

Subject:	Immunohistochemistry		
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I. Policy Description

Immunohistochemistry (IHC) is a very sensitive and specific staining technique that uses anatomical, biochemical, and immunological methods to identify cells, tissues, and organisms by the interaction of target antigens with highly specific monoclonal antibodies and visualization through the use of a biochemical tag or label.¹

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in Section Applicable State and Federal Regulations of this policy document.

1. Code 88342 should be used for the first single antibody procedure and is reimbursed at one unit per specimen, up to four specimens, per date of service.
2. Code 88341 should be used for each additional single antibody per specimen and is reimbursed up to a maximum of 13 units per date of service.
3. Code 88344 should be used for each multiplex antibody per specimen, up to six specimens, per date of service.

Scientific Background

Immunohistochemistry (IHC) is used to identify certain components of tissues or cells (also known as immunocytochemistry) via use of specific antibodies that can be visualized through a staining technique. The premise behind IHC is that distinct tissues and cells contain a unique set of antigens that allows them to be identified and differentiated. The selection of antibodies used for the evaluation of a specimen varies by the source of the specimen, the question to be answered, and the pathologist performing the test.

Importantly, an entirely sensitive and specific IHC marker rarely exists, and therefore, determinations are typically based on a pattern of positive and negative stains for a panel of several antibodies. The four most common IHC staining patterns include nuclear staining, cytoplasmic staining, membrane staining, and extracellular staining.² A single IHC marker approach (other than for pathogens such as cytomegalovirus or BK virus) is strongly discouraged since aberrant expression of a highly specific IHC marker can rarely occur. However, aberrant expression of the entire panel of highly specific IHC markers is nearly statistically impossible.³

Multiplex immunohistochemistry (mIHC) is a particular IHC technique that allows multiple targets in a single tissue to be detected simultaneously; this approach is able to characterize “the tumor microenvironment including vascular architecture and hypoxia, cellular proliferation, cell death as well as drug distribution.”⁴ Hence, mIHC can assist in the development of parameter tumor maps. Other researchers have utilized mIHC for its novel ability to provide quantitative data on different types of tumor-infiltrating immune cells within a single tissue; this may improve cancer patient immunotherapy stratification.⁵

Clinical Utility and Validity

Immunohistochemistry can be used for a variety of purposes including: differentiation of benign from malignant tissue, differentiation among several types of cancer, selection of therapy, identification of the origin of a metastatic cancer, and identification of infectious organisms.⁶ IHC has many uses in the realm of tumor identification, and it has even been clinically used to pinpoint various breast cancer-specific markers, such as progesterone and estrogen receptors, gross cystic duct fluid protein, and mammaglobin.⁷ Further, overexpression of the *HER2* oncogene, a predictive breast cancer biomarker, is often identified via IHC.⁸ In regards to tumor identification, a specific type of IHC, known as pan-Trk IHC, has been shown to positively identify inflammatory myofibroblastic tumors with a nuclear and cytoplasmic staining pattern that may assist in targeted therapy.⁹

Antibodies for use in IHC are available as single antibody reagents or in mixtures of a combination of antibodies. More than 200 diagnostic antibodies are generally available in a large clinical IHC laboratory, and hundreds of antibodies are usually available in research laboratories. The list of new antibodies is growing rapidly with the discovery of new biomarkers by molecular methodologies.¹⁰ Several studies have shown that a relatively low number of antibodies are capable of accurately diagnosing specific cancers and identifying the primary source of a metastasis.¹⁰⁻¹²

Common markers to identify tumor origin:³

Primary Site	Markers
Lung adenocarcinoma	TTF1, napsin A
Breast carcinoma	GATA3, ER, GCDFP15
Urothelial carcinoma	GATA3, UPII, S100P, CK903, p63
Squamous cell carcinoma	p40, CK5/6
RCC, clear cell type	PAX8, RCCma, pVHL, KIM-1

Papillary RCC	P504S, RCCma, pVHL, PAX8, KIM-1
Translocational RCC	TFE3
Hepatocellular carcinoma	Arginase-1, glypican-3, HepPar-1
Adrenal cortical neoplasm	Mart-1, inhibin-a, calretinin, SF-1
Melanoma	S100, Mart-1, HMB-45, MiTF, SOX10
Merkel cell carcinoma	CK20 (perinuclear dot staining), MCPyV
Mesothelial origin	Calretinin, WT1, D2-40, CK5/6, mesothelin
Neuroendocrine origin	Chromogranin, synaptophysin, CD56
Upper GI tract	CDH17, CDX2, CK20
Lower GI tract	CDH17, SATB2, CDX2, CK20
Intrahepatic cholangiocarcinoma	pVHL, CAIX
Pancreas, acinar cell carcinoma	Glypican-3, antitrypsin
Pancreas, ductal adenocarcinoma	MUC5AC, CK17, Maspin, S100P, IMP3
Pancreas, neuroendocrine tumor	PR, PAX8, PDX1, CDH17, islet-1
Pancreas, solid pseudopapillary tumor	Nuclear b-catenin, loss of Ecadherin, PR, CD10, vimentin
Prostate, adenocarcinoma	PSA, NKX3.1, PSAP, ERG
Ovarian serous carcinoma	PAX8, ER, WT1
Ovarian clear cell carcinoma	pVHL, HNF-1b, KIM-1, PAX8
Endometrial stromal sarcoma	CD10, ER
Endometrial adenocarcinoma	PAX8/PAX2, ER, vimentin
Endocervical adenocarcinoma	PAX8, p16, CEA, HPV in situ hybridization, loss of PAX2
Thyroid follicular cell origin	TTF1, PAX8, thyroglobulin
Thyroid medullary carcinoma	Calcitonin, TTF1, CEA
Hyalinizing trabecular adenoma of the thyroid	MIB-1 (unique membranous staining pattern)
Salivary duct carcinoma	GATA3, AR, GCDFFP-15, HER2/neu
Thymic origin	PAX8, p63, CD5
Seminoma	SALL4, OCT4, CD117, D2-40
Yolk sac tumor	SALL4, glypican-3, AFP

Embryonal carcinoma	SALL4, OCT4, NANOG, CD30
Choriocarcinoma	b-HCG, CD10, SALL4
Sex cord–stromal tumors	SF-1, inhibin-a, calretinin, FOXL2
Vascular tumor	ERG, CD31, CD34, Fli-1
Synovial sarcoma	TLE1, cytokeratin
Chordoma	Cytokeratin, S100
Desmoplastic small round cell tumor	Cytokeratin, CD99, desmin, WT1 (N-terminus)
Alveolar soft part sarcoma	TFE3
Rhabdomyosarcoma	Myogenin, desmin, MyoD1
Smooth muscle tumor	SMA, MSA, desmin, calponin
Ewing sarcoma/PNET	NKX2.2, CD99, Fli-1
Myxoid and round cell liposarcoma	NY-ESO-1
Low-grade fibromyxoid sarcoma	MUC4
Epithelioid sarcoma	Loss of INI1, CD34, CK
Atypical lipomatous tumor	MDM2 (MDM2 by FISH is a more sensitive and specific test), CDK4
Histiocytosis X	CD1a, S100
Angiomyolipoma	HMB-45, SMA
Gastrointestinal stromal tumor	CD117, DOG1
Solitary fibrous tumor	CD34, Bcl2, CD99
Myoepithelial carcinoma	Cytokeratin and myoepithelial markers; may lose INI1
Myeloid sarcoma	CD43, CD34, MPO
Follicular dendritic cell tumor	CD21, CD35
Mast cell tumor	CD117, tryptase

Guidelines and Recommendations

Guidelines are lacking regarding the selection and number of antibodies that should be used for most immunohistochemistry evaluations. However, IHC is broadly used for conditions such as cancers, which are mentioned across many different societies. The below section is not a comprehensive list of guidance for immunohistochemistry.

College of American Pathologists (CAP)

The College of American Pathologists has published several reviews in Archives of Pathology & Laboratory Medicine that detail the quality control measures for IHC; further, CAP has also published more than 100 small IHC panels to address the frequently asked questions in diagnosis and differential diagnosis of specific entities. These diagnostic panels are based on literature, IHC data, and personal experience. A single IHC marker approach (other than for pathogens such as cytomegalovirus or BK virus) is strongly discouraged since aberrant expression of a highly specific IHC marker can rarely occur. However, aberrant expression of the entire panel of highly specific IHC markers is nearly statistically impossible.^{3,13}

In 2024, CAP published an update to their guidelines on the principles of analytic validation of immunohistochemical assays. The guidelines include the following recommendations:¹⁴

1. “Laboratories must analytically validate all laboratory developed IHC assays and verify all FDA-cleared IHC assays before reporting results on patient tissues.
2. For initial analytic validation or verification of every assay used clinically, laboratories should achieve at least 90% overall concordance between the new assay and the comparator assay or expected results.
3. For initial analytic validation of nonpredictive laboratory-developed assays, laboratories should test a minimum of ten positive and ten negative tissues. When the laboratory medical director determines that fewer than 20 validation cases are sufficient for a specific marker (eg, rare antigen), the rationale for that decision needs to be documented.
4. For initial analytic validation of all laboratory-developed predictive marker assays, laboratories should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 validation tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.
5. For initial analytic verification of all unmodified FDA-approved predictive marker assays, laboratories should follow the specific instructions provided by the manufacturer. If the package insert does not delineate specific instructions for assay verification, the laboratory should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 verification tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.
6. For initial analytic validation of laboratory-developed assays and verification of FDA-approved or cleared predictive immunohistochemical assays with distinct scoring schemes (eg, HER2, PD-L1), laboratories should separately validate or verify each assay-scoring system combination with a minimum of 20 positive and 20 negative tissues. The set should include challenges based on the intended clinical use of the assay.
7. For laboratory-developed assays with both predictive and nonpredictive applications using the same scoring criteria, laboratories should treat these assays as predictive markers and test a minimum of 20 positive and 20 negative cases.
8. Laboratories should use validation tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically, when possible.
9. For analytic validation of IHC performed on cytologic specimens that are not fixed in the same manner as the tissues used for initial assay validation, laboratories should perform separate validations for every new analyte and corresponding fixation method before placing them into clinical service.
10. A minimum of 10 positive and 10 negative cases is recommended for each validation performed on cytologic specimens, if possible. The laboratory medical director should consider increasing

- the number of cases if predictive markers are being validated. If the minimum of 10 positive and 10 negative cases is not feasible, the rationale for using fewer cases should be documented.
11. If IHC is regularly done on decalcified tissues, laboratories should test a sufficient number of such tissues to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative tissues and the number of predictive and nonpredictive markers to test.
 12. Laboratories should confirm assay performance with at least 1 known positive and 1 known negative tissue when a new antibody lot is placed into clinical service for an existing validated assay (a control tissue with known positive and negative cells is sufficient for this purpose).
 13. Laboratories should confirm assay performance with at least 2 known positive and 2 known negative tissues when an existing validated assay has changed in any one of the following ways:
 1. Antibody dilution
 2. Antibody vendor (same clone)
 3. Incubation or retrieval times (same method).
 14. Laboratories should confirm assay performance by testing a sufficient number of tissues to ensure that assays consistently achieve expected results when any of the following have changed:
 1. Fixative type
 2. Antigen retrieval method (eg, change in pH, different buffer, different heat platform)
 3. Detection system
 4. Tissue processing equipment
 5. Automated testing platform
 6. Environmental conditions of testing (eg, laboratory relocation, laboratory water supply)The laboratory medical director is responsible for determining how many predictive and nonpredictive markers and how many positive and negative tissues to test.
 15. Laboratories should run a full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay.”

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP)

The American Society of Clinical Oncology and the College of American Pathologists currently recommend that “all newly diagnosed patients with breast cancer must have a HER2 test performed.”¹⁵ Also, for those who develop metastatic disease, a HER2 test must be done on tissue from the metastatic site, if available. In less common HER2 breast cancer patterns, as observed in approximately 5% of cases by dual-probe in situ hybridization (ISH) assays, new recommendations have been made to make a final determination of positive or negative HER2 tissue. This new “diagnostic approach includes more rigorous interpretation criteria for ISH and requires concomitant IHC review for dual-probe ISH groups... to arrive at the most accurate HER2 status designation (positive or negative) based on combined interpretation of the ISH and IHC assays;” further, “The Expert Panel recommends that laboratories using single-probe ISH assays include concomitant IHC review as part of the interpretation of all single-probe ISH assay results.”¹⁶

The 2018 update included the following changes from the prior 2013 update, particularly focusing on infrequent HER2 test results that were of “uncertain biologic or clinical significance”:

- “Revision of the definition of IHC 2+ (equivocal) to the original FDA-approved criteria.
- Repeat HER2 testing on a surgical specimen if the initially tested core biopsy is negative is no longer stated as mandatory. A new HER2 test **may** (no longer **should**) be ordered on the excision specimen on the basis of some criteria (such as tumor grade 3).
- A more rigorous interpretation criteria of the less common patterns that can be seen in about 5% of all cases when HER2 status in breast cancer is evaluated using a dual-probe ISH testing. These cases, described as ISH groups two to four, should now be assessed using a diagnostic

approach that includes a concomitant review of the IHC test, which will help the pathologist make a final determination of the tumor specimen as HER2 positive or negative.

The Expert Panel also preferentially recommends the use of dual-probe instead of single-probe ISH assays, but it recognizes that several single-probe ISH assays have regulatory approval in many parts of the world.”¹⁶ The 2018 recommendations were affirmed in 2023.¹⁷

The National Cancer Coalition Network

The NCCN has made numerous recommendations for use of IHC to diagnose and manage various types of cancer. Cancers with clinically useful IHC applications include breast, cervical, various leukemias, and colorectal cancer.

The NCCN states that the determination of estrogen receptor, progesterone receptor, and HER2 status for breast cancer is recommended and may be determined by IHC.¹⁸ Specifically, the guidelines state that “the NCCN Panel endorses the CAP protocol for pathology reporting and endorses the ASCO CAP recommendations for quality control performance of HER2 testing and interpretation of IHC and ISH results.” They also specifically endorse the ASCO/CAP HER2 testing guideline “Principles of HER2 testing,” and state “HR testing (ER and PR) by IHC should be performed on any new primary or newly metastatic breast cancer using methodology outlined in the latest ASCO/CAP HR testing guideline.” Additionally, “PR testing by IHC on invasive cancers can aid in the prognostic classification of cancers and serve as a control for possible false negative ER results. Patients with ER-negative, PR-positive cancers may be considered for endocrine therapies, but the data on this group are noted to be limited.”¹⁸

Further, the NCCN recommendations concerning genetic testing for colorectal cancer state, “the NCCN Panel recommends that, for patients or families where colorectal or endometrial tumor is available, one of two options should be considered for testing among people meeting testing criteria: 1) tumor testing with IHC or MSI, or a comprehensive tumor NGS panel (that includes, at a minimum, the four MMR genes and *EPCAM*, *BRAF* [for CRC only], MSI, and other known familial cancer genes); or 2) germline MGPT that includes at least the four MMR genes and *EPCAM*. The NCCN Panel recommends tumor testing with IHC and/or MSI be used as the primary approach for pathology-lab-based universal screening.”¹⁹ More recently, the NCCN has made additional recommendations to individuals diagnosed with any type of hereditary colorectal cancer (CRC) syndrome; these recommendations state that “all individuals newly diagnosed with CRC have either MSI or IHC testing for absence of 1 of the 4 DNA MMR proteins.”¹⁹

The European Society of Medical Oncology (ESMO)

The ESMO recommends that for cancers of an unknown primary site, “histology and IHC on good quality tissue specimens are required [III, A].”²⁰ Particularly in the context for gastrointestinal carcinomas, ESMO states “Immunohistochemical loss of *BRCA1*-associated protein 1 (BAP1) or AT-rich interactive domain-containing protein 1A (ARID1A) can support the diagnosis but the final decision can only be made in conjunction with clinical and radiological findings.” Other mentions of IHC in their updated 2023 guidelines did not result in any other updated recommendations.²⁰

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Recently, four clinical IHC biomarker assays (PTEN, RB, MLH1, and MSH2) have been validated for use as biomarkers in a nationwide clinical trial; these assays were then approved by the FDA as laboratory-developed tests to assist in the treatment selection of patients in clinical trials.²¹ This shows that IHC assays are currently being utilized with molecular tests to assist in therapeutic decisions.

II. Applicable Codes

Code	Description	Comment
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure	
88342	Immunohistochemistry or immunocytochemistry, per spec; initial single antibody stain	
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure	

III. Definitions

IV. Related Policies

Policy Number	Policy Description
N/A	N/A

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Procedure codes appearing in Reimbursement Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

V. Reference Materials

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VI. Revision History

Revision Date	Summary of Changes
12/03/2025	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria

Disclaimer

Healthfirst's claim edits follow national industry standards aligned with CMS standards that include, but are not limited to, the National Correct Coding Initiative (NCCI), the National and Local Coverage Determination (NCD/LCD) policies, appropriate modifier usage, global surgery and multiple procedure reduction rules, medically unlikely edits, duplicates, etc. In addition, Healthfirst's coding edits incorporate industry-accepted AMA and CMS CPT, HCPCS and ICD-10 coding principles, National Uniform Billing Editor's revenue coding guidelines, CPT Assistant guidelines, New York State-specific coding, billing, and payment policies, as well as national physician specialty academy guidelines (coding and clinical). Failure to follow proper coding, billing, and/or reimbursement policy guidelines could result in the denial and/or recoupment of the claim payment.

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