Immunohistochemistry

Policy Number: AHS –P2018 – Immunohistochemistry	Prior Policy Name and Number, as applicable:
Effective Date: 10/01/2022	

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 though the use of a biochemical tag or label (Fitzgibbons et al., 2014).

II. Related Policies

Policy Number	Policy Title
	Not Applicable

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Medical Policy Statements do not ensure an authorization or payment of services. Please refer to the plan contract (often referred to as the Evidence of Coverage) for the service(s) referenced in the Medical Policy Statement. If there is a conflict between the Medical Policy Statement and the plan contract (i.e., Evidence of Coverage), then the plan contract (i.e., Evidence of Coverage) will be the controlling document used to make the determination.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website https://www.cms.gov/medicare-coverage-database/search.aspx?redirect=Y&from=Overview&from2=search1.asp& or the manual website.

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.

IV. Scientific Background

Immunohistochemistry (IHC) is used to identify certain components of tissues or cells (aka 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 (Tuffaha, Guski, & Kristiansen, 2018). 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 (Lin & Chen, 2014).

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" (Kalra & Baker, 2017). 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 (Hofman et al., 2019).

Clinical Validity and Utility

IHC 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 (Shah, Frierson, & Cathro, 2012). 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 (Hainsworth & Greco, 2017). Further, overexpression of the *HER2* oncogene, a predicative breast cancer biomarker, is often identified via IHC (Yamauchi & Hayes, 2018). 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 (Yamamoto, Nozaki, Kohashi, Kinoshita, & Oda, 2019).

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 (Lizotte et al., 2016). 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 (Le Stang et al., 2019; Lizotte et al., 2016; Prok & Prayson, 2006).

Common markers to identify tumor origin (Lin & Chen, 2014):

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, GCDFP-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

V. 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) (Lin & Chen, 2014; Lin & Liu, 2014)

CAP 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 (Lin & Chen, 2014; Lin & Liu, 2014).

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) (Wolff et al., 2013; Wolff et al., 2018)

ASCO and CAP currently recommend that "all newly diagnosed patients with breast cancer must have a HER2 test performed" (Wolff et al., 2013). 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" (Wolff et al., 2018).

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 2 to 4, 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" (Wolff et al., 2018)

The National Cancer Coalition Network (NCCN, 2021a, 2021b)

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 (NCCN, 2021a). Specifically, the NCCN guidelines state "consistent with the ASCO/CAP guidelines, the NCCN panel considers either IHC or ISH with either a single or dual probe as an acceptable method for making an initial determination of HER2 tumor status." Further, the NCCN recommendations concerning Lynch Syndrome (LS) state, "The panel recommends tumor testing with IHC and/or MSI be used as the primary approach for pathology-lab-based universal screening" (NCCN, 2021b). 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 immunohistochemistry (IHC) testing for absence of 1 of the 4 DNA MMR proteins." (NCCN, 2021b)

The European Society of Medical Oncology (ESMO) (Fizazi et al., 2015)

The ESMO recommends that for cancers of unknown primary, "immunohistochemistry should be applied meticulously in order to identify the tissue of origin and to exclude chemosensitive and potentially curable tumors" (Fizazi et al., 2015).

VI. State and Federal Regulations, as applicable

A search of the FDA Device database on 10/11/2021 for "immunohistochemistry" yielded 12 results. Additionally, 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). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; 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 (Khoury et al., 2018). This shows that IHC assays are currently being utilized with molecular tests to assist in therapeutic decisions.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description

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

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

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
12/01/2021	Initial Effective Date
05/23/2022	Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modifications to the coverage criteria.