare cells in reactive follicles, myoepithelial cells of breast, bile canaliculi, fibroblasts, brush border of kidney and gut	Follicular lymphomas, pre-B-ALL, Burkitt lymphoma, CML, angioimmunoblastic lymphoma RCC (clear cell and papillary), HCC, rhabdomyosarcoma, endometrial stromal sarcoma	Evaluation of follicular center cell lymphomas Evaluation of leukemias Myoepithelial cell marker in breast Endometrial stromal sarcoma (+) vs. leiomyosarcoma (–) (but caldesmon is preferred for this purpose)	
CD11b *(Mac-1)*	Cell surface receptor for the C3bi complement fragment *(Membrane)*	Granulocytes, monocytes, macrophages	Myelomonocytic leukemias		
CD11c	Member of the beta(2) integrin family that mediates adhesion to vascular endothelium, transendothelial migration, chemotaxis, and phagocytosis *(Membrane)*	Myeloid cells, NK cells, dendritic cells, activated lymphoid cells	Hairy cell leukemia, B-cell prolymphocytic leukemia, some B-CLL, marginal zone lymphoma (MALT)		
CD13 *(My 7)*	Aminopeptidase-N, a type II integral membrane metalloprotease functioning in cell surface antigen presentation, receptor for coronaviruses *(Membrane, cytoplasm)*	Granulocytes, macrophages, bone marrow stromal cells, osteoclasts, renal tubules, intestinal brush border, cells lining bile duct canaliculi, endothelial cells, fibroblasts, brain cells	AML, CML with blast crisis, some ALL	Classification of leukemias	Requires frozen tissue

CD15 *(Leu-M1)*	3-fucosyl-N-acetyllactosamine, X-hapten - CHO moiety linked to cell membrane protein *(Membrane and granular perinuclear)*	Granulocytes, monocytes	Reed-Sternberg cells (not LP HD), some large T-cell lymphomas, MF, some leukemias, some epithelial cells (adenocarcinomas), CMV-infected cells	Adenocarcinomas (+) versus mesotheliomas (–) Evaluation of HD	
CD16	Low affinity transmembrane Fc receptor for IgG *(Membrane)*	NK cells, granulocytes, activated macrophages, subsets of T cells	Extranodal NK/T-cell lymphoma, some hepatosplenic T-cell lymphomas		
CD19 *(B4)*	B cell type I integral membrane glycoprotein *(Membrane)*	B cells, follicular dendritic cells, early myelomonocytic cells	pre-B-ALL and B-cell neoplasms (but not plasma cell lesions)	Good pan B-cell marker	Fresh or frozen tissue required
CD20 *(L26, B1, Leu16)*	B cell non-glycosylated phosphoprotein functioning as a receptor during B cell activation and differentiation *(Membrane, cytoplasm)*	B cells, monocytes, not plasma cells	B-cell lymphomas, Reed-Sternberg cells in LP HD, not plasmacytomas	Best pan B-cell marker Evaluation of B-cell lymphomas Evaluation of HD	Under investigation as a target for clinical treatment of B-cell lymphomas L26 is best for formalin-fixed tissue May be preserved in necrotic tissue
CD21 *(B2)*	Type I integral membrane glycoprotein functioning as the receptor for the C3d fragment of complement C3, CR2, receptor for EBV *(Membrane)*	Follicular dendritic cells, mature B cells	Marginal zone (MALT) lymphomas, CLL (B cell), some T-cell ALL, follicular dendritic cell tumors	ID of residual follicular structure in LP HD and other diseases Evaluation of low-grade B-cell lymphomas ID of follicular dendritic cell sarcoma	

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
CD22 *(BL-CAM)*	Type I integral membrane glycoprotein *(Membrane. cytoplasm)*	B cells, precursor B cells	B-cell neoplasms (but not plasma cell lesions)	Pan B-cell marker	
CD23	Membrane glycoprotein, low affinity IgE receptor *(Membrane)*	Subpopulation of peripheral B cells, follicular dendritic cells	CLL, but usually not mantle zone lymphoma, Maltomas, or follicular lymphomas	Evaluation of low-grade B-cell lymphomas	
CD25 *(IL-2 receptor)*	Interleukin-2 receptor *(Membrane, cytoplasm)*	Subpopulation of T cells, myeloid precursors, oligodendrocytes HTLV-1 transformed T and B cells	Hairy cell leukemia, adult T-cell lymphoma/leukemia, some T-cell prolymphocytic leukemia, precursor lymphoblastic lymphoma, and anaplastic large cell lymphoma	Evaluation of cutaneous T-cell lymphomas for potential anti-CD25 therapy Aberrant expression by a subset of neoplastic mast cells	
CD30 *(Ki-1, BERH2)*	Single chain transmembrane glycoprotein, homologous to the nerve growth factor superfamily *(Cytoplasm, membrane and golgi)*	Activated B and T cells, some plasma cells, immunoblasts, interdigitating cells, histiocytes, follicular center cells, decidualized endometrium, reactive mesothelial cells, most other tissues negative	Anaplastic (CD30+) large cell lymphomas, large B-cell lymphoma, primary effusion lymphoma, mediastinal large B-cell lymphoma, Reed-Sternberg cells (not LP HD), enteropathy T-cell lymphoma, peripheral T-cell lymphoma, EBV transformed B cells Embryonal carcinoma, vascular tumors (not KS), some mesotheliomas, rarely carcinomas are positive	Evaluation of anaplastic (CD30+) lymphomas Evaluation of HD (Reed-Sternberg cells are positive except in LP HD) Evaluation of peripheral T-cell lymphoma (large cells may be positive)	
CD33 *(My 9)*	Myeloid specific receptor (sialic acid–binding immunoglobulin-like lectin or Siglec-3) *(Membrane)*	Granulocytes, monocytes	AML	Evaluation of leukemias	Gemtuzumab ozogamicin is a humanized CD33 antibody linked to an antitumor antibiotic calicheaminin for the treatment of AML

CD34 *(HPCA-1, QBEnd10)*	Single chain transmembrane glycoprotein *(Cytoplasm, membrane)*	Lymphoid and myeloid hematopoietic progenitor cells, endothelial cells, some skin cells, myofibroblasts	Acute leukemia Neurofibroma, angiosarcoma,KS, epithelioid hemangioendothelioma, solitary fibrous tumor, DFSP, epithelioid sarcoma, GIST, myofibroblastic tumors	ID of endothelial or myofibroblastic differentiation in tumors Evaluation of angiogenesis Evaluation of the number of blasts in bone marrow in acute leukemia	Not specific for endothelial cells
CD35 *(CR1, C3b/C4b R)*	Transmembrane protein that binds complement components C3b and C4b and mediates phagocytosis *(Membrane)*	Erythrocytes, B cells, a subset of T cells, monocytes, neutrophils, eosinophils, glomerular podocytes, follicular dendritic cells	Marginal zone (MALT) lymphoma, follicular dendritic cell tumors	Detects follicular dendritic cells ID of follicular dendritic cell sarcomas	
CD38	Type II transmembrane glycoprotein with enzymatic action for the formation and hydrolysis of cADPR *(Membrane)*	Immature B and T lymphocytes, thymocytes, mitogen-activated T cells, Ig-secreting plasma cells,monocytes, NK cells, erythroid and myeloid progenitors, brain cells	Acute leukemias, plasma cell lesions Neurofibrillary tangles in Alzheimer's disease	ID of plasma cell lesions	Immunoreactivity may be a poor prognostic marker for patients with CLL
CD43 *(Leu 22, L60)*	Cell surface glycoprotein *(Membrane)*	T cells, macrophages, granulocytes	AML (chloromas), T cell neoplasms, aberrant expression in some low-grade B-cell neoplasms (e.g., mantle cell lymphoma, SLL/CLL, marginal zone lymphoma), some MALT lymphomas	Evaluation of T-cell lymphomas and leukemias Evaluation of low-grade B-cell lymphomas	Less specific than UCHL-1 for T cells
CD45, Leukocyte common antigen *(LCA, CLA)* Note: CLA also refers to a different antigen, HECA-452	Five or more membrane glycoproteins *(Membrane, cytoplasm)*	Lymphocytes, leukocytes, histiocytes, not plasma cells, erythrocytes, platelets	Non-Hodgkin's lymphomas, some anaplastic (CD30+) large cell lymphomas, Reed-Sternberg cells in LP HD (but not other types)	ID of poorly differentiated neoplasms as lymphomas. However, some anaplastic lymphomas and plasmacytomas may be negative	Preserved in necrotic tissue Best general marker for hematologic neoplasms

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
CD45RA *(DPB)*	Restricted form of leukocyte common antigen *(Membrane, cytoplasm)*	B cells, monocytes, some T cells	B-cell neoplasms, Hairy cells (not specific)	Pan B-cell marker that can be used in Zenker's fixed tissue	Not completely specific - other B-cell markers are preferred
CD45RO *(UCHL-1)*	Isoform of CD45 (leukocyte common antigen) *(Membrane, cytoplasm)*	T cells (memory), granulocytes, monocytes	T-cell neoplasms, histiocytic sarcoma, some B-cell lymphomas (plasmacytic, HIV-associated)	Good pan T-cell marker (CD3 is more specific)	
CD56 (*NCAM*)	Neural cell adhesion molecule – cell surface glycoprotein *(Membrane)*	Neurons, astrocytes, Schwann cells, NK cells, subset of activated T cells	Some T/NK-cell lymphomas, plasmacytomas Neuroblastoma	Evaluation of panniculitis-like T-cell lymphoma (both CD56+ and CD56-) and T/NK lymphomas	
CD57 *(Leu 7, HNK-1)*	Lymphocyte antigen that cross reacts with a myelin-associated glycoprotein *(Membrane)*	T cell subsets, NK cells, myelinized nerves, neuroendocrine cells, prostate, pancreatic islets, adrenal medulla	Angioimmunoblastic T-cell lymphoma Nerve sheath tumors (occasional), leiomyosarcoma, synovial sarcoma, rhabdomyosarcoma, neuroblastoma, gliomas, neuroendocrine carcinomas, neurofibromas, some prostate carcinomas	ID of T gamma lymphoproliferative disorder (large granular cell lymphocytic leukemia) ID of neuroendocrine differentiation in tumors Evaluation of NK neoplasms	Not very specific for solid tumors
CD61 *(GPIIIa, platelet glycoprotein IIIa)*	Glycoprotein, receptor for fibrinogen, fibronectin, von Willebrand factor, and vitronectin *(Cytoplasm)*	Megakaryocytes, platelets	Megakaryocytic leukemias	ID of megakaryocytic differentiation	

CD68 *(KP1, CD68-PGM1, Mac-M)*	Intracellular glycoprotein associated with lysosomes *(Cytoplasm, membrane)*	Macrophages, monocytes, neutrophils, basophils, large lymphocytes, Kupffer cells, mast cells, osteoclasts	Some lymphomas, histiocytic sarcomas, APML, Langerhans proliferative disorders Neurofibroma, schwannoma, MPNST, granular cell tumors, PEComa, melanomas, atypical fibroxanthoma, RCC	Best general marker for macrophages, although not specific to this cell type	The antibody PG-M1 does not react with granulocytes
CD74 *(LN2)*	Subunit of MHC II–associated invariant chain *(Membrane)*	B cells, monocytes, histiocytes	B-cell neoplasms, hairy cell leukemia, plasma cell lesions	Pan B-cell marker	
CDw75 *(LN1)*	Sialylated glycoconjugate present in surface Ig-positive B cells *(Membrane, cytoplasm)*	Mature B cells, T-cell subsets, fetal colon, epithelial cells	Reed-Sternberg cells of LP HD (not other types), follicular lymphomas Colon carcinomas (50%), gastric carcinomas	Evaluation of HD	
CD77 *(BLA.36, PK antigen)*	Globotriaosylceramide, glycolipic membrane from Burkitt lymphoma cell line *(Cytoplasm, membrane)*	Tonsillar B cells, dendritic reticulum cells, sinus-lining cells, macrophages, endothelial cell, epithelial cells	HD, Burkitt lymphoma, rarely other B- and T-cell lymphomas	Evaluation of RS cells	
CD79a *(mb-1 protein)*	Heterodimer of mb-1 (CD79a) and B29 (CD79b) polypeptides, B cell antigen receptor *(Membrane)*	B cells, plasma cells	Precursor B-cell ALL, B-cell lymphomas, plasma cell lesions, but not primary effusion lymphoma	Evaluation of B-cell neoplasms (may be the only B-cell marker present)	
CD79b	See above *(Membrane)*			Absent from CLL, hairy cell leukemia	
CD95	Transmembrane glycoprotein member of the nerve growth factor receptor/tumor necrosis factor superfamily – mediates apoptosis *(Membrane)*	Activated T and B cells, epithelial cells	Panniculitis-like T-cell lymphoma (if CD56+)		

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
CD99 *(MIC-2, 12E7, Ewing's sarcoma marker, E2 antigen, HuLy-m6, FMC 29, O13 [different epitope])*	MIC2 gene product – glycoproteins (p30 and p32) involved in rosette formation with erythrocytes (*Membrane* [*immunoreactivity is more specific than cytoplasmic*])	Cortical thymocytes, T lymphocytes, granulosa cells of ovary, pancreatic islet cells, Sertoli cells, some endothelial cells, urothelium, ependymal cells, squamous cells	B and T cell precursor lymphoblastic lymphoma/leukemia PNET/Ewing's sarcoma, chondroblastoma, synovial sarcoma, solitary fibrous tumors, GIST, some alveolar rhabdomyocarcomas, desmoplastic small cell tumors, small cell carcinomas, granulosa cell tumors, yolk sac components of germ cell tumors, Sertoli-Leydig cell tumors, atypical fibroxanthoma, meningioma	Evaluation of lymphoblastic lymphoma/leukemia Thymic carcinomas (lymphocytes +) versus other carcinomas ID of PNET/Ewing's sarcoma (immunoreactivity should be clearly membranous in the majority of the cells)	O13 is the most commonly used antibody Immunoreactivity is highly dependent upon the antigen retrieval system used
CD103	Mucosal integrin alphaEbeta7 with specificity for e-cadherin *(Cytoplasm)*	T cells	Enteropathy type T-cell lymphoma, hairy cell leukemia		Requires frozen tissue or cell suspension
CD117 *(c-kit, stem cell factor receptor)*	Transmembrane tyrosine kinase receptor (ligand is stem cell factor) – apoptosis is inhibited when the ligand is bound *(Cytoplasm, membrane)*	Mast cells, interstitial cells of Cajal (ICC – pacemaker cells of the GI tract found throughout the muscle layers and in the myenteric plexus), epidermal melanocytes, mononuclear bone marrow cells (4%), Leydig cells, early spermatogenic cells, trophoblast, breast epithelium	GIST (>95%), seminomas (>70%), intratubular germ cell neoplasia, mature teratomas (>70%), some melanomas (focal), mast cell tumors, some carcinomas, some brain tumors, some PNET/Ewing's sarcoma, some angiosarcomas AML (>50%), CML in myeloid blast crisis	ID of GIST (+) vs. leiomyomas (–) and schwannomas (–) ID of seminomas ID of mast cells (mastocytosis)	Mast cells are an excellent internal control CD117 positivity does not correlate with mutations and/or oncoprotein activity in tumors not known to have activating mutations and is, in general, not of clinical or therapeutic significance in this setting (e.g., to detect tumors likely to respond to therapy directed against the protein (e.g., Gleevec)

CD123	Alpha chain of the IL-3 receptor *(Membrane)*	Myeloid precursors, macrophages, dendritic cells, mast cells, basophils, megakaryocytes	Plasmacytoid dendritic cell tumors		
CD138 *(Syndecan-1)*	Transmembrane heparin sulphate glycoprotein that interacts with extracellular matrix and growth factors *(Membrane)*	Pre-B cells, immature B cells, Ig-producing plasma cells, basolateral surface of epithelial cells, vascular smooth muscle, endothelium, neural cells	Plasma cell lesions, primary effusion lymphoma, plasma cell component of other B-cell lymphomas Squamous cell carcinomas, other carcinomas	ID of plasma cells and their neoplasms Expression may be diminished or lost in poorly differentiated carcinomas	
CD163 *(M130)*	Endocytic receptor to scavenge haptoglobin and hemoglobin complexes *(Membrane, cytoplasm)*	Tissue macrophages (high expression), monocytes (low expression) including Kupffer cells, Hofbauer cells but not follicular dendritic cells or plasmacytoid monocytes	Neoplasms of histiocytic differentiation Leukemias of monocytic differentiation Synovial type giant cell tumors of the vertebral column Langerhans cell histiocytosis (~60%), benign fibrous histiocytoma (~67%) Littoral cell angioma of the spleen	ID of true histiocytic derivation of tumors	More specific for monocyte/histiocyte derivation than CD68
CD207 *(langerin)*	Langerhans cell specific C-type lectin *(Cytoplasm)*	Langerhans cells of epidermis and epithelia	Langerhans cell histiocytosis		Induces formation of Birbeck granules
Clusterin *(Apolipoprotein J, complement lysis inhibitor, gp80, SGP-2, SP40, TRPM2, T64, ApoJ)*	Multifunctional protein involved in lipid transport, complement regulation, immune regulation, cell adhesion, other functions *(Membrane, cytoplasm, nucleus)*	Many tissues	Anaplastic large cell lymphoma (Golgi pattern) Alzheimer's disease – present in amyloid plaques and cerebrovascular deposits Many types of carcinomas		

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
Cyclin D1 *(PRAD1, bcl-1)*	Cyclin regulating cyclin dependent kinases during G1 in the cell cycle, phosphorylates and inactivates the retinoblastoma tumor suppressor protein *(Nucleus)*	Cycling cells (however, lymphocytes usually express only cyclins D2 and D3)	Mantle cell lymphoma Breast cancer (especially lobular carcinomas and other ER positive carcinomas), esophageal cancer, bladder cancer, lung cancer, HCC, colon carcinoma, pancreatic carcinoma, head and neck squamous cell carcinomas, pituitary tumors, sarcomas Parathyroid adenomas (inversion involving cyclin D1 gene and the parathormone receptor)	ID of mantle cell lymphoma	Involved in t(11;14)(q13;q32) translocation in mantle cell lymphoma
DBA.44 *(HCL)*	B-cell antigen *(Cytoplasm, membrane)*	Mantle zone B cells, some immunoblasts	Hairy cell leukemia (>95%), B-cell lymphomas (30%)	Evaluation of hairy cell leukemia	
Epithelial membrane antigen *(EMA, MUC1, HMFG, DF3, CA 15-3, CA 27.29, PEM, many others)*	Episialin, glycoprotein found in human milk fat globule membranes *(Cytoplasm [more common in malignant cells], membrane [more common in benign cells])*	Epithelial cells, perineurial cells, meningeal cells, plasma cells, usually negative in mesothelial cells, monocytes	Some anaplastic large cell lymphomas (CD30+), plasma cell neoplasms, malignant histiocytosis, erythroleukemia, AML (M4 and M5), LP HD Carcinomas, mesotheliomas, some sarcomas (synovial sarcoma, epithelioid sarcoma), adenomatoid tumor, chordomas, perineurioma, neurofibroma, meningiomas, desmoplastic small round cell tumor, Sertoli cell tumor	ID of epithelial differentiation in tumors – however, keratin is more specific for this purpose. Beware of EMA in some large cell lymphomas Synovial sarcoma typically shows focal positivity	There are over 50 monoclonal antibodies recognizing different glycosylation patterns in normal tissues and tumors[18]

Epstein-Barr virus					
EBV-encoded nonpolyadenylated early RNAs *(EBERS)*	RNA produced by EBV *(Nucleus)*	EBV-infected B cells	All EBV-related tumors	Most sensitive marker for EBV	Detected by in situ hybridization for RNA on paraffin sections
LMP-1	Latent membrane protein *(Membrane)*	EBV-infected B cells	Nasopharyngeal carcinomas, Reed-Sternberg cells (not LP HD), transplant lymphomas, AIDS-related lymphomas, endemic Burkitt lymphoma (rare in sporadic cases)	Evaluation of EBV-related neoplasms	
EBNA 2 *(nuclear antigen 2)*	Nuclear protein *(Nucleus)*	EBV-infected B cells	Transplant-related lymphomas, AIDS-related lymphomas. Not present in Burkitt lymphoma or nasopharyngeal carcinomas	Evaluation of transplant- and AIDS-related lymphomas	
Fascin	Actin bundling protein regulated by phosphorylation *(Cytoplasm)*	Interdigitating reticulin cells from the T-cell zones, dendritic cells, reticular network, histiocytes, smooth muscle, endothelium, squamous cells, splenic sinuses	Reed-Sternberg cells (but not in LP HD) High-grade breast carcinomas	ID of Reed-Sternberg cells in classical HD. Fascin positivity has also been reported in anaplastic large cell lymphoma	
FMC7	Antigen on subgroups of mature B cells, epitope of CD20 *(Cytoplasm)*	B cells	B-cell lymphomas	Not expressed by CLL	Pan B-cell marker Epitope of CD20 but reactivity low in cells with low cholesterol
Glycophorin A *(GPA)*	A glycosylated erythrocyte membrane protein *(Membrane)*	Erythroid elements at all stages	Erythroleukemia	ID of erythroid elements (normal and neoplastic)	
Granzyme B	Neutral serine proteases stored in granules in cytotoxic T cells and in NK cells involved in target cell apoptosis by exocytosis *(Cytoplasm)*	Cytotoxic T cells and NK cells	Some T-cell lymphomas, Reed-Sternberg cells of some cases of EBV-positive HD		

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
Heavy immunoglobulin chains *(G, A, M, D)*	Heavy chain of immunoglobulins *(Cytoplasm [plasma cells], membrane [lymphocytes])*	Plasma cells (G>A>M>D)	Plasma cell tumors (monotypic expression of usually G or A), mantle zone lymphomas and WDLL/CLL may coexpress M and D, lymphoplasmocytic lymphoma (M)	ID of monoclonal populations of plasma or plasmacytoid cells	
HECA-452 *(endothelial cell antigen, cutaneous lymphocyte-associated antigen, CLA)*	Cell surface glycoprotein *(Membrane)*	T cells, more common in cutaneous T cells	Mycosis fungoides and other cutaneous T-cell lymphomas		Note: CLA is also used to refer to CD45
Hemoglobin *(Hb)*	Hemoglobin *(Cytoplasm)*	Erythroid cells	Some leukemias	Marker for erythroid cells	
HHV8	Latent nuclear antigen of human herpes virus type 8 *(Nucleus)*	Absent in normal tissue	Primary effusion lymphoma (PEL), AIDS-associated multicentric Castleman's disease Kaposi's sarcoma (endothelial cells and some perivascular cells)	Evaluation of Kaposi sarcoma and primary effusion lymphoma	
HLA-DR	Major histocompatibility complex Class II gene *(Membrane)*	B lymphocytes, macrophages, Langerhans cells, dendritic cells, activated T cells, some endothelial and epithelial cells	Leukemic myeloblasts		Not very specific for cell type
Light immunoglobulin chains *(lambda [L], kappa [K])*	Light chain of immunoglobulins *(Cytoplasm)*	Plasma cells (normally K > L), B cells	Plasma cell tumors, B-cell lymphomas	ID of monoclonal populations of plasma cells and B cells ID of some types of amyloid	May require frozen tissue for assessment of B lymphoid cells Excellent Ig preservation in plasma cells in B5 or Zenker's fixed tissue

Lysozyme *(Ly)*	Muramidase *(Cytoplasm)*	Circulating monocytes, some tissue macrophages, granulocytes, salivary gland, lacrimal gland, stomach and colon epithelial cells (inflamed or regenerative), apocrine glands, some other epithelial cells	AML with monocytic differentiation, salivary gland tumors, stomach and colon carcinomas	Marker for histiocytes but not specific. May mark activated phagocytic macrophages Evaluation of myeloid leukemias Strongly positive in monocytoid leukemias	Not specific for solid tumor identification
Mast cell tryptase	Serine protease *(Cytoplasm)*	Mast cells	Mast cell neoplasms	ID of mast cell differentiation	
Myeloperoxidase *(MPO)*	Enzyme in primary granules of myeloid cells *(Cytoplasm)*	Myeloid cells, monocytes	AML, chloromas	Classification of leukemias	Can be used with tissue fixed in Zenker's fixative
Oct2 *(Octomer transcription factor)*	Transcription factor of the POU homeodomain family binding to the Ig gene octomer sites regulating B-specific genes *(Nucleus)*	B cells	B-cell lymphomas and leukemias Reed-Sternberg cells in LP HD (but not other types)	Evaluation of HD	Interacts with the transcriptional coactivator BOB.1. BOB.1 and Oct are necessary (but not sufficient) for Ig expression
Perforin	Pore-forming protein in cytoplasmic granules of cytotoxic T cells *(Cytoplasm)*	NK cells, large granular lymphocytes, gamma/delta T cells	NK-cell lymphomas, anaplastic large cell lymphoma	Evaluation of T-cell lymphomas	
TCR *(T-cell antigen receptor, JOVI 1)*	Two polypeptide chains (alpha and beta)	Peripheral T cells	Many T-cell lymphomas	Evaluation of T-cell lymphomas	Alpha/beta and gamma/delta T-cell receptors can be evaluated in frozen tissue

Continued

TABLE 7–43. ANTIBODIES FOR IMMUNOHISTOCHEMISTRY—cont'd

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Hematopathology Markers					
Terminal deoxytransferase *(TdT)*	Enzyme that catalyzes addition of nucleotides to ss DNA *(Nucleus)*	Immature T and B cells	Lymphoblastic lymphoma/ALL	Lymphoblastic lymphoma (+) vs. Burkitt lymphoma (-)	
TIA-1 *(T-cell intracellular antigen)*	A cytolytic granule-associated protein expressed in some CD8+ T cells *(Cytoplasm)*	T cells, mast cells, polymorphonuclear leukocytes, eosinophils	Many T-cell lymphomas	Evaluation of T-cell lymphomas	
traf-1 *(Tumor necrosis factor receptor-associated factor)*	Membrane-bound proteins that activate the nuclear factor-κB (NF-κB) transcription factor resulting in cell proliferation *(Cytoplasm)*	Usually absent	Hodgkin lymphoma, primary mediastinal large B-cell lymphoma	Negative in most DLBCL and ALCL	May interact with LMP1

Notes:
NAME: The most common name used to refer to the marker. The name may refer to the antigen, a CD number, or a specific antibody raised to the antigen. In some cases more than one name is commonly used. Underlined antibodies appear in the tables. Most CD numbers correspond to a specific gene product. However, some correspond to antigens from post-translational modifications. For example, CD15 (LeuM1) is a carbohydrate side chain linked to a protein.
ALTERNATE NAME: This list includes abbreviations, antibody names (sometimes recognizing different epitopes), or other terms for the marker.
ANTIGEN: The antigen recognized by the antibody.
LOCATION: The normal location of the antigen. In some cases, only certain locations of the antigen are considered a positive result (e.g., nuclear immunoreactivity for estrogen receptor, membrane immunoreactivity for HER2/neu).
NORMAL CELLS AND TISSUES: The presence of the marker in normal cells and tissues. These cells serve as important internal positive controls. Abnormal positive immunoreactivity is also an important control for the specificity of the study.
TUMORS: The tumors in which immunoreactivity is typically expected. Refer to the Tables for additional information.
USES: The most common uses for the marker. Different pathologists and institutions will often have preferences for the use of certain markers.
COMMENTS: Additional comments regarding the marker.
Additional information on CD antigens can be found at http://www.ncbi.nlm.nih.gov/prow/guide/45277084.htm.
Abbreviations: AD, Alzheimer's disease; AIDS, acquired immunodeficiency syndrome; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; APML, acute promyelogenous leukemia; BM, basement membrane; CML, chronic myelogenous leukemia; CMV, cytomegalovirus; DFSP, dermatofibrosarcoma protuberan; EBV, Epstein Barr virus; FISH, fluorescence in situ hybridization; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; HD, Hodgkin's disease; HNPCC, hereditary non-polyposis colorectal cancer; ID, identification; KS, Kaposi's sarcoma; LP HD, lymphocyte predominant Hodgkin's disease; MF, mycosis fungoides; MPNST, malignant peripheral nerve sheath tumor; NK, natural killer; PIN, prostatic intraepithelial neoplasia; PNET, primitive neuroectodermal tumor; RCC, renal cell carcinoma; RS, Reed Sternberg; TCC, transitional cell carcinoma.

TABLE 7–44. ALTERNATIVE NAMES FOR ANTIGENS

LOOKING FOR?	FIND IT UNDER:	LOOKING FOR?	FIND IT UNDER:
1D5	Estrogen receptor (G)	B4	CD19 (H)
6F/3D	Beta-amyloid	B72.3	B72.3 (G)
12E7	CD99 (G, H)	bcl-1	Cyclin D1 (H)
34βE12	Keratins (G)	bcl-2	bcl-2 (H, G)
38.13	CD77(H)	B-cell specific activator protein	BSAP (H)
70 kD NF	Neurofilaments (G)	BER-EP4	BER-EP4 (G)
200 kD NF	Neurofilaments (G)	BERH2	CD30 (G, H)
903	Keratins--34βE12 (G)	Beta-amyloid	Beta-amyloid (G)
A (blood group antigen)	Blood group antigens (G)	Beta-catenin	Beta-catenin (G)
A (Ig heavy chain alpha)	Heavy chain immuno-globulins (H)	Beta-2 microglobulin	Beta-2 microglobulin (G)
A32 antigen	CD146 (G)	BG8	BG8 (G)
A103	MELAN-A (G)	B-HCG	Human chorionic gonadotropin (G)
AAT	Alpha 1-antitrypsin (G, H)	BLA.36	CD77 (H)
ACH	Alpha-1 antichymotrypsin (H)	BL-CAM	CD22 (H)
AE1/AE3	Keratins (G)	Blood group antigens	Blood group antigens (G, H)
AFP	Alpha-fetoprotein (G)	BR-2	Gross cystic disease fluid protein-15 (G)
Alpha 1-antitrypsin	Alpha 1-antitrypsin (G, H)	BRST-2	Gross cystic disease fluid protein-15 (G)
Alpha 1-antichymotrypsin	Alpha 1-antichymotrypsin (H)	C3b/C4bR	CD35 (H)
Alpha 1-fetoprotein	Alpha fetoprotein (G)	C5b-9	C5b-9 (G)
Alpha fetoprotein	Alpha fetoprotein (G)	c-kit	CD117 (G)
Alpha-methylacyl-CoA racemase	AMACR (G)	CA 15-3	Epithelial membrane antigen (G, H)
Alpha smooth muscle actin	Alpha smooth muscle actin (G)	CA 19-9	CA 19-9 (G)
AMACR	AMACR (G)	CA 27.28	Epithelial membrane antigen (G, H)
Amyloid	Beta-amyloid (G)	CA 72-4	B72.3 (G)
Androgen receptor	Androgen receptor (G)	CA125	CA125 (G)
Apolipoprotein J	Clusterin (H)	CA19-9	CA19-9 (G)
AR	Androgen receptor (G)	Calcitonin	Calcitonin (G), Hormones (G)
B (blood group antigen)	Blood group antigens (G)	Caldesmon	Caldesmon (G)
B1	CD20 (H)	Calgranulin	MAC 387 (G)
B2	CD21 (H)		

Continued

TABLE 7–44. ALTERNATIVE NAMES FOR ANTIGENS—cont'd

LOOKING FOR?	FIND IT UNDER:	LOOKING FOR?	FIND IT UNDER:
CALLA	CD10 (G, H)	CD38	CD38 (H)
CALP	Calponin (G)	CD43	CD43 (H)
Calponin	Calponin (G)	CD44v3	CD44v3 (G)
Calprotectin	MAC 387 (G)	CD45	CD45 (H)
Calretinin	Calretinin (G)	CD45RA	CD45RA (H)
CAM5.2	Keratins (G)	CD45Ro	CD45Ro (H)
Carbohydrate antigen 19-9	CA19-9 (G)	CD56	CD56 (H)
Carcinoembryonic antigen	Carcinoembryonic antigen (G)	CD57	CD57 (G)
CD1a	CD1a (H)	CD61	CD61 (H)
CD2	CD2 (H)	CD68	CD68 (G, H)
CD3	CD3 (H)	CD74	CD74 (H)
CD4	CD4 (H)	CDw75	CDw75 (H)
CD5	CD5 (G, H)	CD77	CD77 (H)
CD7	CD7 (H)	CD79a	CD79a (H)
CD8	CD8 (H)	CD79b	CD79b (H)
CD10	CD10 (G, H)	CD95	CD95 (H)
CD11b	CD11b (H)	CD99	CD99 (G, H)
CD11c	CD11c (H)	CD117	CD117 (G)
CD13	CD13 (H)	CD141	CD141 (G)
CD15	CD15 (G, H)	CDX	CDX (G)
CD16	CD16 (H)	CDKN2	p16 (G)
CD19	CD19 (H)	CDP	Gross cystic disease fluid protein-15 (G)
CD20	CD20 (H)	CEA	Carcinoembryonic antigen (G)
CD21	CD21 (H)	c-erbB2	HER-2/neu (G)
CD22	CD22 (H)	Chromogranin A	Chromogranin A (G)
CD23	CD23 (H)	c-kit	CD117 (G)
CD25	CD25 (H)	CLA	CD45 (H) or HECA-452 (H)
CD30	CD30 (G, H)	CLDN1	Claudin (G)
CD31	CD31 (G)	Clusterin	Clusterin (H)
CD33	CD33 (H)	Collagen IV	Collagen IV (G)
CD34	CD34 (G, H)	Common acute leukemia antigen	CD10 (G, H)
CD35	CD35 (H)		

TABLE 7–44. ALTERNATIVE NAMES FOR ANTIGENS—cont'd

LOOKING FOR?	FIND IT UNDER:	LOOKING FOR?	FIND IT UNDER:
Complement lysis inhibitor	Clusterin (H)	Factor XIIIa	Factor XIIIa (G)
CR1	CD35 (H)	Fascin	Fascin (H)
Cyclin D1	Cyclin D1 (H)	Fast myosin	Myosin heavy chain (G)
Cystic fibrosis antigen	MAC 387 (G)	Fibronectin	Fibronectin (G)
D (Ig heavy chain delta)	Heavy chain immunoglobulins (H)	Fli-1	Fli-1 (G)
		FMC7	FMC7 (H)
DBA.44	DBA.44 (H)	FMC 29	CD99 (G, H)
Desmin	Desmin (G)	Friend leukemia integrin-site 1	Fli-1 (G)
DF3	Epithelial membrane antigen (G, H)	FVIII:g	Factor VIII (G)
DPB	CD45RA (H)	G (Ig heavy chain gamma)	Heavy chain immunoglobulins (H)
E2 antigen	CD99 (G, H)		
EBERS	Epstein-Barr virus (G, H)	Gal-3	Galectin-3 (G)
EBNA	Epstein-Barr virus (G, H)	Galectin-3	Galectin-3 (G)
E-cadherin	E-cadherin (G)	Gastrin	Hormones (G)
EGFR	EGFR (G)	GCDFP	Gross cystic disease fluid protein-15 (G)
EM ACT	HHF-35 (G)	GFAP	Glial fibriallary acidic protein (G)
EMA	Epithelial membrane antigen (G)		
E-MEL	HMB-45 (G)	Glial fibrillary acidic protein	Glial fibriallary acidic protein (G)
Endothelial cell antigen	HECA-452 (H)	Glucagon	Hormones (G)
Ep-CAM	BER-EP4 (G)	Glucose transporter 1	GLUT-1 (G)
Epidermal growth factor receptor	EGFR (G)	GLUT-1	GLUT-1 (G)
		GPIIIa	CD61 (H)
Epithelial membrane antigen	Epithelial membrane antigen (G, H)	gp80	Clusterin (H)
Epithelial specific antigen	BER-EP4 (G)	gp200	RCC (G)
Epstein-Barr virus	Epstein-Barr virus (G, H)	GPA	Glycophorin A (H)
ER	Estrogen receptor (G)	Granzyme B	Granzyme B (H)
erbB2	HER-2/neu (G)	Gross cystic disease fluid disease-15	Gross cystic disease fluid protein-15 (G)
ESA	BER-EP4 (G)		
Estrogen receptor	Estrogen receptor (G)	H (blood group antigen)	Blood group antigens (G)
Ewing's sarcoma marker	CD99 (G, H)	H222	Estrogen receptor (G)
Factor VIII related antigen	Factor VIII (G)	Hb	Hemoglobin (H)
FVIII:RAg	Factor VIII (G)	HBME-1	HBME-1 (G)

Continued

TABLE 7–44. ALTERNATIVE NAMES FOR ANTIGENS—cont'd

LOOKING FOR?	FIND IT UNDER:	LOOKING FOR?	FIND IT UNDER:
h-caldesmon	Caldesmon (G)	IL-2 receptor	CD25 (H)
H-CAM	CD44v3 (G)	Inhibin-alpha subunit	Inhibin-alpha subunit (G)
HCG	Human chorionic gonadotropin (G)	Insulin	Hormones (G)
HCL	DBA.44 (H)	J5	CD10 (G, H)
HBME-1	HBME-1 (G)	JOVI 1	TCR (H)
Heavy chain immuno-globulins	Heavy chain immunoglobulins (H)	K (Ig lighit chain kappa)	Light chain immunoglobulins (H)
HECA-452	HECA-452 (H)	Keratin 5/6	Keratins (G)
Hematopoietic progenitor cell, class 1	CD34	Keratin 7	Keratins (G)
Hemoglobin	Hemoglobin (H)	Keratin 20	Keratins (G)
HepPar-1	HepPar-1 (G)	Keratins	Keratins (G)
Hepatocyte paraffin-1	HepPar-1 (G)	Ki-1	CD30 (G, H)
HER-2/neu	HER-2/neu (G)	Ki-67	Ki-67 (G)
HHF-35	HHF-35 (G)	kip2	p57 (G)
HHV8	HHV8 (H)	Kit	CD117 (G)
HLA-DR	HLA-DR (H)	KP-1	CD68 (G, H)
HMB-45	HMB-45 (G)	L (Ig light chain lambda)	Light chain immunoglobulins (H)
HMFG	Epithelial membrane antigen (G, H)	L1 antigen	MAC 387 (G)
hMLH1	hMLH1 (G)	L26	CD20 (H)
hMSH2	hMLH1 (G)	L60	CD43 (H)
HNK-1	CD57 (G)	Laminin	Laminin (G)
HP1	HepPar-1 (G)	LCA	CD45 (H)
HPCA-1	CD34 (G, H)	Leu 1	CD5 (H)
HPL	Human placental lactogen (G)	Leu 2	CD8 (H)
HuLy-m6	CD99 (G, H)	Leu 3	CD4 (H)
Human chorionic gonadotropin	Human chorionic gonadotropin (G)	Leu 5a + b	CD2 (H)
Human herpes virus 8	HHV8 (G, H)	Leu 7	CD57 (G, H)
Human mutL homologue 1	hMLH1 (G)	Leu 9	CD7 (H)
Human mutS homologue 2	hMLH1 (G)	Leu16	CD20 (H)
Human placental lactogen	Human placental lactogen (G)	Leu 22	CD43 (H)
		Leukocyte common antigen	CD45 (H)
		Leu-M1	CD15 (G, H)

TABLE 7–44. ALTERNATIVE NAMES FOR ANTIGENS—cont'd

LOOKING FOR?	FIND IT UNDER:	LOOKING FOR?	FIND IT UNDER:
Light chain immunoglobulins	Light chain immunoglobulins (H)	MSH2 or MSH6	hMLH1
LFA-2	CD2 (H)	MTS1	p16 (G)
LMP-1	Epstein-Barr virus (G, H)	MUC1	Epithelial membrane antigen (G, H)
LN1	CDw75 (H)	MUC18	CD146 (G)
LN2	CD74 (H)	Muscle common actin	HHF-35 (G)
Lysozyme	Lysozyme (H, G)	Muscle specific actin	HHF-35 (G)
M (Ig heavy chain mu)	Heavy chain immunoglobulins (H)	My 7	CD13 (H)
Mac-1	CD11b (H)	My 9	CD33 (H)
MAC 387	MAC 387 (G)	Myeloperoxidase	Myeloperoxidase (H)
Mac-M	CD68 (G, H)	Myf-4	Myf-4 (G)
MART 1	MELAN A (G)	MyoD1	MyoD1 (G)
Mast cell tryptase	Mast cell tryptase (H)	Myogenin	Myf-4 (G)
mb-1	CD79a (H)	Myoglobin	Myoglobin (G)
MCAM	CD146 (G)	Myosin heavy chain	Myosin heavy chain (G)
ME491	CD63 (G)	NCAM	CD56 (H)
MELAN-A	MELAN-A (G)	Neprilysin	CD10 (G, H)
Melanoma antigen recognized by T cells	MELAN-A (G)	NEU N	NEU N (G)
Melanoma-associated antigen	CD63 (G)	Neurofilaments	Neurofilaments (G)
Melanoma cell adhesion molecule	CD146 (G)	Neuron specific enolase	Neuron specific enolase (G)
Melanoma-specific antigen	HMB-45 (G)	NFP	Neurofilaments (G)
MELCAM (or Mel-CAM)	CD146 (G)	NKI-betab	HMB-45 (G)
MIB-1	Ki-67 (G)	NKI/C3	CD63 (G)
MIC-2	CD99 (G, H)	NSE	Neuron specific enolase (G)
MLH1	hMLH1	O13	CD99 (G, H)
MN-4	CD146 (G)	OC125	CA125 (G)
MNF-116	Keratin--Pan-K (G)	Oct2	Oct2 (H)
MPO	Myeloperoxidase (H)	Octomer transcription factor	Oct2 (H)
MRF4	Myf-4 (G)	p16	p16 (G)
MSA	HHF-35 (G)	$p27^{kip1}$	$p27^{kip1}$ (G)
		p53	p53 (G)
		p57	p57 (G)

Continued

TABLE 7–44. ALTERNATIVE NAMES FOR ANTIGENS—cont'd

LOOKING FOR?	FIND IT UNDER:	LOOKING FOR?	FIND IT UNDER:
p63	p63 (G)	S-Endo-1	CD146 (G)
P504S	AMACR (G)	SGP-2	Clusterin (H)
PAN-K	Keratins (G)	SMA	Alpha smooth muscle actin (G)
PAP	Prostate acid phosphatase (G)	SM-ACT	Alpha smooth muscle actin (G)
PECAM-1	CD31 (G)	Smad4	DPC4 (G)
PEM	Epithelial membrane antigen (G, H)	SM-MHC	Myosin heavy chain (G)
Perforin	Perforin (H)	Somatostatin	Hormones (G)
PGM1	CD68 (G, H)	SP40	Clusterin (H)
PgR	Progesterone receptor (G)	Stem cell factor receptor	CD117 (G)
PK antigen	CD77 (H)	Synaptophysin	Synaptophysin (G)
Placental alkaline phosphatase	Placental alkaline phosphatase (G)	Syndecan-1	CD138 (H)
PLAP	Placental alkaline phosphatase (G)	Synuclein-1	Synuclein-1 (G)
Platelet glycoprotein IIIa	CD61 (H)	T3	CD3 (H)
PMS2	hMLH1	T4	CD4 (H)
Podoplanin	D2-40	T6	CD1a (H)
PR	Progesterone receptor (G)	T8	CD8 (H)
PRAD1	Cyclin D1 (H)	T11	CD2 (H)
PrAP	Prostate acid phosphatase (G)	T64	Clusterin (H)
Prealbumin	Prealbumin (G)	TAG-72	B72.3 (G)
Progesterone receptor	Progesterone receptor (G)	Tau	Tau (G)
Prostate acid phosphatase	Prostate acid phosphatase (G)	T cell antigen receptor	TCR (H)
Prostate specific antigen	Prostate specific antigen (G)	T cell intracellular antigen	TIA-1 (H)
PSA	Prostate specific antigen (G)	TCR	TCR (H)
QBEnd10	CD34 (G, H)	TdT	Terminal deoxytransferase (H)
Renal cell carcinoma marker	RCC (G)	TE	CD2 (H)
ret	ret (G)	Terminal deoxytransferase	Terminal deoxytransferase (H)
RCC	RCC (G)	TH	CD4 (H)
rT3	CD2 (H)	Thrombomodulin	CD141 (G)
S-100	S-100 (G)	Thyroglobulin	Thyroglobulin (G)

TABLE 7–44. ALTERNATIVE NAMES FOR ANTIGENS—cont'd

LOOKING FOR?	FIND IT UNDER:	LOOKING FOR?	FIND IT UNDER:
Thyroid transcription factor 1	TTF-1 (G)	Tumor necrosis factor receptor–associated factor	traf-1 (H)
TIA-1	TIA-1 (H)	UCHL-1	CD45Ro (H)
TM	CD141 (G)	UEA 1	Ulex (G)
traf-1	traf-1 (H)	Ulex	Ulex (G)
Transthyretin	Prealbumin (G)	Vimentin	Vimentin (G)
TRPM2	Clusterin (H)	von Willebrand's factor	Factor VIII (G)
TTF-1	TTF-1 (G)	VWF	Factor VIII (G)
TTR	Prealbumin (G)	Wilms' tumor 1 protein	WT1 (G)
Tumor-associated glycoprotein 72	B72.3 (G)	WT1	WT1 (G)

G, general markers; H, hematopathology markers.

ELECTRON MICROSCOPY

Indications for EM Studies.

- Diagnostic renal biopsies for glomerular disease
- Adenocarcinoma versus mesothelioma (see Table 7-36)
- Difficult to classify tumors (Tables 7-45 and 7-46)
- Nerve (e.g., toxic or drug-induced neuropathy) and muscle biopsies (e.g., inclusion body or nemaline myopathy)
- Bullous skin diseases (e.g., epidermolysis bullosa)
- Ciliary dysmorphology (primary ciliary dyskinesia or Kartagener syndrome)
- Endomyocardial biopsies (e.g., adriamycin toxicity, amyloid, nemaline myopathy)
- Liver biopsies for microvesicular fat in acute fatty liver of pregnancy
- Small bowel biopsies to look for pathogens (e.g., Whipple disease)
- Congenital, inherited, and metabolic diseases (e.g., ceroid lipofuscinoses)
- Prion diseases

Method. Ultrastructural details of tissues are lost rapidly. Therefore, fresh tissue must be fixed rapidly and well for EM. Tissues are usually fixed in special fixatives for EM to preserve lipids and glycogen (e.g., 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4).

1. Place a small fragment of tissue in a drop of fixative on a cutting surface.
2. Cut the tissue into multiple tiny fragments, each no greater than 0.1 cm in any dimension.
3. Place the tissue into the vial of fixative. Shake the vial to make sure all the tissue fragments are covered by fixative.

Note: If tissue from a small biopsy is found to be nondiagnostic on H&E, any tissue saved for EM should be retrieved for examination by light microscopy.

Results. A separate electron microscopy report is usually issued. The results should be incorporated into the final diagnosis.[19]

TABLE 7–45. ELECTRON MICROSCOPIC FEATURES OF POORLY DIFFERENTIATED TUMORS

TUMOR	ULTRASTRUCTURE	ADDITIONAL TESTS	COMMENTS
Carcinoma	Well-developed desmosomes (pentalayered with a dense central line in the intracellular space) with intermediate filament attachment. Tonofilaments and bundles of filaments (keratin). Adenocarcinomas: • Intercellular lumina (but also present in vascular tumors) • Microvilli • Intracellular lumina (mucin vacuoles in signet ring cells) Squamous cell carcinomas • Numerous intermediate filaments (keratin) and desmosomes	IHC: Cytokeratins are present in almost all carcinomas if broad spectrum antibodies are used. EMA is present in almost all carcinomas, but is less specific and sensitive. Additional markers can be used to identify specific carcinomas.	Other tumors can also be keratin positive and have desmosomes, filaments, and cytokeratin (mesothelioma, meningioma, synovial sarcoma, and epithelioid sarcoma)
Melanoma	Melanosomes in various stages of development – indicative of a melanin-forming cell type. Abnormal pleomorphic melanosomes may be present in melanomas. Desmoplastic melanomas lack melanosomes.	IHC: S100, HMB45, MART-1 HMB45 and MART-1 may be absent in non-epithelioid melanomas. The HMB-45 epitope (gp100) is present in immature melanosomes or premelanosomes, but is not specific to these structures.	Melanosomes are also seen in clear cell sarcoma, pigmented schwannomas, PEComa, and other rare tumors. Mature forms can be taken up by melanophages, keratinocytes, and carcinomas.
Lymphoma	No specific features are present. The cells lack cellular junctions and there is a paucity of cytoplasmic organelles.	IHC: LCA	LCA may be absent in 30% of anaplastic (ALK1) lymphomas. These tumors can be EMA (+) but are keratin (-).
Sarcoma	Some types have specific diagnostic features of cell type (e.g., neural, smooth muscle, striated muscle). No well-developed desmosomes.	IHC: May be helpful for identifying specific types.	Keratin negative except for synovial sarcoma and epithelioid sarcoma (or rarely in other types).

TABLE 7–46. CELLS, TUMORS, AND STRUCTURES WITH CHARACTERISTIC FINDINGS BY ELECTRON MICROSCOPY

TUMOR	EM FINDINGS	CORRELATIONS AND OTHER DIAGNOSTIC TESTS
Alveolar soft part sarcoma	Rhomboid, rod-shaped, or spiculated crystals in a regular latttice pattern.	The characteristic cytoplasmic crystals are composed of monocarboxylate transporter 1 (MCT1) and its chaperone CD147. These proteins are found in many other cell types and are not specific for this tumor. Cytogenetics: t(X;17) creates a ASPL-TFE3 fusion protein. IHC: TFE3 positive (as well as rare pediatric renal tumors with the same translocation). Nuclear immunoreactivity is not present in other tumors or normal tissues. Histo: The crystals are PAS with diastase positive.

TABLE 7–46. CELLS, TUMORS, AND STRUCTURES WITH CHARACTERISTIC FINDINGS BY ELECTRON MICROSCOPY—cont'd

TUMOR	EM FINDINGS	CORRELATIONS AND OTHER DIAGNOSTIC TESTS
Amyloid	Non-branching fibrils, 7.5 to 10 nm in width and up to 1 micron in length.	May be present associated with plasma cell tumors, medullary carcinoma of the thyroid, Alzheimer's disease, or as an isolated finding (primary amyloidosis). IHC: Can be used to identify specific types of amyloid (e.g., lambda or kappa chains, beta2 microglobulin, calcitonin, tau)
Bronchioloalveolar carcinoma of the lung (BAL)	Lamellar (surfactant) "myelin-like" granules in the supranuclear cytoplasm (typical of Type II pneumocytes). Clara-like electron-dense granules in supranuclear cytoplasm. Intranuclear inclusions comprised of parallel microtubular arrays. These features can also be seen in metastatic BAL.	Cytogenetics: These carcinomas are less likely to be associated with smoking and have fewer cytogenetic changes. Bronchioloalveolar carcinomas or adenocarcinomas with features of BAL are more likely to respond to Iressa (38%) as compared to other lung carcinomas (14%) due to specific mutations in EGFR. Mucinous BAL has intranuclear inclusions but generally lacks the other EM features.
Chordoma	Desmosomes, large vacuoles, glycogen, dilated ER, cytoplasmic invaginations, and intermediate filaments The physaliphorous (= having bubbles or vacuoles) appearance is due to dilated ER, glycogen, and cytoplasmic invaginations.	IHC: Keratin (corresponds to intermediate filaments), EMA, S100
Clear cell sarcoma	Melanosomes in various stages of development. Glycogen (resulting in clear cytoplasm).	Cytogenetics: t(12;22) EWS;ATF1 fusion protein IHC: S100, HMB45
Dense core granules	Dense core granules (vesicle bound by a single membrane with a dense center – 60 to 300 nm) – cytoplasmic organelles involved in regulated exocytosis of cell products. Examples: Pancreatic beta cells (insulin): angular crystalline inclusions Pheochromocytoma (epinephrine and norepinephrine): Large, pleomorphic, often clear or only partially filled Carcinoid: • Foregut – small, round • Midgut – larger, pleomorphic • Hindgut – mixed	Found in tumors of neuronal or neuroendocrine origin. Vesicles are comprised of granins (predominantly chromogranin A, chromogranin B, and secretogranin II) and various peptide hormones and transmitters, ATP, and biogenic amines IHC: Chromogranin A (most specific). Specific products of tumors can also be detected. Note: Prostate cancers and breast cancers can also show strong chromogranin positivity and can be mistaken for neuroendocrine tumors, particularly at metastatic sites.
Desmoplastic small round cell tumor	Numerous desmosomes and tight junctions, numerous cell processes, large number of organelles (mitochondria and RER), microfilaments, small neurosecretory granules	Cytogenetics: t(11;22) EWS;WT1 fusion protein IHC: Keratin, desmin, WT-1, actin, EMA, NSE
Endothelial cells	Weibel-Palade bodies (cigar-shaped membrane-bound structures filled with tubules in parallel arrays). Intracytoplasmic lumina may be present in normal cells and in epithelioid vascular neoplasms.	Weibel-Palade bodies are frequently absent in tumors arising from endothelial cells (e.g., angiosarcomas). IHC markers are more sensitive to detect endothelial derivation. The membranes are formed by P-selectin and the tubules contain FVIII. IHC: Vascular markers (CD34, CD31, FVIII)
Ewing sarcoma (PNET)	Homogeneous cell population characterized by the lack of specialized features, large pools of glycogen, no organelles, no extracellular matrix, variable numbers of neurosecretory granules and cell processes.	Cytogenetics: t(11;22) EWS;FLI1 fusion protein (and other less common variants) IHC: CD99. FLI1 is also present, but is less specific. Histo: PAS +/– diastase can detect glycogen, but is not currently used for diagnosis.

Continued

TABLE 7–46. CELLS, TUMORS, AND STRUCTURES WITH CHARACTERISTIC FINDINGS BY ELECTRON MICROSCOPY—cont'd

TUMOR	EM FINDINGS	CORRELATIONS AND OTHER DIAGNOSTIC TESTS
Granular cell tumor	Numerous lysosomes (filled with tubular, vesicular, and amorphous material), phagosomes, and granules (correlating with the "granular" cytoplasm), reduplicated basal lamina surrounding groups of cells.	IHC: S100, inhibin, CD68, calretinin
Langerhans cell histiocytosis	Birbeck granules (rod-or tennis racket-shaped) structures of variable length with a central periodically striated lamella.	May serve as a reservoir for Langerin (a transmembrane type II Ca2+-dependent lectin) and CD1a in the endosomal recycling compartment. IHC: CD1a, Langerin, S100
Mast cells	Lameller or scroll-like membrane pattern, granules of variable size.	IHC: CD117 (c-kit), mast cell tryptase
Medullary carcinoma of the thyroid	Numerous neurosecretory granules (calcitonin) associated with stromal amyloid (calcitonin).	Cytogenetics: Mutations in the RET gene (sporadic and germline) IHC: Calcitonin (in tumor cells and amyloid), chromogranin
Merkel cell carcinoma	Neurosecretory granules in processes or along cell membranes (subplasmalemmal).	IHC: Chromogranin, NSE, cytokeratin 20
Mesothelioma	Elongated, serpiginous, and branched microvilli (generally 10 to 16 length: 1 width) apical without a glycocalyx or actin rootlets.	Cytogenetics: Characteristic chromosome deletions and loss of 9 and 22 IHC: Calretinin, WT-1
Neuroblastoma	Cellular processes with microtubules (neuropil), dense core granules, Homer-Wright rosettes (the center is comprised of a tangle of cell processes), synaptic vesicles, no glycogen	Cytogenetics: Changes are linked to prognosis IHC: Chromogranin, NSE, NFP, synaptophysin
Oncocytoma	Numerous mitochondria packed in the cytoplasm (correlating with the granular appearance of the cytoplasm). In contrast, chromophobe renal cell carcinoma has fewer mitochondria and more microvesicles.	Cytogenetics: Monosomy with loss of X or Y, 11q13. Chromophobe carcinomas have different cytogenetic changes. IHC: RCC is negative in oncocytomas but positive in 45% to 50% of chromophobe renal cell carcinomas.
Perineurioma	Long cell processes wrapping around adjacent cells	IHC: Claudin-1 (a component of tight junctions), EMA
Rhabdoid tumor of the kidney	Large paranuclear whorls of intermediate filaments (corresponding to cytokeratin and vimentin) and occasional tonofilaments	Cytogenetics: hSNF5/INI1 deletions and mutations on chromosome 22 IHC: Cytokeratin (+), vimentin (+), absence of INI1 nuclear protein
Rhabdomyosarcoma	Parallel thick (12 to 15 cm) and thin (6 to 8 nm) myosin-actin filaments, Z-bands, filament ribosomal complexes. Spider cells may be seen in cardiac tumors (clear cytoplasm divided by cytoplasmic processes and cross striations formed by leptofibrils).	Cytogenetics: Characteristic changes in alveolar and embryonal types IHC: Muscle markers (HHF-35, desmin, myf4)
Schwannoma	Basal lamina prominent, often reduplicated. Luse bodies (long spacing collagen, extracellular), myelin figures, long cell processes wrapping around collagen, may rarely have melano-somes (melanotic schwannoma)	Cytogenetics: Deletion of 2q (NF2 inactivation) IHC: S100

See Tables 7-8, 7-9, 7-22, and 7-47 for additional information.

SNAP FROZEN TISSUE

Frozen tissue is useful for staining (some antibodies only detect antigens in frozen tissue), enzyme studies (muscle biopsies), and to save tissue for DNA or RNA studies.

Indications. All specimens with a question of a lymphoproliferative disorder, sarcomas, unusual tumors, muscle biopsies.

Methods. Small (approximately 0.5 × 0.5 × 0.3 cm^3) portions of tissue are placed in a clean specimen container moistened with a small amount of normal saline until they can be frozen. Specimens should be snap frozen using liquid nitrogen or dry ice and stored at –20°C.

Results. The results of studies on frozen tissue are usually incorporated into the surgical pathology report.

IMMUNOFLUORESCENCE

Like immunoperoxidase studies, immunofluorescence (IF) detects antigens in tissues. However, because amplification of the signal is not used, it is better suited for precise localization of antigen/antibody complexes in tissues or for determining the deposition pattern of immune complexes (e.g., linear vs. granular). Thus, it is most useful for the investigation of diseases related to immune complex deposition such as glomerular diseases and bullous diseases of the skin.

Tissue for IF may be snap frozen (see instructions earlier) or stored in special fixatives for IF. If the specimen is not frozen, special care must be taken to ensure that the biopsy is kept moist in a sealed container.

- **Direct IF:** Uses antibodies to detect antigens in the patient's **tissues**.
- **Indirect IF:** Uses control tissues to detect antibodies (e.g., anti-BM) in the patient's **serum**.

Indications. Some skin biopsies (e.g., lupus, pemphigus, pemphigoid, and dermatitis herpetiformis), all diagnostic nontransplant renal biopsies, some transplant renal biopsies, identification of amyloid in cardiac biopsies, and the evaluation of vasculitis in nerve biopsies.

Method. Tissue must be submitted fresh.

Results. The results of the examination are usually incorporated into the surgical pathology report.

Immunofluorescence of Skin Lesions

- **SLE (lupus band test)**: linear or granular staining along dermal epidermal junction for multiple immunoreactants in about 80% of cases (most commonly IgG and less often IgM or C3). The specificity increases with the number of positive immunoreactants. Uninvolved sun-exposed skin shows positivity in most patients with active systemic lupus. Uninvolved skin in patients with discoid lupus is usually negative for this test.
- **Herpes gestationis:** perilesional skin shows linear BM zone C3 and sometimes IgG.
- **Dermatitis herpetiformis:** granular IgA at tips of dermal papillae of uninvolved skin.
- **Pemphigus:** IgG and C3 between epidermal cells creating a net-like pattern. In pemphigus vulgaris, a split just above the basal cell layer creates a "tombstone" appearance to the row of basal cells at the base of the vesicle. In pemphigus foliaceus and related disorders, the split occurs near the granular cell layer.
- **Pemphigoid:** Ig and C3 along basement membrane but not between cells. Indirect IF reveals an anti-BM antibody.

MOLECULAR GENETIC PATHOLOGY

Molecular genetic pathology is the newest subspeciality in pathology with board certification. Molecular diagnostics incorporates many types of techniques for the investigation of genetic alterations in cells and viruses (e.g., Southern blotting, PCR analysis, FISH). It has applications in three main areas:

Inherited diseases:
- Identification of inherited diseases (e.g., cystic fibrosis, hemochromatosis, Factor V Leiden, Prothrombin 20210A, Fragile X syndrome).
- Identification of genes conferring susceptibility to diseases (e.g., microsatellite instability [MSI], BRCA1 and 2)

Infectious diseases:
- Detection of organisms
- Identification of specific organisms
- Quantitation of viral infection (e.g., HIV viral load)

Cancer:
- Identification of specific genetic alterations associated with tumors
- Identification of gene mutations associated with susceptibility to treatment (e.g., EGFR mutations in lung cancer, c-kit mutations in GIST)
- Identification of clonality in hematolymphoid proliferations
- Detection of minimal residual disease after treatment

These studies are especially helpful for hematolymphoid proliferations that are difficult to classify because of the frequent and characteristic rearrangements that occur in many of these disorders. Unlike cytogenetics, the cells need not be viable. However, it is preferable that the nucleic acids are relatively intact. Southern blot and RNA-based PCR (RT-PCR) assays are best performed on fresh or frozen tissues. Formalin-fixed,

paraffin-embedded tissue is amenable to DNA-based PCR assays. Some fixatives (e.g., Bouin's) cause extensive breakage of DNA and may preclude genetic analysis of the tissue.

Indications.

- B-cell proliferations – clonal rearrangements of the immunoglobulin heavy and light chain genes; specific translocations
- T-cell proliferations – rearrangements of the γ and β T-cell receptor genes
- Leukemias
- Post-transplant lymphoproliferative disorders – clonal populations of EBV-infected cells
- Oligodendrogliomas – PCR-based LOH analysis for 1p/19q deletions.
- Colon cancers possibly associated with hereditary non-polyposis colorectal carcinoma syndrome (HNPCC): microsatellite instability (MSI) testing of colon cancers occurring in patients 50 years of age or younger.
- Human papilloma virus testing: cervical PAP smears, squamous cell carcinomas of the head and neck (see subsequent section).
- GIST – most have mutations in the KIT tyrosine kinase gene. A smaller group (5% to 7%) have mutations in the KIT-homologous tyrosine kinase PDGFRA. About 10% to 15% of GISTs are negative for KIT and PDGFRA mutations (termed "wild-type GISTs"). The specific type of mutation is correlated with prognosis and the respone to specific types of treatment.
- Lung adenocarcinoma – some cancers have mutations in EGFR that predict response to the tyrosine kinase inhibitor gefitinib.

Method of Submitting Tissue. Fresh or frozen tissue (e.g., snap frozen tissue) as well as fluids may be used. Cytologic preparations can be used for FISH. Paraffin blocks can also be used.

Results. The results are usually either reported separately or incorporated into the surgical pathology report.

CYTOGENETICS

Cytogenetic studies have been demonstrated to be useful in several areas important to pathology:

- **Tumor classification:** Particularly sarcomas (e.g., Ewing's sarcoma and synovial sarcoma), lymphomas, leukemias, kidney tumors, brain tumors, and other unusual tumors.
- **Benign vs. malignant lesions:**
  - Reactive mesothelial cells vs. mesothelioma
  - Lipoma vs. liposarcoma
- **Prognosis:** Neuroblastoma, oligodendroglioma, multiple myeloma, chronic lymphocytic leukemia.
- **Treatment:** Amplification of HER2/neu to predict response to Herceptin.
- **Research:** Translocations are common to many tumors and usually identify genes important to the pathogenesis of the tumor

Cells may be cultured to perform complete karyotype analysis or tissues can be analyzed for specific chromosomal alterations by fluorescence in situ hybridization (FISH).

FISH studies can be performed on cultured cells, cytology specimens, touch preparations, and paraffin-embedded tissues.

Indications.

- **For karyotype analysis:** Soft tissue tumors, mesotheliomas (tissue or pleural fluid), unusual tumors, poorly differentiated tumors, all subcutaneous lipomas >10 cm or of unusual gross appearance, all deep-seated lipomas (subfascial, intramuscular, intraabdominal, retroperitoneal, clinically apparent cord tumors), unusual uterine masses.
- **For FISH:** Oligodendroglioma, neuroblastoma.

Method for Submitting Tissue. Tissue for karyotyping must be fresh, viable, and relatively sterile. However, tissue may be submitted even if it has not been handled under strictly sterile conditions (contamination is not usually a problem). If specimens are to be held overnight, the tissue should be minced (into 0.1 cm cubes) in a sterile specimen container, covered with culture medium, and held overnight in the refrigerator. Fluids may also be submitted for analysis (especially pleural effusions with a suspicion of mesothelioma).

Results. The results of the cytogenetic analysis should be incorporated into the final diagnosis or reported separately (Tables 7-47 and 7-48).

Text continues on page 178.

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Adenoid cystic carcinoma	6q translocations and deletions		>50%	
Adrenal cortical carcinomas		2p16 loss	>90%	This area is close to the region associated with Carney complex type 2.
		17p13 LOH	85%	These changes are less common in localized tumors (25% to 35%) but, if present, such tumors are more likely to metastasize. The 11p15 imprinted region is also involved in Beckwith-Wiedemann syndrome.
		11p15 LOH with duplication of the active paternal allele leading to IGF-II overexpression	85%	
Aggressive angiomyxoma	t (12q15)	HMGA2 involvement	>20%	
Alveolar soft part sarcoma	t(X;17)(p11.2;q25)	ASPL-TFE3 fusion	>90%	TFE3 can be detected by IHC. This translocation is also present in rare papillary-like renal tumors in young adults (see "Renal tumors").
Aneurysmal bone cyst	t(16;17)(q22;p13)	CDH11-USP6 fusion	20%	
	t(17p13.2)	USP6 fusions	>50%	
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	FUS-ATF1 fusion	Unknown	
	t(12;22)(q13;q12)	EWSR1-ATF1 fusion		EWSR1-ATF1 translocation also present in clear cell sarcoma
	t(2;22)(q33;q12)	CREB1-EWSR1 fusion		
Bizarre parosteal osteochondromatous proliferation (Nora's lesion)	t(1;17)(q32;q21)			A breakpoint in 1q32 was found in 100% of lesions. A breakpoint in 17q21 was found in 4 or 5 cases.
	t(1;17)(q42;q23)			Only one case with different breakpoints
Breast carcinoma		HER2/neu amplification	15-20%	Detected by FISH (gene amplification) or IHC (protein overexpression). Positive carcinomas are more likely to respond to Herceptin.[a]
		BRCA1 and BRCA2 germline mutations	<5%	Patients are more likely to be young and have multiple carcinomas. BRCA1 carcinomas are frequently high grade, have "medullary" features, and lack ER, PR, HER2. BRCA2 carcinomas have no specific pathologic features.
	t(12;15)	ETV6-NTRK3 fusion	100% (secretory carcinoma)	This translocation is found in secretory carcinomas. The same translocation is found in infantile fibrosarcoma and cellular mesoblastic nephroma.

Continued

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Carcinoma of the upper aerodigestive tract in children	t(15;19)(q13;p13.2)	BRD4-NUT fusion		Patients with this translocation have a poor prognosis.
Chondromyxoid fibroma	Deletion of 6q		>75%	
Clear cell hidradenoma	t(11;19)(q21;p13)	MECT1-MAML 2 fusion		Same translocations as mucoepidermoid carcinoma and Warthin tumor
Clear cell sarcoma	t(12;22)(q13;q12)	EWSR1-ATF1 fusion	>75%	
	t(2;22)(q33;q12)	EWSR1-CREB1 fusion	Unknown	
Colon carcinoma		hMLH1 and hMSH2 mutations	15% of sporadic carcinomas	95% of HNPCC patients have germline mutations in these genes. Absence can be detected by IHC or by PCR assays for microsatellite instability. Mutations are correlated with characteristic clinical, pathologic, and treatment response features.
		EGFR (HER1) overexpression	82% of all carcinomas	Approximately 23% of patients treated with cetuximab[b] and chemotherapy respond.
		APC mutations	80% of all carcinomas	Also present as a germline mutation in familial adenomatous polyposis syndrome.
		LKB1/STK11 LOH	~ 15%	Germline mutations occur in some cases of Peutz-Jeghers syndrome. Mutations appear to be rare in sporadic colon carcinoma but LOH is observed in some.
		DPC4 (Smad4 or MADH4) mutations (18q21.1)	10–20%	Germline mutations in occur in some cases of juvenile polyposis syndrome. Mutations in sporadic carcinomas are uncommon.
Desmoplastic fibroblastoma	t(2;11)(q31;q12)	Unknown		
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	EWSR1-WT1 fusion	>95%	WT1 can be detected by IHC.
Dermatofibrosarcoma protuberans/ giant cell fibroblastoma	+r(17;22)(q21;q13) t(17;22)(q21;q13),	COL1A1-PDGFB fusion COL1A1-PDGFB fusion	>75%	The same translocation is present in giant cell fibroblastoma, but without formation of a ring chromosome.

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Endometrial stromal sarcoma	t(7;17)(p15;q21)	JAZF1-JJAZ1 fusion	30%	
	t(6;7)(p21;p15)	JAZF1-PHF1 fusion	10%	
	t(6;10)(p21;p11)	EPC1-PHF1 fusion	Rare	
Epithelioid hemangioendothelioma	t(1;3)((p36.3;q25)			
Epithelioid sarcoma	t/del(22q11.2)	INI1 deletion, mutations		Absence can be detected by IHC.
Ewing sarcoma/ PNET	t(11;22)(q24;q12)	EWSR1-FLI1 fusion	>80%	FLI1 can be detected by IHC but is not specific for Ewing's. FISH can detect fusion genes. The type of fusion is correlated with prognosis (e.g., Type I exon 6 has >100 month survival, Type II exon 5 has 2 year survival).
	t(21;22)(q22;q12)	EWSR1-ERG fusion	5-10%	
	t(2;22)(q33;q12)	EWSR1-FEV fusion	<5%	
	t(7;22)(p22;q12)	EWSR1-ETV1 fusion	<5%	
	t(17;22)(q12;q12)	EWSR1-E1AF fusion	<5%	
	inv(22)(q12)(q12)	EWSR1-ZSG fusion	<5%	
	t(16;21)(p11;q22)	FUS-ERG fusion	Unknown	
	t(2;22)(q31;q12)	EWSR1-SP3 fusion		
	t(2;16)(q33;p11)	FUS-FEV fusion		
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	EWS-NR4A3 fusion	>75%	
	t(9;17)(q22;q11)	TAF2N-NR4A3 fusion	<10%	
	t(9;15)(q22;q21)	TCF12-NR4A3 fusion	<10%	
	t(3;9)(q11;q22)	TGF-NR4A3 fusion		
Fibromatosis (desmoid)	Trisomies of 8 and 20		30%	
	Deletion of 5q21	APC inactivation	10%	
		Beta-catenin mutations	50%	Nuclear beta-catenin can be detected by IHC.
Fibromyxoid sarcoma, low grade	t(7;16)(q32-34; p11.2)	FUS-CREB3L2 fusion	96%	
	t(11;16)(p11;p11)	FUS-CREB3L1 fusion	Rare	

Continued

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Fibrosarcoma, infantile	t(12;15)(p13;q25)	ETV6-NTRK3 fusion	>75%	The same translocation is seen in cellular mesoblastic nephroma and secretory breast carcinoma.
	Trisomies 8, 11, 17,20		>75%	
Gastrointestinal stromal tumor	Monosomies 14 and 22		>75%	
	Deletion of 1p		>25%	
		KIT or PDGFRA mutation	>90%	CD117 (KIT) is detected by IHC and is useful for diagnosis. Gleevec[c] is effective against tumors with activating mutations in either gene. The type of mutation correlates with treatment response.
Germ cell tumors	Isochromosome 12p		>80-90%	Includes all histologic subtypes in males and dysgerminomas of ovary
		KIT mutations	25-70%	Seminomas
Giant cell tumor	Telomeric changes		>50%	
Giant cell tumor, diffuse type (PVNS)	t(1;2)(p13;q37)	COL6A3-CSF1 fusion	>25%	
	Trisomies 5 and 7		Unknown	
Glioblastoma multiforme (anaplastic mixed glioma)		EGFR (HER1) amplification	40%	Detected by ISH. IHC is not helpful for detecting overexpression. The co-deletion of 1p36 and 19q13.3 is absent.
Hepatoblastoma	Trisomies 2q and 20		>75%	
Hibernoma	11q13 rearrangement		>50%	
Inflammatory myofibroblastic tumor	t(1;2)(q22;p23)	TPM3-ALK fusion	Unknown	ALK can be detected by IHC in one third of cases. There are other partners for ALK fusion.
	t(2;19)(p23;p13)	TPM4-ALK fusion		
	t(2;17)(;23;q13)	CLTC-ALK fusion		
	t(2;2)(p23;q13)	RANB2-ALK fusion		
Leiomyoma, uterine	t(12;14)(q15;q24) Deletion of 7q Deletion of 1p	HMGA2 rearrangement	40%	Uterine leiomyosarcomas have more complex karyotypes. These tumors are cellular and have gene expression patterns similar to leiomyosarcomas.

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Lipoblastoma	8q12 rearrangement	PLAG1 oncogene	>80%	
	Polysomy 8			
Lipoma				
Typical	t(12q15)	HMGA2 rearrangement	40%	
	t(6p21)	HMGA1 rearrangement	10%	
	t(8q12)	PLAG1	5%	
	Deletion 13q		5%	
Spindle cell or pleomorphic	Deletion of 13q or 16q		>75%	
	t(11;16)(q13;p12-13)		Unknown	
Liposarcoma				
Well-differentiated	Ring /giant markers (12q13-q15)	HMGA2, MDM2 amplification	>75%	Similar ring/giant markers are seen in dedifferentiated liposarcomas, with additional aberrations
Myxoid/round cell	t(12;16)(q13;p11) t(12;22)(q13;q12)	FUS-DDIT3(CHOP) fusion EWS-CHOP fusion	>75% <5%	
Pleomorphic	Complex		90%	
Dedifferentiated	Ring /giant markers (12q13-q15)	HMGA2, MDM2 amplification		
Lung adenocarcinomas that respond to gefitinib (most in women, nonsmokers, with features of BAC)	Fewer changes than seen in carcinomas associated with smoking	EGFR - small deletions or amino acid substitutions	10-20% of all lung carcinomas	Mutations predict response (gain of function) to the tyrosine kinase inhibitor gefitinib (Iressa).[d] 40% to 80% of lung carcinomas show EGFR overexpression by IHC, but only carcinomas with specific mutations respond to gefitinib.
Medulloblastoma	Isochromosome 17q		>25%	
Meningioma	Monosomy 22		90%	
	1p deletion		25%	

Continued

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Mesothelioma	Deletion of 1p	? BCL10 inactivation	>50%	Cytogenetic changes are less complex than those seen in carcinomas. Cytogenetic analysis of cytologic specimens (e.g., pleural fluid) can be of value if larger biopsies are not available.
	Deletion of 9p	p15, p16, and p19 inactivation	>75%	
	Deletion of 22q	NF2 inactivation	>50%	
	Deletions of 3p and 6q		>50%	
Mucoepidermoid carcinoma	t(11;19)(q21;p13)	MECT1-MAML 2 fusion	>50%	Same translocation as Warthin tumor and clear cell hidradenoma
Neuroblastoma	Hyperdiploid, no 1p deletion		40%	Good prognosis
	1p deletion		40%	Poor prognosis
	Double minute chromosomes	N-MYC amplification	>25%	Poor prognosis
Oligodendroglioma	Co-deletion of 1p36 and 19q13.3		50%	Useful for diagnosis and to predict response to radiation and/or chemotherapy. EGFR amplification is absent.
	9p21 deletion	CDKN2A (p16) deletion		Occurs in some anaplastic oligodendrogliomas. Poor prognostic factor.
Osteochondroma	Deletion of 8q	EXT1 inactivation	>25%	
Osteosarcoma				
Low grade	Ring chromosomes		>50%	
High grade	Complex	RB and P53 inactivation	>80%	
Pheochromocytoma				
Sporadic (70%)		Losses on 1p	>80%	
Hereditary (30%)		Germline mutations in RET, VHL, NF1, SDHB, SDHD, MEN2A, MEN2B	>90% of hereditary cases	Patients are more likely to be young (<50), have multiple tumors, and have a family history of pheochromocytoma, paraganglioma, or medullary carcinoma of the thyroid.
Pleomorphic adenoma (salivary)	t(3;8)(p21;q12)	CTNNB1-PLAG1 fusion	>50%	
	t(8q12)	PLAG1 fusions	<20%	
	t(9;12)(p12;q15)	NFB1-HMGA2 fusion		

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Prostate cancer	t(21;21)(q22.2;q22.3)	TMPRS2-ERG fusion	Unknown	
	t(7;21)(p21.2;q22.2)	TMPRSS2-ETV1 fusion	Unknown	
Renal Tumors				
Clear cell carcinoma	Deletion of 3p		90%	
		VHL	70%	Deletion or inactivation
Papillary carcinoma – adult	Trisomies 3, 7, 12, 16, 17, and 20		>90%	
		KIT mutations	>90%	CD117 (c-kit) present by IHC in cytoplasm and is associated with activating mutations.
Xp11.2 or TFE3 translocation carcinomas (young adults [<1% of all adults] and children [30% to 50% of pediatric cases], female > male, 10% to 15% have prior treatment)	t(X;17)(p11.2;q25.3)	ASPL/TFE3 fusion		Tumors have voluminous cytoplasm. The ASPL-TFE3 fusion is also present in alveolar soft part sarcoma. TFE3 and TFEB can be detected by IHC.
	t(X;1)(p11.2;q21)	PRCC/TFE3 fusion		
	t(X;1)(p11.2;p34)	TFE3-PSF fusion		
	inv(X)(p11.2q12)	TFE3/*NonO* fusion		
	t(6;11)(p21.1;q12)	TFEB-*Alpha* fusion		Tumors have a more solid, compact architecture, less voluminous cytoplasm, less frequent psammoma bodies and hyaline nodules, and less prominent nucleoli.
Oncocytoma	-1, -X or -Y		>25%	
	11q13 rearrangement		>25%	
Chromophobe carcinoma	Monosomies 1, 2, 3, 6, 10, 13, 17, and 21		>75%	CD117 (c-kit) is present by IHC on membranes, but activating mutations have not been detected.
Mesoblastic nephroma	t(12;15)(p13;q25)	ETV6-NTRK3 fusion		The same translocation is seen in infantile fibrosarcoma and secretory breast carcinoma.
Retinoblastoma	13q14 deletion	RB1 inactivation	>75%	40% of cases are due to germline mutations in RB1.
	Isochromosome 6p		25%	

Continued

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Rhabdoid tumor of the kidney and atypical teratoid/rhabdoid tumor (AT/RT)	Normal karyotype	hSNF5/INI1 (22q11.2) deletions and mutations	>90%	Infants and children with both tumors have a germline mutation in INI1 (rhabdoid predisposition syndrome). Choroid plexus carcinomas are also associated with non-function of this gene (70%).
	del/t(22)(q11.2)	hSNF5/INI1 deletions and mutations		
Rhabdomyosarcoma				
Alveolar	t(2;13)(q35;q14)	PAX3-FKHR fusion	>75%	Poor 4-yr survival if metastatic (8%)
	t(1;13)(p36;q14), double minutes	PAX7-FKHR fusion	10-20%	Better 4-yr survival if metastatic (75%)
	t(2:2)(q35;23)	PAX3-NCOA1 fusion	rare	
	t(X;2)(q35;q13)	PAX3-AFX fusion	rare	
Embryonal	Trisomies 2q, 8, and 20		>75%	
		LOH 11p15.5	>75%	
Schwannoma and perineurioma	Deletion of 22q	NF2 inactivation	>80%	5% of cases of vestibular schwannomas are associated with neurofibromatosis type 2 (germline NF2 mutations).
Subungual exostosis	t(X;6)(q13-14;q22)	COL4A5 and COL12A1 involvement		
Synovial sarcoma				
Monophasic	t(X;18)(p11;q11)	SYT with SSX1, SSX2, or SSX4 fusion	>90%	
Biphasic	t(X;18)(p11;q11)	SYT-SSX1 fusion	>90%	

TABLE 7–47. COMMON CYTOGENETIC AND GENETIC CHANGES IN SOLID TUMORS OF DIAGNOSTIC OR THERAPEUTIC SIGNIFICANCE—cont'd

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Thyroid carcinoma				
Papillary	10q11 rearrangement	RET/PTC rearrangements	20%	Most common in younger patients (avg 26) and radiation-associated carcinomas, all have psammoma bodies
		RAS mutations	15%	All follicular variant, rare LN mets, 6 women to 1 man, larger tumors
	1q21 rearrangement	NTRK1 fusion oncogenes	>10%	
		BRAF point mutation	44%	Older patients (avg 48), some anaplastic and poorly differentiated carcinomas (15% are tall cell), higher stage, more extrathyroidal invasion
Follicular	t(2;3)(q13;p25)	PAX8-PPARG fusion	>40%	45% have RAS mutations
Medullary				
Sporadic (75%)		RET activating mutations	>90%	
Hereditary (25%)		Germline RET, MEN2A, or MEN2B mutations	>90%	Indication for screening for pheochromocytoma and screening family members
Wilms tumor, pediatric	Deletion 11p13	WT1 inactivation	25%	Germline mutations occur in several syndromes. WT1 mutations also occur in sporadic tumors.
	Trisomy 12		40%	
		WTX		WTX is inactivated in approximately one-third of Wilms tumors

[a]Trastuzumab (Herceptin) = a monoclonal antibody directed against the HER2/neu receptor. Patients are selected for treatment by testing carcinomas with IHC or FISH.
[b]Cetuximab (C225, Erbitux) = a monoclonal antibody directed against the EGFR receptor. A test has been approved by the FDA for the determination of EGFR (DakoCyomation, EGFR PharmDX). This test is not used for lung carcinomas.
[ca]Imatinib mesylate (STI571, Gleevec, Glivec) is a small molecule tyrosine kinase inhibitor that may be used for the treatment of tumors overexpressing tyrosine kinases:
Bcr-Abl tyrosine kinase: CML, ALL (Ph+)
KIT tyrosine kinase: GIST, systemic mastocytosis, some types of AML
PDGFR kinase: CMML, chronic eosinophilic leukemia, rare cases of GIST
The KIT protein (CD117) is encoded by the c-kit proto-oncogene and is a transmembrane receptor protein with tyrosine kinase activity. Mutations may render KIT independent of its ligand, SCF (stem cell factor). Mutated proteins may or may not respond to therapy with Imatinib. Wild-type KIT and KIT with mutations in the juxtamembrane domain (the intracellular segment between the transmembrane and tyrosine kinase domains) are found in GISTs and are sensitive to imatinib. Other tumor types are associated with mutations in the enzymatic domain and the altered protein is generally not sensitive to imatinib. Overexpression of the protein is detected by IHC.
[d]Gefitinib (Iressa) = a tyrosine kinase inhibitor effective against a small subset of lung adenocarcinomas with specific activating mutations in EGFR. IHC for EGFR is not helpful for identifying carcinomas likely to respond to treatment.
For additional information on specific genes, see Online Mendelian Inheritance in Man (OMIM; www.ncbi.nlm.nih.gov).

TABLE 7–48. COMMON CYTOGENETIC CHANGES IN LYMPHOMAS AND LEUKEMIAS

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
Chronic Leukemias and Mastocytosis				
CML (Ph[1])	t(9;22)(q34;q11.2)	BCR-ABL fusion (usually p210, but also p190 and p230 fusion proteins)	90-95%	Philadelphia chromosome. Also present in 5% of children and 15-30% of adults with ALL and 2% of patients with AML. Treated with the ABL tyrosine kinase inhibitor imatinib (Gleevec)[a]. Mutations in BCR-ABL are associated with resistance. RT-PCR is used to detect minimal residual disease.
	Other variants or cryptic translocations	BCR-ABL fusion (usually p210, but also p190 and p230 fusion proteins)	5-10%	
CML, accelerated phase or blast phase	Additional changes: extra Ph, +8, or i(17)(q10)		80%	May be myeloid (70%) or lymphoid (30%).
Juvenile myelomonocytic leukemia		PTPN11 mutation	35%	11% of patients have neurofibromatosis type 1.
		NF1 mutation	20%	
		NRAS and KRAS2 mutations	20%	
Chronic eosinophilic leukemia	t(5;12)(q33;p13)	ETV6 (also called TEL) - PDGFRB fusion	Rare	With eosinophilia. Excellent response to imatinib.[a]
	t(5q33)	Several PDGFRB fusions	?rare	Excellent response to imatinib.[a]
	Cryptic del(4)(q12) – interstitial 800 kb deletion	FIP1L1-PDGFRA fusion	~ 50%	The fusion protein is an activated tyrosine kinase. Excellent response with the tyrosine kinase inhibitor imatinib.[a]
		FIP1L1-PDGFRA mutation (T6741)		Detected by FISH. Homologous to the resistance-inducing T3151 mutation in BCR-ABL.
	t(4q12)	Several PDGFRA fusions	?rare	
Stem cell leukemia-lymphoma syndrome	t(8;13)(p11;q11-12)	FGFR1-ZNF198 fusion	Unknown	Features of both lymphoma and eosinophilic MPD.
	t(8p11)	Several FGFR1 fusions	Rare	
Classic MPD				
Polycythemia		JAK2V617F	95%	
		JAK2 exon 12 mutation	5%	
Essential thrombocythemia		JAK2V617F	50%	
		MPLW515L/K	1%	
Primary myelofibrosis		JAK2V617F	50%	
		MPLW515L/K	5%	

TABLE 7–48. COMMON CYTOGENETIC CHANGES IN LYMPHOMAS AND LEUKEMIAS—cont'd

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
Chronic Leukemias and Mastocytosis				
Unclassified MPD	t(9;21)(p24;p13)	ETV6-JAK2		
	t(9;22)(p24;q11.2)	BCR-JAK2 fusion		
	t(8;9)(p22;p24)	PCM1-JAK2 fusion		
Systemic mastocytosis		c-KIT point mutations (Asp816Val)	100%	CD117 (c-kit) is detected by IHC in normal and abnormal mast cells. The most common mutations do not result in proteins sensitive to imatinib.
	Cryptic del(4)(q12) – interstitial 800 kb deletion	FIP1L1-PDGFRA fusion	~60% of patients with eosinophilia	Found in mastocytosis with associated eosinophilia. These patients do not have the typical c-KIT mutation. Excellent response to treatment with imatinib [a].
Acute Myeloid Leukemia				
AML	Normal karyotype		40-50%	
		FLT3 (13q12) internal tandem duplications (ITD, 20%) or point mutations (7%)	20 -30%	More common in monocytic AML (M5), less common in myeloblastic leukemia with maturation (M2) or erythroleukemia (M6). Less common in AML with cytogenetic changes (10%). Poor prognostic factor. Results in an activated tyrosine kinase. Current trials are evaluating response to a kinase inhibitor – PKC412.
		Partial tandem duplication MLL (11q23)	10%	Exon 2 through 6 also carry FLT3-IDT mutation.
		High expression BAALC (8q22.3)		Adult younger than 60 yrs with de novo AML, unfavorable prognostic impact Independent adverse prognostic factor for resistance to initial induction chemotherapy.
		CEBPA (19q13.1) mutation	4-15%	Favorable prognostic significance.
		NPM1(5q35) mutation	45-62%	Cytoplasmic localization of nucleophosmin 956dupTCTG in exon 12 (type A). Female, higher WBC, low/absence CD34+, 40% also carry FLT3-IDT or TKD mutation. Patients with NPM1 mutations lacking FLT3-IDT had significantly better CR rates.
		Overexpression ERG (21q22)		Adverse prognosis.

Continued

TABLE 7–48. COMMON CYTOGENETIC CHANGES IN LYMPHOMAS AND LEUKEMIAS—cont'd

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
Acute Myeloid Leukemia				
AML (M1, M2, or M4)	t(6;9)(p23;q34)	DEK-CAN fusion	1% of all AML	Poor prognosis.
		FLT3 ITDs	90% of this AML type	
AML with t(8;21) (M2)	t(8;21)(q22;q22)	AML1(RUNX1)-ETO fusion	5-12% of AML	30% of cases of AML with karyotypic abnormalities and maturation in neutrophilic lineage. Usually younger patients, good prognosis
		c-KIT mutations	~50% of this AML type	Response to imatinib[a] untested.
		Other RUNX1 fusion		Toxic exposure.
Acute promyelocytic leukemia (M3, M3v.)	t(15;17)(q22; q11-12)	PML-RARA fusion	5-8% of AML (95-100% of APML)	Abnormal promyelocytes predominate. Usually occurs in adults in mid life. Treatment with all trans-retinoic acid acts to differentiate the cells. Favorable prognosis.
	t(11;17)(q23;p21)	PLZF-RARA fusion		
	t(5;17)(q34;q12)	NPM1-RARA fusion		
	t(11;17)(p13;q21)	NUMA-RARA fusion		
		FLT3 ITDs	32% of APML	
AML with inv(16) or t(16;16)	inv (16)(p13)(q22) t(16;16)(p13;q22) del(16q) Other rare variants or cryptic translocations	CBFB-MYH11 fusion	10-12% of AML (100% of M4EO)	Monocytic and granulocytic differentiation and abnormal eosinophils in the marrow. Usually younger patients. Favorable prognosis.
		c-KIT mutations	~50% of this AML type	Response to imatinib[a] untested.
AML with 11q23 abnormalities	11q23 abnormalities	MLL fusion with numerous different partners (86)	5-6% of AML	Usually associated with monocytic features. Occurs in infants and in patients after therapy with topoisomerase II inhibitors. Intermediate prognosis.
AML and MDS, therapy related	5q-/7q-/12p-/20q-			Occurs after alkylating agents and/or radiation, usually 5 to 6 years after treatment. Poor prognosis.
	t(9;11), t(11;19), t(6;11)	MLL balanced translocations		Occurs after DNA-topoisomerase II inhibitors, usually 3 years after treatment. Long-term prognosis unknown.
	t(21q22)	Other RUNX1 fusion		

TABLE 7–48. COMMON CYTOGENETIC CHANGES IN LYMPHOMAS AND LEUKEMIAS—cont'd

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
B Cell				
B lymphoblastic leukemia/ lymphoma (ALL)				
	t(9;22)(q34;q11.2)	BCR-ABL fusion (usually p190 [esp. in children], but also p210 protein)	5% of childhood ALL, 20-25% of adult ALL	Philadelphia chromosome. Poor prognosis.
	t with 11q23	MLL rearrangements		Poor prognosis. Usually infants.
	t(12;21)(p13;q22)	ETV6(TEL)-AML1 fusion	> 50% of childhood ALL or hyperdiploid	Good prognosis. This translocation is not detected by standard cytogenetics. Detected by FISH.
	t(1;19)(q23;p13.3)	PBX1-E2A fusion	5-6%	Pre-B-ALL; most common translocation in childhood. Unfavorable but modified by therapy.
	Hypodiploid			Poor prognosis.
	Hyperdiploid >50			Good prognosis (= DNA Index 1.16 to 1.6).
	t(5;14)(q31;q32)	IL3-IGH fusion		Poor prognosis.
	t(8;14)(q24;q32)	MYC-IGH fusion		Good prognosis.
	t(2;8)(p12;q24)	IGK-MYC fusion		Good prognosis.
	t(8;22)(q24;q11)	MYC-IGL fusion		Good prognosis.
	t(17;19)(q21;p13)	HLF-E2A fusion		Poor prognosis.
	t(4;11)(q21;q23)	MLL-AF4 fusion		Poor prognosis.
ALL, therapy related				Similar to therapy-related AML.
Small lymphocytic lymphoma/CLL	trisomy 12		16%	Usually do not have I_gV_H mutations. Aggressive clinical course.
	del(11q22-23)	ATM deletion	18%	Poor prognosis. Detected by FISH.
	del(13q14)	D13S319 deletion	55%	Usually do have I_gV_H mutations. Long term survival. Detected by FISH.

Continued

TABLE 7–48. COMMON CYTOGENETIC CHANGES IN LYMPHOMAS AND LEUKEMIZAS—cont'd

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
B Cell				
	del(17p)	P53 deletion	7%	Worse prognosis. Detected by FISH.
		I_gV_H not mutated	40-50%	Worse prognosis (< 8 year median survival).
		I_gV_H (mutated, > 2% difference in nucleotide sequence) ZAP70	50-60%	Better prognosis (median survival > 24 years).
Lymphoplasmacytic lymphoma (Waldenström macroglobulinemia)	6q deletion		50% if in bone marrow	Detected by FISH. Not specific for LPL.
Mantle cell lymphoma	t(11;14)(q13;q32)	CCND1-IGH fusion ATM point mutations	>95%	Overexpression of cyclin D1 detected by IHC.
Marginal zone lymphoma (MALT)	+3		60%	
	t(1;14)(p21;q32)	BCL10-IGH fusion		
	t(11;18)(q21;q21)	API2-MALT1 fusion	25-50%	
	t(11;14)(q21;q32)	MALT1-IGH fusion		
Splenic	del(7q21)			
Follicular lymphoma	t(14;18)(q32;q21)	IGH-BCL-2 fusion	70-95%	
	t(2;18)(p12;q21)	IGK-BCL-2 fusion	Rare	
Burkitt lymphoma and Burkitt-like lymphoma	t(8;14)(q24;q32)	MYC-IGH fusion	85%	
	t(2;8)(p12;q24)	MYC-IGK fusion	Rare	
	t(8;22)(q24;q11)	MYC-IGL fusion	Rare	
	t(8q24)	Other MYC fusion		
Mediastinal (thymic) large B-cell lymphoma	9p+	REL amplification		
Diffuse large B-cell lymphoma	t(3q27)	BCL6 translocations with many partners	30%	BCL6 is detected by IHC in most cases, BCL2 in some cases.
	t(14;18)(q32;q21) trisomy 18	BCL2-IGH fusion	20-30%	

TABLE 7–48. COMMON CYTOGENETIC CHANGES IN LYMPHOMAS AND LEUKEMIAS—cont'd

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
B Cell				
Hairy cell leukemia				No consistent changes.
Hodgkin lymphoma				No consistent changes.
Primary effusion lymphoma				No consistent changes.
Plasmacytoma/ myeloma	t(11;14)(q13;q32)	CCND1-IGH fusion		Best prognosis.
	t(6;14)(p21;q32)	CCND3-IGH fusion		
	t(4;14)(p16;q32)	FGF23-IGH fusion		Adverse prognosis.
	t(14;16)(q32;q23)	IGH-MAF fusion		Adverse prognosis.
	Monosomy 13/13q-		15-40%	
T Cell				
Precursor lymphoblastic leukemia/ lymphoblastic lymphoma	Translocations involving TCR alpha, beta, delta, and gamma and partner genes MYC, TAL1, RBTN1, RBTN2, HOX11, and LCK		30%	
	del(1)	TAL1 (small deletion)	25%	Adolescents.
	t(1;14)	TAL1-TCRdelta fusion	>30%	Adolescents.
	t(5;14)	HOX11L2-TCRdelta fusion		Young children.
	del(9p)	CDKN2A deletion		
T-cell prolymphocytic leukemia	inv(14)(q11;q32)	TCRα/β-TCL1 & TCL1b fusion	80%	
	t(14;14)(q11;q32)	TCRα/β-TCL1 & TCL1b fusion	10%	
	t(7;14)(q35;q32.1)	TCRβ-TCL 1A fusion	70-80%	
	Chrom 8 abnormalities			
Adult T-cell lymphoma/ leukemia				No consistent changes.

Continued

TABLE 7–48. COMMON CYTOGENETIC CHANGES IN LYMPHOMAS AND LEUKEMIAS—cont'd

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
T Cell				
Mycosis fungoides and Sezary syndrome				No consistent changes.
Peripheral T-cell lymphoma, NOS				No consistent changes.
Hepatosplenic T-cell lymphoma	i(7q)(q10)		100%	
Panniculitis-like T-cell lymphoma				No consistent changes.
Angioimmunoblastic lymphoma	Trisomy 3, trisomy 5, + X			
Enteropathy-type T-cell lymphoma				No consistent changes.
Anaplastic large cell lymphoma (CD30+)	t(2;5)(p23;q35)	NPM1-ALK fusion protein (p80)	70-80%	ALK detected by IHC in nucleus, nucleolus, and cytoplasm.
	t(2p23)	Several ALK fusions		ALK detected by IHC in cytoplasm.
Extranodal NK/T-cell lymphoma, nasal type				No consistent changes.
Blastic NK-cell lymphoma				No consistent changes.

[a]Imatinib mesylate (STI571, Gleevec, Glivec) is a small molecule tyrosine kinase inhibitor that may be used for the treatment of tumors overexpressing tyrosine kinases:
Bcr-Abl tyrosine kinase: CML, ALL (Ph+)
KIT tyrosine kinase: GIST, systemic mastocytosis, some types of AML
PDGFR kinase: CMML, chronic eosinophilic leukemia, rare cases of GIST
The KIT protein is encoded by the c-kit proto-oncogene and is a transmembrane receptor protein with tyrosine kinase activity. Mutations may render KIT independent of its ligand, SCF (stem cell factor). Mutated proteins may or may not respond to therapy with Imatinib. Wild-type KIT and KIT with mutations in the juxtamembrane domain (the intracellular segment between the transmembrane and tyrosine kinase domains) are found in GIST's and are sensitive to imatinib. Other tumor types are associated with mutations in the enzymatic domain and the altered protein is generally not sensitive to imatinib.
For additional information on specific genes, see Online Mendelian Inheritance in Man (OMIM; www.ncbi.nlm.nih.gov).

Tumors and Diseases Associated with Germline Mutations (Tables 7-49 and 7-50)

The following features are suggestive of hereditary susceptibility to cancer:

- Two or more close relatives on the same side of the family with cancer
- Evidence of autosomal dominant transmission
- Early development of cancer in the patient and relatives (in general, <50 years of age)
- Multiple primary cancers
- Multiple types of cancers
- Unusual pathologic features of tumors (Table 7-49)
- A constellation of tumors suggestive of a specific syndrome (Table 7-50)

Pathologists can aid in the detection of hereditary carcinomas by being aware of the types and pathologic characteristics of carcinomas associated with these syndromes. Patients with germline mutations are important to identify in order to:

- Screen patients for other common tumors or other components of the disease
- Consider prophylactic surgery or preventive interventions
- Offer screening to family members at risk and genetic counseling

Text continues on page 184.

TABLE 7–49. PATHOLOGIC FEATURES OF TUMORS AND DISEASES SUGGESTIVE OF A GERMLINE MUTATION

TYPE OF TUMOR	% OF CASES RELATED TO KNOWN GERMLINE MUTATIONS	SYNDROMES/GENES INVOLVED	CLUES FOR THE PATHOLOGIST
Adrenocortical carcinoma in children	50% to 100%	Li-Fraumeni, Beckwith-Wiedemann, MEN1	Unusual occurrence in a child.
Angiomyolipoma of kidney	20%	Tuberous sclerosis	Patients may be screened for other features of tuberous sclerosis.
Basal cell carcinoma	Rare if solitary	Nevoid basal cell carcinoma syndrome	Risk of a mutation is increased if multiple or if tumor occurs at <30 years of age.
Breast cancer, poorly differentiated, ER negative*	>25% if <35 years old, <10% if >35 years old	BRCA1	BRCA1 cancers are more likely to have "medullary" features, and be ER- PR- HER2/neu -. BRCA1 mutation more likely if patient has a family history or has bilateral cancer.
Breast cancer, male	4% to 14%	BRCA2	Cancers are of no specific type.
Colorectal carcinoma, poorly differentiated, mucinous, or with prominent lymphocytic infiltrate	~ 10–15% overall, ~80% if patient is <40	HNPCC	HNPCC carcinomas are more likely right -sided (two-thirds), poorly differentiated ("medullary"), mucinous, signet ring, lymphocytic infiltrate. IHC for MSH2 and MLH1 can be used to detect many, but not all, cases, but MLH1 may also be absent in sporadic cases.
GI neuroendocrine tumors: Somatostatinoma PPoma Non-functioning Gastrinoma Glucagonoma VIPoma Insulinoma Carcinoid	 45% 18-44% 18-44% 20-25% 1-20% 6% 4-5% Rare	MEN1 mutations	MEN1 mutations are also found in 15% to 70% of sporadic neuroendocrine tumors.
Hirschsprung disease	20-40%	MEN2A (RET mutations in codons 609, 618, 620)	
Juvenile (hamartomatous) polyps	Rare if solitary	Juvenile polyposis syndrome	Suspect JPS if there are >5 polyps, if present throughout the GI tract, or if there is a family history of juvenile polyps.
Medullary carcinoma of the thyroid	25%	MEN2A, MEN2B, familial medullary carcinoma (RET mutations)	May be multiple and associated with C cell hyperplasia. Cancers in occur in children in MEN2B and in young adults in MEN2A.
Medulloblastoma	Rare (?)	Nevoid basal cell carcinoma syndrome	If <3 years of age or of desmoplastic type, risk of mutation is increased.
Myxoma, cardiac	<5%	Carney complex	Increased likelihood if multiple, right-sided, and/or recurrent and in young patients (<30).
Neurofibromas	~10% if solitary but >90% if plexiform	Neurofibromatosis type 1	Increased risk if there are ≥2 neurofibromas or one plexiform neurofibroma.

Continued

TABLE 7–49. PATHOLOGIC FEATURES OF TUMORS AND DISEASES SUGGESTIVE OF A GERMLINE MUTATION—cont'd

TYPE OF TUMOR	% OF CASES RELATED TO KNOWN GERMLINE MUTATIONS	SYNDROMES/GENES INVOLVED	CLUES FOR THE PATHOLOGIST
Ovarian carcinoma	Rare	BRCA1, BRCA2	Increased risk if there is a history of breast cancer. BRCA1-associated carcinomas are more likely to be serous in type.
Pheochromocytoma	30% of all cases, 59% if patient is <18, 84% if bilateral	MEN2A, MEN2B, VHL, Isolated familial pheochromocytoma	Multiple tumors, hyperplasia of the medulla.
Primary pigmented nodular adrenocortical disease (PPNAD)	>90%	Carney complex (25% have PPNAD)	May present with Cushing syndrome. Most are associated with germline mutations, but patients may not have other manifestations of the Carney complex.
Retinoblastoma	40% of all cases, 100% if bilateral or with a positive family history	RB mutations (13q14.1-q14.2)	
Rhabdomyoma of heart in infants	50%	Tuberous sclerosis	
Sarcoma, children	7-33%	Li-Fraumeni, basal cell nevus syndrome, neurofibromatosis type 1, pleuropulmonary blastoma syndrome	
Sebaceous carcinoma	~10% if ocular, 40% if above the chin, 80% if elsewhere	HNPCC	Increased likelihood if the tumor has cystic degeneration or features of keratoacanthoma. Usually due to germline MSH2 mutations.
Schwannoma, psammomatous melanotic	>50%	Carney complex	Higher likelihood if patient is young (<30 years) and/or multiple tumors present.
Schwannoma, vestibular	5%	Neurofibromatosis type 2	Risk is increased if the patient is <30 or if there is bilateral involvement. Sporadic cases almost all have somatic NF2 mutations.
Sertoli cell tumor, large-cell calcifying	25-35%	Carney complex, Peutz-Jeghers	Most are bilateral and multifocal in young patients. Rarely malignant. Present in 30% of males with Carney complex.
Trichilemmoma, facial, multiple	~80%	PTEN	Sporadic tumors also have loss of PTEN which can be shown by IHC.
Wilms tumor	10-15%	Germline mutations in WT1 (11p13)	Nephrogenic rests are present and may be extensive. 5% to 10% of cases associated with germline mutations are multicentric or bilateral. Associated with WAGR syndrome (Wilms tumor, aniridia, GU anomalies, mental retardation) and Denys-Drash syndrome.

*See Lakhani SR, et al, The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 20:2310-2318, 2002, for additional information relating pathologic characteristics to risk of a BRCA1 mutation.

TABLE 7–50. HEREDITARY SYNDROMES ASSOCIATED WITH MULTIPLE TUMORS

SYNDROME	GERMLINE MUTATIONS	TUMORS (% OF PATIENTS DEVELOPING TUMOR)	COMMENTS
Beckwith-Wiedemann syndrome	11p15 abnormalities (loss of methylation, uniparental disomy, mutations in CDKN1C)	Wilms tumor, neuroblastoma, hepatoblastoma, adrenocortical carcinoma, rhabdomyosarcoma	Macrosomia, macroglossia, visceromegaly, ear creases and pits, omphalocele, hypoglycemia.
Bloom syndrome	BLM (RecQL3), 15q26.1	Acute leukemia, lymphoma, gastrointestinal adenocarcinoma (20% of patients develop a malignancy)	Characteristic appearance, café-au-lait spots, telangiectasias. Carcinomas do not have a specific appearance.
BRCA1 and 2	BRCA1 (17q21), BRCA2 (13q12.3)	Breast (85%), ovary (BRCA1 63%, BRCA2 27%), prostate carcinoma, others	BRCA1 breast cancers are more often poorly differentiated, have medullary features, are ER- PR- HER2/neu -, and have p53 mutations. Ovarian carcinomas are generally serous (90%), high grade, and bilateral. BRCA2 cancers do not have specific pathologic features.
Carney complex	Type 1 (CNC1) (30% of patients): PRKAR1A (17q23-24) Type 2 (CNC2) (40% of patients): locus at 2p16 30% of patients do not have identified mutations	Myxomas (cardiac, cutaneous, breast), primary pigmented nodular adrenocortical disease (25%), large-cell calcifying Sertoli cell tumors (> 90% males), multiple thyroid nodules or carcinoma (75%), growth hormone producing pituitary adenoma (10%), psammomatous melanotic schwannoma (10%), breast duct adenomas, osteochondromyxoma of bone Pigmented skin lesions (lentigos, blue nevi (especially epithelioid blue nevus), café-au-lait spots)	The skin lesions characteristically involve the vermillion border of the lip and the intercanthal portion of the eye. Myxomas of the heart can involve all chambers (sporadic tumors usually involve the left atrium) and frequently recur.
Carney triad	Unknown	Gastric gastrointestinal stromal tumor, pulmonary chondroma, extra-adrenal paraganglioma Also esophageal leiomyomas and adrenocortical tumors	Most patients are young and female. Only 22% have all three tumors. Most family members are not affected.
Familial adenomatous polyposis (FAP; including Gardner syndrome and Turcot syndrome)	APC (5q21-22)	Colorectal carcinoma, upper GI carcinoma, desmoid, Gardner fibroma, osteoma, thyroid, brain (1/3 to 2/3 are medulloblastomas – Turcot syndrome)	
Familial gastrointestinal stromal tumor syndrome	KIT (4q12) PDGFRA	GIST, often multiple (> 90% lifetime risk), hyperplasia of the interstitial cells of Cajal	GIST may occur at younger ages.
Familial medullary thyroid carcinoma	RET mutations in codons 10, 11, 13, 14 (10q11.2)	Medullary thyroid carcinoma	Cancers usually occur in adults.

Continued

TABLE 7–50. HEREDITARY SYNDROMES ASSOCIATED WITH MULTIPLE TUMORS—cont'd

SYNDROME	GERMLINE MUTATIONS	TUMORS (% OF PATIENTS DEVELOPING TUMOR)	COMMENTS
Hereditary diffuse gastric cancer syndrome	CDH1 (e-cadherin) (16q22.1)	Signet ring cell carcinoma of the stomach (67% men, 83% women), lobular carcinoma of the breast (39% women)	50% of sporadic signet ring cell carcinomas have CDH1 somatic mutations and all show loss of e-cadherin by IHC.
Hereditary non-polyposis syndrome* ("Lynch syndrome" but first patients were described by Warthin)	Mismatch repair genes: MSH2 (2p22-p21) (40%), MLH1 (3p21.3) (40%), MSH6 (2p16) (5 to 7%), PMS2 (7p22) (rare)	Colon carcinoma (80%), endometrial carcinoma (20 to 60%), ovarian carcinoma (9 to 12%), stomach carcinoma (11 to 19%), hepatobiliary tumors (2 to 7%), transitional cell carcinoma (4 to 5% - esp ureter and renal pelvis), small bowel tumors (1 to 4%), lymphoma (rare) Sebaceous skin tumors, adenomas, epitheliomas, carcinoma, keratoacanthomas (Muir-Torre – usually MSH2)	Colon carcinomas are more likely (overall, 66%) to be on the right side, poorly differentiated ("medullary"), mucinous, signet ring, or undifferentiated, with a prominent lymphocytic infiltrate. IHC can be used to detect the absence of MSH2 (usually due to germline mutations) and MLH1 (can be due to germline mutations, epigenetic changes (methylation), or less commonly, somatic mutations) in many patients. IHC is 92% sensitive for MSI and 100% specific. MSI testing is also used.
Juvenile polyposis syndrome	MADH4 (or SMAD4) (18q21.10 (15%) or BMPR1A (10q22.3) (25%)	Hamartomatous (juvenile) polyps, GI carcinomas	
Li-Fraumeni	p53 (17p13.1), rarely CHEK2 (22q12.1)	Sarcomas, breast cancer, leukemia, osteosarcomas, brain tumors, adrenocortical carcinoma, others	
MEN1	MEN 1 (11q13)	Pituitary adenoma, pancreatic islet cell tumors, parathyroid adenomas, adrenocortical tumors, carcinoids, lipomas	MEN1 mutations also occur in 15 to 70% of sporadic neuroendocrine tumors.
MEN2A	RET exon 10 and 11 missense mutations (10q11.2)	Medullary thyroid carcinoma (95%), hyperplasia of the parathyroids (15-30%), pheochromocytoma (50%), ganglioneuromatosis of GI tract. Subsets of patients have Hirschsprung disease or cutaneous lichen amyloidosis	Specific mutations correlate with age at development of medullary thyroid carcinoma.
MEN2B	RET missense mutation in exon 16 (10q11.2)	Medullary thyroid carcinoma (100%), pheochromocytoma (50%) Mucosal neuromas of lips and tongue,	Marfanoid habitus, distinctive facies.
Nevoid basal cell carcinoma syndrome (Gorlin syndrome)	PTCH (9q22.3)	Basal cell carcinomas (90%), odontogenic keratocysts (90%), cardiac or ovarian fibromas (20%), medulloblastoma in childhood (5%)	Macrocephaly, skeletal anomalies, palmar or plantar pits, calcification of falx (90%).
Neurofibromatosis type 1	NF1 (17q11.2)	Neurofibromas (esp plexiform) (100%), optic gliomas, adrenal ganglioneuromas, pheochromocytoma (0.1 – 6%), MPNST (10%), leukemia, ganglioneuromatosis of the GI tract	Café-au-lait macules (95%), iris hamartomas (Lisch nodules), axillary freckling.

TABLE 7–50. HEREDITARY SYNDROMES ASSOCIATED WITH MULTIPLE TUMORS—cont'd

SYNDROME	GERMLINE MUTATIONS	TUMORS (% OF PATIENTS DEVELOPING TUMOR)	COMMENTS
Neurofibromatosis type 2	NF2 (22q12.2)	Bilateral vestibular schwannomas (100%, 40% have lobular pattern), schwannomas of other nerves, meningiomas (50%, often fibroblastic)	
Peutz-Jeghers (Hamartomatous polyp syndrome)	LKBI/STK11 (19p13.3)	Colon, breast, stomach, pancreas, small bowel, thyroid, lung, uterus, sex cord stromal tumors, calcifying Sertoli cell tumors Hamartomatous polyps of GI tract	Perioral pigmentation.
Pheochomocytoma or paraganglioma, familial	SDHB (1p36.1-p35) SDHD (11q23) SDHC (1q21) (paraganglioma)	Pheochromocytoma, paraganglioma	Patients are more commonly young (< 40), with multifocal adrenal tumors, or extra-adrenal disease. SDHD is imprinted and only confers susceptibility after paternal transmission.
PTEN hamartoma syndrome (including 80% of Cowden's syndrome, 50-60% of Bannayan-RIley-Ruvalcaba syndrome)	PTEN (10q23.31)	Breast cancer (25 to 50%), thyroid carcinoma (10% - especially follicular), endometrial carcinoma (5 to 10%), hamartomatous polyps of GI tract Multiple facial trichilemmomas, acral keratosis, oral papillomatous lesions, mucosal lesions	Macrocephaly (megalencephaly, 97th percentile), Lhermitte-Duclos disease.
Tuberous sclerosis	TSC1 (9q34), TSC2 (16p13,3)	Subependymal glial nodules (90%), cortical or subcortical tubers (70%), angiomyolipoma of kidney (70%), lymphangiomyomatosis of lung (1 to 6%), rhabdomyoma of heart (47 to 67%) Skin lesions (100%, including myomelanotic macules, multiple facial angiofibromas, shagreen patch, fibrous facial plaque, ungual fibroma)	Seizures (80%), developmental delay or retardation (50%).
Von Hippel-Lindau (VHL)	VHL (3p26-p25)	Hemangioblastomas (retinal, cerebellar, spinal cord) (80%), renal cell carcinoma (40%), renal cysts, pancreatic cysts, pheochromocytoma, endolymphatic sac tumors (10%), epididymal cystadenomas	

*MSI-H is found in 11% of sporadic colon carcinomas and has histologic features similar to patients with germline mutations. There is reduced response to 5-FU but better survival. >5 fold increased risk of metachronous cancers.
For additional information on most syndromes, see http://www.genetests.org/and Online Mendelian Inheritance in Man (OMIM; www.ncbi.nlm.nih.gov).

Although the sporadic forms of cancers, in general, far outnumber cases associated with germline mutations, in some cases the appearance or site of a carcinoma is highly suggestive of a known syndrome and further investigation may be warranted.

ANALYTICAL CYTOLOGY (FLOW CYTOMETRY)

Flow cytometers analyze populations of thousands of dissaggregated cells as they pass by stationary detectors. Cell size and cytoplasmic granularity can be measured as well as DNA content and the presence or absence of immunohistochemical markers added to the cell suspension. Newer techniques can analyze three or more features simultaneously to divide cells into unique populations. DNA content can be used to determine the number of cells in S-phase (a measure of proliferation - S-phase fraction). Because cells are not visualized by this technique, one must be sure to submit only lesional tissue.

Indications for Ploidy and S-Phase Analysis.

- Hydatidiform moles – complete (diploid), partial (triploid)
- Some carcinomas – DNA ploidy and S-phase have been reported to be of prognostic significance for some carcinomas (e.g., colon, breast, and prostate) but is not routinely performed at all institutions or used by all oncologists.

Indications for Cell Surface Marker Analysis.

- Lymphomas and leukemias

Method for Submitting Tissue. Single cell suspensions are necessary for analysis. For fresh tissues, cells must be viable. Fresh tissue (approximately 0.3 to 0.5 cm^3) is placed in a specimen container and kept moist with HBSS. Tissues can be held overnight in a refrigerator.

Formalin-fixed paraffin-embedded sections may also be used for DNA ploidy analysis by the Hedley method, although the results are not as satisfactory due to nuclear fragmentation.

Results. The results are usually incorporated into the final surgical pathology report.

CYTOLOGIC PREPARATIONS FROM SURGICAL SPECIMENS

Cytologic preparations of surgical specimens often add additional information.

- **Intraoperative diagnosis:** Touch preps or smears are especially valuable for:
  - Infectious cases (to avoid contamination of the cryostat and aerosolization of infectious agents)
  - Neuropathology cases – for diagnosis and for the performance of cytogenetic (FISH) analysis.
  - Tumors (for excellent cytologic detail, especially lymphomas and papillary carcinomas of the thyroid)
- **Special stains:** Stains for microorganisms can be performed the same day on cytologic smears of specimens from critically ill patients. Do not submit air-dried smears of infectious cases for staining as the unfixed material may constitute a hazard to laboratory personnel. Fat is dissolved during routine processing, but can be demonstrated with fat stains on air dried slides.
- **Genetic studies (FISH):** In touch preparations nuclei are intact, unlike tissue sections in which only partial nuclei are present. This feature makes these preparations superior for techniques such as FISH and image analysis.

Comparing cytology preparations and the corresponding surgical specimen is always a useful exercise in learning the comparative morphology of these techniques.

SPECIMEN RADIOGRAPHY

Specimen radiographs are often preferable over patient radiographs:

- A permanent record of the radiograph can be kept with the case.
- A radiograph of the specimen may reveal more details of the underlying process (e.g., fewer structures may be present to complicate the appearance).
- There may have been a significant time interval between the patient radiograph and the surgical excision.
- The radiograph will often indicate important sites to examine histologically (tumor invasion into a rib or microcalcifications in a breast biopsy).
- The specimen radiograph can confirm that the clinical lesion was removed.

Indications.

- Tumors of bone and cartilage
- Tumors invading into bone
- Avascular necrosis
- All bioprosthetic heart valves (to document the degree of calcification)
- Breast biopsies or mastectomies performed for mammographic lesions that cannot be located grossly. Paraffin blocks of breast tissue can be radiographed if microcalcifications were seen by specimen radiography but not in histologic sections and were not identified prior to processing. Clips placed after core needle biopsy are also easily identified.

Calcifications can dissolve in formalin over several days. If the demonstration of calcifications is important (e.g., mammographically detected calcifications) it is preferable to process the tissue within 1 to 2 days. If processing is to be delayed, the tissue can be stored in ethanol.

Method. Radiographic equipment is available in radiology departments and in some pathology departments. The specimen may be placed on a piece of wax paper (to keep the surfaces clean) lying on the film. Specimens can be radiographed after decalcification (not all calcium is removed) but best results are obtained on fresh undecalcified specimens. Lungs should not be inflated prior to radiography.

If the specimen is small, two exposures at different settings or at different angles may be useful. Lead sheets can be used to allow two exposures on one piece of film.

If the film is too dark (overexposed), the exposure is too high and a lower setting should be tried. If the film is too light (i.e., unexposed) the exposure is too low and a higher setting is indicated.

Special injection techniques with radiocontrast media are available for unusual specimens (e.g., a recipient lung with pulmonary hypertension, vascular ectasia of the bowel).

Octreotide and Sentinel Nodes. Labeled compounds are sometimes used to localize certain types of tumors (generally neuroendocrine) or sentinel lymph nodes. The patient is injected with the isotope prior to surgery and the surgeon uses a hand held probe to identify the labeled tissue. The amount of radioactivity in the tissue is small and generally does not pose a hazard to pathologists handling the tissue and does not need special disposal methods. However, each pathology department should consult with their radiation safety department to ensure appropriate handling of such tissues. In some cases, if a gross lesion is not present corresponding to the area of octreotide uptake, specimens can be imaged using a gamma camera.

Results. The radiographs are documented in the gross description and any information gained from the radiograph is incorporated into the surgical pathology report.

TISSUE FOR RESEARCH — TUMOR BANK

The pathology department is a unique resource for researchers who need human tissues. The pathologist plays a key role as patient advocate and diagnostician in order to provide appropriate human tissues for biologic research. Most hospitals have a policy that allows the release of tissue for research **if it would otherwise be discarded**. Therefore, tissue is never provided for research until all necessary tissue has been taken for diagnosis. Tissue from primary diagnostic breast biopsies and open lung biopsies without gross lesions must not be given away. It is in the best interest of the patient that a pathologist evaluate the specimen rather than have tissue given away by nonpathologists who are not aware of what is needed for diagnosis.

Indications. By request of researchers who have obtained permission from the hospital Institutional Review Board (IRB). Patients must provide specific consent. In some cases, all patient identification will need to be removed from the specimen.

MICROBIOLOGICAL CULTURE AND SMEARS

The investigation of infectious disease by culture is complementary to its investigation by histologic sections (Tables 7-51 to 7-53).

TABLE 7–51. IDENTIFICATION OF INFECTIOUS DISEASES

CULTURE	HISTOLOGIC SECTIONS
Can be performed on aspirates, swabs, fluids, or tissues.	Requires surgical excision of tissues.
Cultures amplify the number of organisms present, allowing them to be recognized.	Organisms may be rare, or not seen in tissue sections.
The specific organism can be identified and tested for drug susceptibility.	Categories of organisms can be recognized but specific identification may not be possible.
Some organisms cannot be cultured.	Many organisms can be identified that will not grow in culture or that require long culture times (e.g., TB).
It may be difficult to exclude contamination for a positive culture.	Morphologic evidence of an inflammatory response provides evidence for a clinical infection. The location of the infection may be of diagnostic importance (e.g., cellulitis vs. necrotizing fasciitis or superficial colonization of devitalized tissue vs. deep infections involving viable tissues). The use of special studies, such as PCR and other molecular assays, may be warranted if the tissue pattern of injury is indicative of a potential organism despite lack of culture evidence and/or lack of organisms seen on tissue sections.

Indications.
- Suspected infectious processes, either by clinical data or by frozen section
- Suspected sarcoid to exclude an infectious process

Method. Tissue is kept as sterile as possible. Suture removal kits are a convenient source of sterile scissors and forceps. Serially section the specimen to determine if there are focal lesions. Place representative sections in a sterile specimen container making sure to retain a duplicate piece of tissue for histology. Label with the patient's name and unit number, patient's physician, type of specimen, collection date, and time of collection (required for Joint commission accreditation).

Results. The results are generally reported by the microbiology laboratory. Communication with the microbiology laboratory and staff is essential to correlate histologic results with microbiology results from the same specimen.

Reports. The results of culture of surgical specimens are usually reported in a separate report.

TABLE 7-52. FUNGI: HISTOLOGIC APPEARANCE IN SURGICAL SPECIMENS

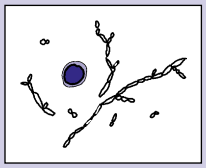
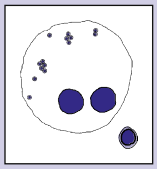
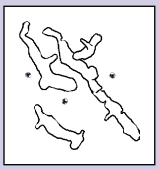
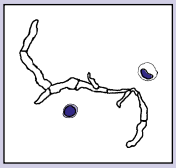
HISTOLOGIC APPEARANCE OF FUNGUS		STAINS	USUAL SITES OF INFECTION (PATIENT GROUPS)	TISSUE RESPONSE
***CANDIDA* SPECIES (*C. ALBICANS*, 60%; *C. TROPICALIS, C. PARAPSILOSIS, C. KRUSEI*)**				
	3- to 4-μm round yeast forms True hyphae with acute angle branching (5-10 μm) Budding yeast (single bud) Extracellular	MSS (+) PAS (+) Gram (+) (unlike most other fungi)	Skin/oral: superficial Larynx (thrush) (immunocompromised; newborn) Vagina (pregnancy; DM; antibiotic use) Esophagus (HIV; malignancy) Invasive/systemic: kidney, liver, lung, heart valves (immunocompromised) Oral, vaginal, disseminated (immunocompromised; antibiotic therapy; elderly; denture wearing)	Fungi on surface of epithelium, Cl, +/– eosinophils Ulcer, pseudomembrane, dirty necrosis, PMNs, MP, GC, occasional GRAN AI
***CANDIDA GLABRATA* ("TORULOPSIS")**				
	Similar to other *Candida* but no true hyphae Only small yeast forms are usually present		Urogenital tract (immunocompromised) Bloodstream (immunocompromised)	Similar to *C. albicans*
HISTOPLASMA CAPSULATUM				
	2- to 5-μm yeast forms Unequal budding Often intracellular in histiocytes or PMNs (looks like leishmania but with no kinetoplast—one dot only) Very rare short pseudohyphae Giemsa may give false impression of a capsule	MSS (+) PAS (+) Mucicarmine (–) FM (–) Giemsa (+)	Lung (Mississippi and Ohio River valleys): usually an incidentally found fibrocaseous nodule in lung, lymph node, liver, or spleen (patients are not immunocompromised) Disseminated (e.g., gastrointestinal, lung, lymph node) (immunocompromised)	NEC GRAN or old resolved GRAN with calcifications, degenerate fungal forms Predominantly intracellular organisms, MP, GRAN, not AI

TABLE 7–52. FUNGI: HISTOLOGIC APPEARANCE IN SURGICAL SPECIMENS—cont'd

HISTOLOGIC APPEARANCE OF FUNGUS		STAINS	USUAL SITES OF INFECTION (PATIENT GROUPS)	TISSUE RESPONSE
ZYGOMYCETES (INCLUDING THE GENERA *MUCOR, RHIZOPUS, RHIZOMUCOR, ABSIDIA*, AND *CUNNINGHAMELLA*)				
	Large hyphae, irregular in width, infrequently septate hyphae, 6-50 μm Right angle branching Budlike or bulbous projections No yeast forms Usually seen without stains	MSS (weak) PAS (weak) H&E (+)	Skin (primary or hematogenous) (DM) Rhinocerebral (DM, leukemia, dialysis) Lung (immunocompromised) GI or bladder	Invasion of BVs with infarction, hemorrhage, perineural invasion, little host response or AI (all patients are immunocompromised)
***ASPERGILLUS* SPP. (*A. FUMIGATUS*, 90%; *A. FLAVUS, A. NIGER, A. TERREUS, A. NIDULANS*)**				
	Septate hyphae, 45-degree branching, 3-8 μm, evenly contoured Fruiting bodies (pigmented) seen only in necrotic tissue with air exposure (rarely in invasive lesions) Dilated and distorted hyphae are present in chronic lesions	MSS (+) PAS (+) Gram (weak) IHC and ISH available	Nose/sinus: Invasive (immunocompromised) Noninvasive Allergic mucin Bronchopulmonary: Superficial (allergic bronchopulmonary aspergillosis) Bronchocentric granulomatosis Aspergilloma: fungal ball in preexisting necrotic cavity (not immunocompromised) Invasive: target lesions (necrotic areas with peripheral hemorrhage) (immunocompromised) Skin: usually secondary to hematogenous spread	Ulceration, AI, fungi in viable tissue Mat of fungal hyphae, little or no inflammation Eosinophils and hyphae in mucin, Charcot-Leyden crystals Bronchiolitis or alveolitis, eosinophils, mucoid impaction with fragmented fungal hyphae Circumferential GRAN of small airways, mucin impaction with fragmented fungal hyphae, eosinophils Little tissue response, CI. Conidiophores (fruiting bodies) may be present if in contact with air. *Aspergillus niger* is associated with calcium oxalate production causing thrombosis and ischemic necrosis Invasion of arteries with thrombosis and infarction, may be +/– host response; may resolve with GRAN Microabscesses and NEC GRAN

Continued

TABLE 7–52. FUNGI: HISTOLOGIC APPEARANCE IN SURGICAL SPECIMENS—cont'd

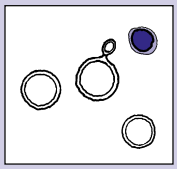
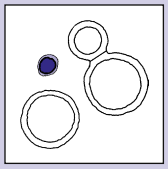
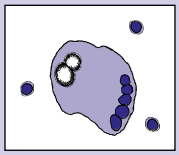
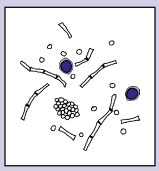
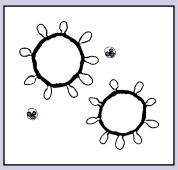
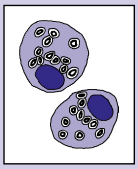
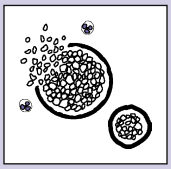
HISTOLOGIC APPEARANCE OF FUNGUS		STAINS	USUAL SITES OF INFECTION (PATIENT GROUPS)	TISSUE RESPONSE
CRYPTOCOCCUS NEOFORMANS				
	2-15 μm, spherical or oval narrow-based unequal buds, prominent capsule (mucicarmine positive); variable size and shape is characteristic Single or multiple buds, rare pseudohyphae. Capsule-deficient forms (mucicarmine negative) may be present. However, FM is usually positive.	PAS (+) Gram (weak) MSS (+) FM (+) AB (+ capsule) Mucicarmine (+ capsule) ICH available	Lungs: symptomatic pneumonia that may affect both lungs. Can also form residual fibrocaseous granulomas (rarely calcify). Meningoencephalitis (soap-bubble lesions): CSF (India ink positive, but antigen test is better) (immunocompromised); the antigen test cross-reacts with *Trichosporon* species. Disseminated (any organ) (immunocompromised)	Solitary, well-circumscribed focus of yeast forms surrounded by GRAN and GC Fungi in Virchow-Robin space, little host response Little host response
BLASTOMYCES DERMATITIDIS				
	8-20 μm spheres with broad-based budding Double contour thick capsule Often in giant cells Multiple nuclei No hyphae	MSS (+) PAS (+) Mucicarmine (+/–) FM (–)	Lung (Mississippi and Ohio River valleys) (residual nodules are much rarer than for *Histoplasma* or *Coccidioides* species) Skin: fleshy fungating ulcers, may be verrucous Disseminated (bones, GI, CNS, prostate, liver, spleen, kidney)	Usually solitary focus of GRAN, rarely calcified Pseudoepitheliomatous hyperplasia hyperkeratosis, microabscesses, AI (intraepithelial), GRAN
DEMATIACEOUS FUNGI (BROWN PIGMENTED; >100 SPECIES)				
	1.5- to 5-μm brown to black yeast "copper penny," may be present in giant cells, often in pairs Sclerotic bodies (septated cells) and septate hyphae	H&E (+) MSS (+) PAS (+)	Chromoblastomycosis usually due to traumatic introduction of fungi by thorn or splinter Phaeohyphomycosis (cutaneous phaeomycotic cyst) usually due to traumatic introduction of fungi by thorn or splinter Mycetoma (chronic tumor-like lesion with draining sinuses) Systemic (cerebral)—rare	Hyperkeratosis, microabscesses, PMNs, and GRAN; verrucous appearance, sclerotic bodies present Subcutaneous cystic granulomatous nodule, surface not involved, hyphae usually present GRAN, AI, hyaline granules Abscesses
DERMATOPHYTES (*TRICHOPHYTON, MICROSPORUM, EPIDERMOPHYTON* SPECIES)				
	Septate hyphae and yeast forms ("spaghetti and meatballs")	MSS (+) PAS (+) FM (+)	Skin (tinea corporis or "ringworm," tinea pedis or athlete's foot), hair (tinea capitis), and nails (tinea unguium or onychomycosis) Disseminated (immunocompromised)—very rare	Chronic dermatitis and spongiosis, little host response, superficial fungal forms

TABLE 7–52. FUNGI: HISTOLOGIC APPEARANCE IN SURGICAL SPECIMENS—cont'd

HISTOLOGIC APPEARANCE OF FUNGUS		STAINS	USUAL SITES OF INFECTION (PATIENT GROUPS)	TISSUE RESPONSE
***PARACOCCIDIOIDES BRASILIENSIS* (SOUTH AMERICAN BLASTOMYCOSIS)**				
	5- to 25-μm double-walled yeast forms that reproduce by gemmulation: 10- to 60-μm "ship's wheel"	MSS (+) PAS (+)	Skin ulceration (trauma with soil, residency in South America) Upper and lower respiratory tract Disseminated (lymph nodes, liver, spleen, gastrointestinal, genitourinary, bones, adrenal, CNS)	Ulcers or vegetations, AI, GRAN, eosinophils Ulcers or vegetations, AI, GRAN, eosinophils, followed by fibrosis Ulcers or vegetations, AI, GRAN, eosinophils
SPOROTHRIX SCHENCKII				
	Round or oval 2- to 6-μm yeast, often in GC Unequal narrow-based budding Elongated cigar-shaped forms No hyphae	MSS (+) PAS (+)	Skin (nodular lymphangitic cutaneous sporotrichosis): red papule that ulcerates and local lymphadenitis (farmers and gardeners, exposure to cats) Extracutaneous: bones and joints, lung—very rare	AI and NEC GRAN, hyperkeratosis, pseudoepitheliomatous hyperplasia, intraepithelial abscesses (looks like blastomycosis) Cavitary pneumonitis in one upper lobe
***COCCIDIOIDES* SPECIES (*C. IMMITIS, C. POSADASII*)**				
	20- to 200-μm nonbudding thick-walled spherules containing 2- to 5-μm endospores Hyphae may rarely be found in pulmonary cavities	MSS (+) PAS (+) Mucicarmine (–) FM (–)	Lung (San Joaquin valley, SW and W): often seen as residual fibrocaseous nodules—organisms may be rare or absent Systemic (meninges, bone, adrenal CNS, liver) (immunocompromised, DM, elderly, pregnant)	Single focus, may calcify, AI or GRAN (no GCs) GRAN: if spherules are unruptured AI: if spherules ruptured and endospores released May be hazardous to laboratory workers if cultured

AB, Alcian blue; AI, acute inflammation; BV, blood vessel; CNS, central nervous system; CSF, cerebrospinal fluid; DM, diabetes mellitus; FM, Fontana Masson; GC, giant cell; GI, gastrointestinal; GRAN, granulomas; IHC, immunohistochemical methods; ISH, in situ hybridization methods; MP, macrophage; MSS, silver stain (similar to GMS—Grocott-Gomori methenamine silver); NEC GRAN, necrotizing granulomas; PAS, periodic acid–Schiff; PMNs, polymorphonuclear leukocytes; (+), positive; (-), negative.
Data from Lerone DH. Medically Important Fungi: A Guide to Identification, 4th ed. Washington, DC: ASM Press, 2004; and Chandler FW, Watts JC. Pathologic Diagnosis of Fungal Infections. Chicago: ASCP Press, 1987.

TABLE 7-53. VIRUSES: HISTOLOGIC APPEARANCE AND ASSOCIATED NEOPLASMS

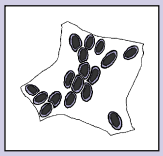
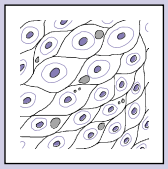
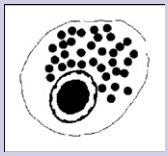
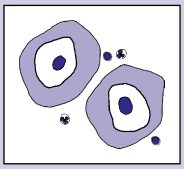
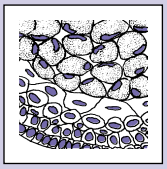
HISTOLOGIC APPEARANCE OF VIRUS		HOST REACTION	COMMON SITES (OR CELLS) OF INVOLVEMENT	ASSOCIATED NEOPLASMS/ VALUE OF TESTING FOR VIRUS	TESTS TO IDENTIFY VIRUS
HERPES SIMPLEX VIRUS (HSV I AND II) (ds DNA)					
	Multinucleated squamous cells, hepatocytes, pneumocytes, microglial cells, or placenta Glassy nuclei with chromatin compressed at nuclear membrane Intranuclear eosinophilic inclusions with a clear halo and thick nuclear membrane (Cowdry A*)	Vesicles or ulcerated surface with CI and AI Necrosis of organs in neonates or immunocompromised patients	Squamous mucosa of esophagus, cervix, or anus Skin Lung Temporal lobe (diagnosed by PCR of CSF)	No associated neoplasms	IHC for viral proteins (does not distinguish types I and II) ISH/PCR
VARICELLA-ZOSTER VIRUS (VZV, ds DNA)					
	Similar to HSV	Similar to HSV	Skin: rarely biopsied Lung	No associated neoplasms	IHC (can distinguish VZV from HSV)
SMALLPOX VIRUS (ds DNA)					
	Eosinophilic cytoplasmic inclusions (Guarnieri bodies), ballooning degeneration of epithelial cells†	Multilocular vesicles that coalesce, perivascular lymphocytic infiltrate, AI	Skin GI tract All organs in severe forms	No associated neoplasms	IHC electron microscopy: fluid from vesicles can be used to detect viral particles PCR Report immediately to the CDC!
CYTOMEGALOVIRUS (CMV; ds DNA)					
	Enlarged cells with amphophilic intranuclear (with a surrounding halo) and basophilic intracytoplasmic inclusions	Ulcerated mucosa with CI and AI Endothelial cells with thrombosis and infarction Interstitial pneumonitis	Esophagus, colon, lung, adrenal, heart, liver, placenta	No associated neoplasms	IHC (>40% of U.S. population is infected; significance of [+] in normal-appearing cells is unclear) ISH/PCR

TABLE 7–53. VIRUSES: HISTOLOGIC APPEARANCE AND ASSOCIATED NEOPLASMS—cont'd

HISTOLOGIC APPEARANCE OF VIRUS	HOST REACTION	COMMON SITES (OR CELLS) OF INVOLVEMENT	ASSOCIATED NEOPLASMS/ VALUE OF TESTING FOR VIRUS	TESTS TO IDENTIFY VIRUS
HUMAN PAPILLOMAVIRUS (HPV, MORE THAN 200 TYPES; ds DNA)				
 Koilocytosis (irregular nuclear enlargement with perinuclear clearing), disrupted keratohyaline granules, hyperkeratosis, parakeratosis	Squamous cells with acanthosis, papillomatosis, and coarse clumped keratohyaline granules	Verruca vulgaris: hands, oral cavity, larynx (HPV 2), EV (HPV 5 and 8) Verruca plana: foot (HPV 5 and 8) Condyloma acuminatum: external genitalia (HPV 6 and 11) Cervix: LSIL, HSIL, cancer (HPV 16 and 18) Head and neck cancer (especially tonsil) (HPV 16)	SCC of cervix, tonsil, anogenital, papillomas of skin and other sites, cervical adenocarcinoma Value of viral testing: cervical cancer screening Metastatic SCC: probable primary site‡ HPV+ tonsillar carcinomas have a better prognosis	ISH/PCR IHC for p16 (cellular protein overexpressed in >90% of HPV infections, especially HPV 16 and 18)
EPSTEIN-BARR VIRUS (EBV; ds DNA)				
No diagnostic features Infected B cells may have a plasmacytoid immunoblastic appearance In oral hairy leukoplakia, the epithelial cells have a foamy balloon cell appearance	Lymphocytic infiltrate, peripheral lymphocytosis	B cells, oropharyngeal epithelial cells, gastric mucosa, smooth muscle	Lymphomas,§ nasopharyngeal carcinoma, lymphoepithelioma-like gastric carcinoma, EBV-associated smooth muscle tumors, IPFDT Value of viral testing: diagnosis of HD; diagnosis of nasopharyngeal carcinoma EBV+ gastric carcinoma has a better prognosis	IHC for LMP-1 (nasopharyngeal carcinomas, HD [not LP], transplant lymphomas, AIDS-related lymphomas, endemic Burkitt lymphoma) and EBNA 2 (transplant lymphomas, AIDS-related lymphomas) ISH/PCR for EBER-1 and -2 RNA
MOLLUSCUM CONTAGIOSUM (ds DNA)				
 Intracytoplasmic molluscum bodies in squamous cells of granular layer	Acanthosis of skin	Skin: umbilicated nodules	No associated neoplasms	

Continued

TABLE 7–53. VIRUSES: HISTOLOGIC APPEARANCE AND ASSOCIATED NEOPLASMS—cont'd

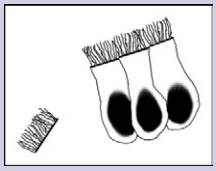
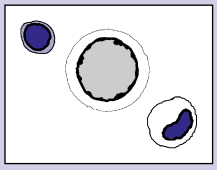
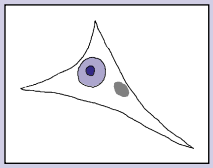
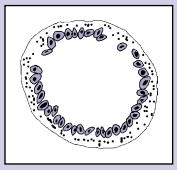
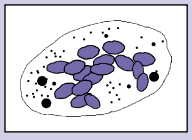
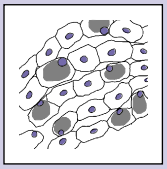
HISTOLOGIC APPEARANCE OF VIRUS	HOST REACTION	COMMON SITES (OR CELLS) OF INVOLVEMENT	ASSOCIATED NEOPLASMS/ VALUE OF TESTING FOR VIRUS	TESTS TO IDENTIFY VIRUS
ADENOVIRUS (ds DNA)				
 Alveolar lining cells and bronchial epithelial cells with inclusions filling enlarged nuclei ("smudge cells")	Necrotizing bronchiolitis and pneumonia Diffuse alveolar damage No GCs	Lung and other organs	No associated neoplasms	ISH/PCR (rarely biopsied; may be cultured)
PARVOVIRUS B19 (ss DNA)				
 Large glassy nucleus of nucleated red blood cells	None	Bone marrow, placenta, fetus, and sites of EMH	No associated neoplasms	ISH/PCR
RABIES VIRUS (ss RNA)				
 Negri bodies: cytoplasmic round to oval or bullet-shaped eosinophilic inclusions	Little inflammation present	Neurons or Purkinje cells	No associated neoplasms	ISH/PCR IHC
MEASLES VIRUS (ss RNA)				
 GC with eosinophilic nuclear and cytoplasmic inclusions, Warthin-Finkeldey cells (multinucleated giant cells)	GCs in tracheobronchial mucosa, alveoli, and lymphoid tissue	Lung: diffuse alveolar damage Brain: subacute sclerosing panencephalitis or measles inclusion body encephalitis	No associated neoplasms	ISH/PCR (rare in vaccinated populations; rarely biopsied)
RESPIRATORY SYNCYTIAL VIRUS (RSV; ss RNA)				
 Intracytoplasmic eosinophilic inclusions	Syncytial GCs, diffuse alveolar damage, CI	Lung	No associated neoplasms	Rarely biopsied; clinical tests are available
MERKEL CELL POLYOMA VIRUS (MCV, MCPYV; ds DNA)				
No specific features	No specific host response	Skin	Merkel cell carcinoma	Clinical tests not yet available
JC VIRUS, POLYOMAVIRUS (ds DNA)				
Intranuclear basophilic inclusions in oligodendroglia	Demyelination without inflammation	Subcortical white matter (progressive multifocal leukoencephalopathy)	No associated neoplasms	IHC ISH/PCR (can be performed on CSF)

TABLE 7–53. VIRUSES: HISTOLOGIC APPEARANCE AND ASSOCIATED NEOPLASMS—cont'd

	HISTOLOGIC APPEARANCE OF VIRUS	HOST REACTION	COMMON SITES (OR CELLS) OF INVOLVEMENT	ASSOCIATED NEOPLASMS/ VALUE OF TESTING FOR VIRUS	TESTS TO IDENTIFY VIRUS
BK VIRUS, POLYOMAVIRUS (ds DNA)					
	Decoy cells (tubular or urothelial cells with inclusions) in urine Nuclear enlargement in tubules in late phase of infection	PVAN Tubular cell necrosis, interstitial inflammation, fibrosis	Urinary tract (transplanted kidneys)	No associated neoplasms	IHC ISH/PCR (can be used on urine or bladder washings) Electron microscopy–specific intranuclear inclusions
HEPATITIS B VIRUS (HBV; ds DNA)					
	Cells with ground-glass cytoplasm displacing the nucleus	Chronic active hepatitis, cirrhosis Apoptotic bodies	Hepatocytes	Hepatocellular carcinoma	IHC for core antigen (HBcAg) and surface antigen (HbsAg) ISH/PCR
HEPATITIS C VIRUS (ss RNA)					
	No diagnostic features	Chronic active hepatitis, steatosis, intralobular inflammation, plasma cells, cirrhosis	Hepatocytes	Hepatocellular carcinoma	IHC ISH/PCR
KAPOSI SARCOMA–ASSOCIATED HERPESVIRUS (KSHV, HUMAN HERPESVIRUS-8 [HHV-8]; ds DNA)					
	No diagnostic features	None	Vascular endothelial cells, lymphocytes	Kaposi sarcoma, multicentric Castleman disease, HHV-8–related lymphomas, primary effusion lymphoma Value of testing for virus: diagnosis of Kaposi sarcoma	ISH/PCR Note: most primary effusion lymphomas are also EBV+
HUMAN T-CELL LEUKEMIA VIRUS (HTLV-1; ds DNA)					
	Lymphocytes with condensed chromatin and a convoluted polylobated nucleus ("flower cells")	No specific reaction	T cells	Adult T-cell leukemia Value of testing for virus: diagnosis in patients seronegative for HTLV-1	ISH/PCR

Continued

TABLE 7–53. VIRUSES: HISTOLOGIC APPEARANCE AND ASSOCIATED NEOPLASMS—cont'd

HISTOLOGIC APPEARANCE OF VIRUS	HOST REACTION	COMMON SITES (OR CELLS) OF INVOLVEMENT	ASSOCIATED NEOPLASMS/ VALUE OF TESTING FOR VIRUS	TESTS TO IDENTIFY VIRUS
HUMAN IMMUNODEFICIENCY VIRUS (HIV; ss RNA)				
	No specific changes	No specific reaction	CD4+ T cells	Immunosuppression increases risk for other virus-associated neoplasms	Diagnosis usually made by serology

*Cowdry type B inclusions were described as smaller inclusions associated with polio. However, this finding has not been validated and is not currently used for diagnosis.

†Monkeypox has a similar appearance. Intracytoplasmic inclusions are characteristic of smallpox and are not seen in HSV or VZV infections. It may be difficult to distinguish smallpox, HSV, and VZV by H&E alone (Nuovo GJ, Plaza JA, Magro C: Rapid diagnosis of smallpox infection and differentiation from its mimics. Diagn Mol Pathol 12:103-107, 2003).

‡The presence of HPV in metastatic carcinoma to a lymph node suggests a primary in the tonsil (if in the head and neck) or cervix (if abdominal).

§Burkitt lymphoma, classic Hodgkin lymphoma (but not lymphocyte-predominant Hodgkin lymphoma), AIDS-associated B-cell lymphoma, plasmablastic lymphoma, post-transplantation lymphoproliferative disorder, lymphomatoid granulomatosis, methotrexate-associated B-cell lymphoma, severe combined immunodeficiency–associated B-cell lymphoma, Wiskott-Aldrich syndrome–associated B-cell lymphoma, X-linked lymphoproliferative disorder–associated B-cell lymphoma, EBV-positive diffuse large B-cell lymphoma of the elderly, peripheral T-cell lymphoma, extranodal NK/T-cell lymphoma, nasal type, virus-associated hemophagocytic syndrome T-cell lymphoma. Angioimmunoblastic T-cell lymphoma is associated with EBV+ B cells. However, the neoplastic T cells are negative for EBV.

AI, acute inflammation; CI, chronic inflammation; CDC, Centers for Disease Control and Prevention; CSF, cerebrospinal fluid; ds, double-stranded; EBER, EBV-associated nonpolyadenylated early RNAs; EBNA 2, EBV nuclear antigen 2; EMH, extramedullary hematopoiesis; EV, epidermodysplasia verruciformis; GC, multinucleated giant cells; GI, gastrointestinal; HD, Hodgkin disease; HSIL, high-grade squamous intraepithelial lesion; IHC, immunohistochemistry uses antibodies to visualize either viral proteins (e.g., LMP-1 or HBcAg) or cellular proteins increased during infection (e.g., p16) on tissue sections; IPFDT, inflammatory pseudotumor-like follicular dendritic cell tumor; ISH/PCR, viral nucleic acids are amplified by polymerase chain reaction (PCR) and either quantified or visualized on tissue sections (in situ hybridization); LMP-1, latent membrane protein 1; LNA-1, latency-associated nuclear antigen (or LANA1); LP, lymphocyte predominant HD; LSIL, low-grade squamous intraepithelial lesion; PVAN, polyomavirus-associated nephropathy; SCC, squamous cell carcinoma; ss, single-stranded.

Data from Eyzaguirre E, Haque AK: Application of immunohistochemistry to infections. Arch Pathol Lab Med 132:424-431, 2008; McLaughlin-Drubin ME, Munger K: Viruses associated with human cancer. Biochim Biophys Acta 1782:127-150, 2008; Nuovo GJ: The utility of in situ-based methodologies including in situ polymerase chain reaction for the diagnosis and study of viral infections. Hum Pathol 38:1123-1136, 2007; Slifka MK, Hanifin JM: Smallpox: the basics. Dermatol Clin 22:263-274, 2004.

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