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Proposed LCD - Special Histochemical Stains and Immunohistochemical Stains (DL35986)

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CMS National Coverage Policy

Title XVIII of the Social Security Act (SSA), §1862(a)(1)(A), states that no Medicare payment shall be made for items or services that "are not reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member." 42 Code of Federal Regulations (CFR) §410.32 Diagnostic x-ray tests, diagnostic laboratory tests, and other diagnostic tests: Conditions.

Coverage Guidance

Coverage Indications, Limitations, and/or Medical Necessity

This policy does not designate specific special histochemical stains (aka special stains) and/or immunohistochemical (IHC) stains that should be used in the differential diagnosis of tissues or neoplasms because this information is readily available in textbooks and various scientific publications. This policy identifies the medically necessary criteria for the use of special stains and/or IHC stains and addresses, based on claims review, the scenarios that may be driving medically unnecessary over-utilization or incorrect billing of these services including:

- Reflex templates or pre-orders for special stains and/or IHC stains prior to review of the routine hematoxylin and eosin (H&E) stain by the pathologist; or
- Use of special stains and/or IHC stains without clinical evidence that the stain is actionable or provides the treating physician with information that changes patient management, or
- Use of added stains when the diagnosis is already known based on morphologic evaluation of the primary stain.

The surgical pathology report is expected to designate the specific block(s) upon which IHC testing is performed, the reason and results for IHC testing, the specific markers, and whether single antibody(ies) or a cocktail of antibodies is utilized. A statement alone in the pathology report that states "IHC confirms the diagnosis" will not be covered as reasonable and necessary.

Background

Routine hematoxylin and eosin (H&E) staining is the corner stone of tissue-based microscopic diagnosis. Thin sections of tissue are stained with H&E to visualize the tissue morphology. Hematoxylin dye stains the cell nuclei blue and the eosin dye stains other structures pink/red. H&E staining provides excellent detail required for tissue-based diagnosis and is NOT a separate~~ly billable~~ service, as ~~reimbursement for~~ pathology services include~~s~~ routine H&E staining. ~~At least one lab has touted~~ "Acid hematoxylin" ~~as a special stain for purposes of billing Medicare and private payers~~ is not

a special stain given that all hemotoxylin stains are acidic and that this stain has never been recognized by the Biological Stain Commission. It is ~~incorrect coding to present~~ **not reasonable and necessary** to claim~~s for~~ this stain as a special stain. Hematoxylin and eosin (H&E) staining is included ~~in the billing CPT code and is not a separately billable service.~~ **as part of the pathology services**.

Special stains are called "special" because they are dyes used to stain particular tissues, structures or pathogens such as bacteria that may not be visible by routine H&E staining. Special stains can identify whether a substance is present or absent, where the substance is located in the tissue specimen, and frequently, how many or how much of a substance is present. There are special stains to identify bacteria, yeast and fungi; for connective tissue, muscle, collagen, lipid and fibrin; for nuclei acids; and multi-purpose stains to identify basement membranes, mucins, and various other cellular constituents. Two major AMA CPT coding categories for special stains are recognized: One is specifically for microorganisms; the second code is for all other purposes (not microorganisms) and specifically excludes detection of enzyme constituents.

IHC is a powerful tool for identifying substances and cells in tissue sections using the specificity of antigen-antibody reactions, where the antibody is linked to a colored indicator (stain) that can be seen with a microscope. More than 400 distinct antibody targets are currently available with varying sensitivity and specificity for a given target. A major use of IHC is to identify poorly differentiated malignant neoplasms (tumors) such as a carcinoma, lymphoma, melanoma and sarcoma. Some IHC stains are useful in determining the primary site of a metastatic neoplasm, and others are used to guide specific therapies (e.g., Her2 IHC to determine potential response to trastuzumab).

Medical Necessity of Services Performed

There are many different relationships that exist in the ~~providing the~~ provision of pathology services in the United States. Some physicians, groups, laboratories and hospitals submit global claims for the services described in this policy. In other instances, there are separate individuals or entities providing the professional ~~(-26)~~ and the technical services ~~(-TC)~~. It is the obligation of each billing party to recognize that they are responsible for the medical necessity of the charges submitted. For example, when a physician or physician group ~~bills for~~ **performs** the professional component of services described in this policy and another entity bills for the technical services, it is the obligation of each entity to independently assure the medical necessity of the services rendered ~~and billed~~ **by each entity.**

Special Stains/IHC Medical Necessity

~~The IOM, Benefit Policy Manual~~ **The CMS Internet-Only Manual, Pub. 100-02, Medicare Benefit Policy Manual** (CPT15, §80.6.5) specifies "…there may be additional tests, such as special stains, that the pathologist may need to perform, even though they have not been specifically requested by the treating physician/practitioner. The pathologist may perform such additional tests under the following circumstances:

- Services are **medically necessary** so that a complete and accurate diagnosis can be reported to the treating physician/practitioner
- **Results** of the tests are communicated to and are used by the treating physician/practitioner in the treatment of the beneficiary; and
- Pathologist **documents** in his/her report **why** additional testing was done."

The above citation means that reflex templates or pre-orders for special stains and/or IHC stains prior to review of the routine hematoxylin and eosin (H&E) stain by the pathologist are not reasonable and necessary. A pathologist must first review the H&E stain prior to ordering special stains or IHC.

Exceptions do exist and are recognized standards of care in the practice of pathology. These exceptions include but are not limited to renal, liver, and neuromuscular biopsies, and for the suspicion of an infectious disease, particularly in an immune compromised patient. In certain clearly defined circumstances, it may be reasonable to perform some IHC on sentinel lymph nodes when the frozen sections show they are free of tumor.

The medical necessity for the special stain or IHC studies, and the results of the stain or IHC, must be documented in the surgical pathology report.

IHC for Breast Pathology

The clinical care of patients with breast cancer depends upon the accurate diagnosis and the assessment of biomarkers. Hormone receptor assays and Her2 testing are recommended on all primary **invasive** breast cancers, and on recurrent or metastatic cancers. At the current time, there is no recommendation for Her2 testing on in situ breast lesions outside of a clinical trial. While there are a number of promising additional biomarkers, such as Ki-67, PI3K and gene expression assays, the College of American Pathologists (CAP), the American Society of Clinical Oncologists (ASCO) and the National Comprehensive Cancer Network (NCCN) have not recognized these markers in patient treatment pathways.[1-4]

Estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (Her2) are well-established prognostic markers in invasive breast cancer management. The triple negative breast carcinoma subtype (ER-/PR-/Her2-) has been associated with worse overall prognosis in comparison with other subtypes in study populations consisting of ethnic minorities and young women.[1]

Ki-67 expression is a biomarker for proliferation and has been associated with response to therapy, but methods of measurement are controversial. In December, 2013, the CAP reported that there is “a lack of consensus on scoring, definition of low versus high expression, an appropriate cut point for positivity, or which part of the tumor should be scored (e.g., leading edge, hot spots, overall average).[3] There is also paucity of data on the effects of pre-analytical variables (e.g., ischemic time, length of fixation, antigen retrieval) on Ki-67 staining. For these reasons, routine testing of breast cancers for Ki-67 expression is not currently recommended by either ASCO or the NCCN.[5-6] ~~Consequently, Ki-67 is not reasonable and necessary for breast cancer and will not be covered by Medicare.~~

More recent evidence identifies the use The use of the PharmDx Ki-67 (MIB-1) by Agilent Technologies (formerly Dako; Santa Clara, Ca) as a companion diagnostic shown to define a high-risk population which along with high risk clinicopathologic features (nodal status, tumor size, and grade). This is used to identify patients with an even greater risk of recurrence and thus has prognostic value in the population of patients with Estrogen Receptor positive (ER+), Her2 negative (HER2-) lymph node positive high risk breast cancer for use of the Cyclin-dependent 4 and 6 (CDK 4/6) inhibitor abermaciclib (Eli Lilly and Company)

as adjuvant therapy in addition to endocrine therapy. With 19 months of median follow up time abemaciclib + endocrine therapy (ET) resulted in a 29% reduction in the risk of developing an invasive disease-free survival (IDFS) event [hazard ratio (HR) = 0.71, 95% confidence interval (CI) 0.58-0.87; nominal P = 0.0009]. At the additional follow-up analysis, with 27 months median follow-up and 90% of patients off treatment, IDFS (HR = 0.70, 95% CI 0.59-0.82; nominal P < 0.0001) and DRFS (HR = 0.69, 95% CI 0.57-0.83; nominal P < 0.0001) benefit was maintained. The absolute improvements in 3-year IDFS and distant relapse free survival (DRFS) rates were 5.4% and 4.2%, respectively. Whereas a high centrally determined Ki-67 index defined as greater than or equal to 20% was prognostic for recurrence in this treatment setting it was not predictive of the treatment effect as abemaciclib benefit was observed regardless of Ki-67 index. Safety data were consistent with the known abemaciclib risk profile7. This is supported by updates to NCCN Guidelines and International Ki67 workgroup 6,8. Outside of this exception, Ki-67 is not considered reasonable and necessary for breast cancer and consequently will not be covered by Medicare.

The clinical utility of testing for hormone receptors in in-situ breast cancer differs from those of invasive disease. ~~Guidelines and the peer reviewed literature support the use of ER testing for in-situ breast neoplasia and PR testing only when the ER status is negative (Lester, personal communication)~~. **2020 Asco/CAP Guidelines for Ductal Carcinoma in-situ (DCIS) testing state: "ER testing in cases of newly diagnosed DCIS (without associated invasion) is recommended to determine potential benefit of endocrine therapies to reduce risk of future breast cancer. PR testing is considered optional" [recommendation 4, and subsequent discussion].[5] This is supported by the peer reviewed literature which support the use of ER testing for in-situ breast neoplasia. The addition of PR testing should be determined in those settings where it has been deemed reasonable and necessary and its relevance has been documented in the pathology report and individual patient. Clinical guidelines have not been established for the use of Her2 or other biomarkers in patients with non-invasive breast neoplasia.[5]** Clinical guidelines have not been established for the use of Her2 or other biomarkers in patients with non-invasive breast neoplasia.[5]

In the absence of professional guidelines based on proven scientific literature, standing orders from clinicians for such tests as Ki-67 and EGFR on every breast cancer are not reasonable and necessary, and are not a covered Medicine service.

~~In addition,~~ Basal phenotype markers (eg, IHC for CK5) are not routinely necessary. Neither are IHC stains such as E-cadherin, p27, or high molecular weight cytokeratin to distinguish ductal from lobular differentiation **reasonable and** necessary on every breast case, nor are myoepithelial cell markers such as p63 or smooth muscle myosin heavy chain **routinely** necessary on every case. **The use of these markers should be determined by the pathologist when there are ambiguous histologic/morphologic findings on H&E and the distinction between lobular and ductal differentiation or usual ductal hyperplasia (UDH) versus atypical ductal hyperplasia (ADH) and Ductal Carcinoma in Situ (DCIS) are critical to the clinical management of the patient and its rationale is documented in the pathology report.[9].**

Special Stains and/or IHC for GI Pathology

Pathologists are often called upon to microscopically diagnose abnormalities seen on

endoscopic exam of the esophagus, stomach, duodenum and colon. Biopsy specimens constitute an important diagnostic patient service. Most normal and abnormal conditions of these organs can be detected by the **use** of routine H&E stain **alone**.

Only the pathologist may determine the medical necessity of a special stain. Ordering special stains or IHC stains prior to review of the routine H&E stain is not reasonable and necessary. For most esophageal, gastric and duodenal specimens, it is not reasonable or necessary to perform special stains such as alcian blue – periodic acid Schiff (AB-PAS), or other mucin stains, such as diastase – PAS (D-PAS), or IHC stains such CDX-2 to determine if clinically meaningful intestinal metaplasia is present. In addition, it is not usually reasonable and necessary to perform special stains or IHC to determine the presence of H. pylori organisms.[10-12]

Other examples of special stains or IHC that are not reasonable and necessary on every specimen include:

- Esophagus – fungal stains, trichrome, D-PAS, CDX-2 or other mucin stains
- Gastric – AB-PAS, D-PAS, CDX-2 or other mucin stains, or special stains or IHC for H. pylori, or neuroendocrine markers such as synaptophysin or chromogranin
- Duodenum – AB-PAS, D-PAS, CD3, and trichrome, or other mucin stains
- Colon – CD3, p53 trichrome
- Hyperplastic polyps – Ki67, CK20, p53, CEA, BRAF
- Tubular or tubulovillous adenoma – Ki-67, CK20, CEA, p53, MMR

If special stains or IHC are needed in addition to the routine H&E for gastric specimens, specific documentation to justify the medical necessity for the stain is required in the pathology report. Cases that may require special stains or IHC include but are not limited to the following:

- Detection of H pylori in an appropriate milieu when organisms are not seen on H&E stained slides;
- Evaluating atrophic gastritis for evidence of autoimmune etiology and for enterochromaffin-like (ECL) cell hyperplasia/carcinoid tumor
- Characterizing a carcinoma, lymphoma, melanoma or sarcoma
- Defining a GIST tumor and to distinguish it from mimics
- Ki-67 by IHC in the differential diagnosis of certain neuroendocrine tumors of the gut

Scientific data demonstrates that the combined number of gastric biopsies requiring special stains or IHC is roughly 20% of biopsies received and examined in a pathology practice. GI specialty practices with a large GI referral base or GI consultant pathologists may sometimes exceed this relative number of special stains/IHC, but one would not expect to see routine high utilization of special stains or IHC. **To check utilization, we encourage providers to perform a self-audit on the number of separate gastric biopsies as compared to ancillary stains. The ancillary stain group should be lass than 20% of the total gastric biopsies submitted. Providers that exceed the 20% criteria may be subject to additional action.** [13-14]

Over-utilization of special stains has also been observed with duodenal biopsies where CD3 and AB/D-PAS are reportedly used to help exclude intraepithelial lymphocytosis

and gastric metaplasia. Both of these conditions, if present, are easily recognizable on H&E morphology. Mucin stains such as AB-PAS or DPAS would be reasonable and necessary in limited circumstances, and rarely is CD3 warranted on duodenal biopsies which show villous architectural abnormalities.

Architectural and histologic features define colonic polyps including hyperplastic, inflammatory, and adenomatous lesions. Special stains and/or IHC stains are not reasonable and necessary for colon polyps despite textbooks noting, for example, thickened subepithelial collagen demonstrated by trichrome or collagen staining in hyperplastic polyps, or carcinoembryonic antigen (CEA) overexpression in hyperplastic polyps. While the information is of academic interest, special stains are not reasonable and necessary to make the diagnosis of various colonic polyps.

Lynch Syndrome (LS) is a genetic predisposition to colorectal cancer (CRC) and certain other malignancies as a result of an autosomal dominant germline mismatch repair (MMR) gene mutation. There is benefit in identifying an asymptomatic individual with LS as it allows for early and intensive surveillance to detect colon polyps, which can prevent malignancies and reduce the risk of premature death".[15]

- LS tumor screening for **microsatellite instability (MSI)/**DNA mismatch repair (MLH1, MSH2, MSH6 and PMS2) by qualitative IHC ~~and/or microsatellite instability (MSI~~ is considered medically necessary and covered by Medicare for ~~the following indications~~ **individuals with newly diagnosed colorectal cancer or endometrial cancer.**

- ~~All individuals with colorectal cancer diagnosed at age ≤70 years of age, and those > 70 years of age who meet the revised Bethesda guidelines OR~~
- ~~Individuals with endometrial cancer~~

No definitive **or clearly superior** algorithm for LS screening has been recommended. ~~However, if IHC is done first and is abnormal, MSI testing is not warranted. If IHC is normal, MSI may be warranted. IHC testing Lynch syndrome is qualitative and does not require the use of tumor morphometry.~~ **Microsatellite instability (MSI) testing or immunohistochemical (IHC) testing (with or without *BRAF V600E* mutation testing) for MLH1, MSH2, MSH6 and PMS2 of the tumor tissue are examples of preliminary testing strategies that could be used to select patients for subsequent diagnostic testing. Diagnostic testing involves MMR gene mutation (and deletion/duplication) testing of the proband, usually using a blood sample. Lynch syndrome is most commonly caused by mutations in the two MMR genes MLH1 and MSH2 and less commonly by mutations in MSH6 and PMS*. T*he presence of a BRAF mutation essentially excludes LS as virtually 100% of individuals with LS do not carry the BRAF mutation. The use of BRAF mutation testing by IHC is usually restricted to CRC cases with absent staining for MLH1.[16]**

If IHC is normal and there is clinical evidence to consider additional testing. MMR gene mutation testing may be warranted. IHC testing for LS is qualitative and does not require the use of tumor morphometry for evaluation.

Special Stains and/or IHC for Prostate Pathology

The accuracy of the pathologic diagnosis of prostate cancer is critical for optimal patient

care. The diagnosis can usually be made on morphologic features such as growth pattern, nuclear atypia and the absence of basal cells. However, it may be difficult to reach a firm diagnosis by routine H&E stain for small foci of cancer in needle biopsies because many benign conditions can mimic prostate cancer.[17]

The immunohistochemical diagnosis of prostate cancer largely depends on panels of markers because no absolutely specific and sensitive marker for prostate cancer has yet been identified. These panels usually include at least one basal cell marker, such as high-molecular-weight cytokeratin (HMWCK) or p63, and the prostate cancer-specific marker, alpha-methyl-CoA-Racemase (AMACR). Although AMACR is considered a useful IHC marker for prostate cancer, because of non-standardized immunostaining protocols, interpretation criteria and heterogeneous staining pattern, there is wide variation in the sensitivity and specificity of AMACR immunoreactivity in prostate biopsies. Furthermore, because AMACR expression has been demonstrated in high-grade **prostatic intraepithelial neoplasia** (PIN), atypical adenomatous hyperplasia/adenosis and nephrogenic adenoma, it is recommended that AMACR is best restricted to the evaluation of morphologically highly suspicious foci in which negative immunoreactivity of basal cell markers alone is insufficient to establish a diagnosis of cancer.[18-19]

PTEN and MYC may provide some prognostic information but neither is part of any standard treatment protocol and neither should be routinely performed.[20] ERG is another IHC that is more likely to be positive in cancer than in benign tissue, but it does not add information to conventional PIN4 testing.[21] Similarly, neuroendocrine markers, such as IHC for synaptophysin, may be indicated in cases of recurrent/metastatic prostate carcinoma that have undergone small cell transformation after hormone therapy. The latter marker is only necessary for high grade, undifferentiated tumors and should not be used routinely.[17]

PIN4 is an IHC cocktail of CK5/14, p63 and P504S that is used primarily to differentiate normal and neoplastic epithelial tissues. In prostate tissue, CK5 and CK14 are detected in basal cells of normal glands and PIN which is a precursor lesion to prostatic adenocarcinoma. However, expression of CK5 and CK14 is not identified in invasive prostatic adenocarcinoma. P63 is detected in nuclei of basal epithelium in normal prostate glands but is not expressed in malignant prostate tumors. Because P504S (aka AMACR) is not specific for prostatic adenocarcinoma, the use of PIN4 is best restricted to evaluation of morphologically highly suspicious foci.

It is not reasonable and necessary to ~~bill for~~ **perform** IHC testing (either single antibody or antibody cocktails) on cases with morphologically negative cores. It is not reasonable and necessary to ~~bill for~~ **perform** IHC testing in a negative or a suspicious core biopsy when obvious prostate cancer is present in other cores. While the pathologist may choose to confirm a suspicious focus in one or more cores in a case where the diagnosis of cancer has already been made, it is not a Medicare covered service because it provides no additional actionable information to the treating physician.

Prostate cases that may require reasonable and necessary IHC staining include but are not limited to the following:

- Indeterminate/suspicious focus and no other cores are positive for cancer;
- Single worrisome core with minimal % tumor (roughly <5%);

- Worrisome core(s) contralateral to a positive core(s):
    - In a multi-part biopsy with Gleason 3+3=6 cancer in 1 part, and atypical small acinar proliferation (ASAP) suspicious for Gleason 3+3=6 cancer in other part(s); the number of positive biopsy sites and % core involvement of these sites can affect therapeutic choices for active surveillance (AS), focal therapy or surgery
    - In a multi-part biopsy with 4+3=7 or 4+4=8 cancer in 1 part, and ASAP suspicious for the same grade cancer in other part(s); workup is justified since the extent of high-grade cancer affects treatments
- Identify tumor invasion of adjacent structures
- Determine origin of undifferentiated/poorly differentiated neoplasm, such as bladder vs prostate
- Other unexpected results when specific cell stains would be necessary

Prostate cases when IHC workup is **Not Reasonable and Necessary** include the following:

- In a multi-part biopsy with ≥ **=**3+4=7 cancer in 1 part, and ASAP suspicious for 3+3=6 cancer in other part(s), because stains are unlikely to change treatment; or
- In a multi-part biopsy with ≥ **=**4+3=7 cancer in 1 part, and "~~atypical~~ cribriform **glands that include a differential of Intraductal Carcinoma of Prostate (ICD-P) "atypical cribriform lesson" (ACL)** suspicious for intra-ductal carcinoma versus invasive, Gleason pattern 4 cancer in other part(s), because intra-ductal carcinoma is almost always closely associated with invasive high-grade cancer **and the results will not change the overall highest Gleason grade/Grade group for the case and may not change treatment.**

The International Society of Pathology (ISUP) recommendations state that at the current time, there are no prognostic IHC or molecular studies that are recommended to be routinely performed on biopsy or resection specimens.[22] ~~The surgical pathology report is expected to designate the specific block(s) upon which IHC testing is performed, the reason for IHC testing, the specific markers, and whether single antibody(ies) or a cocktail of antibodies is utilized. A statement alone in the pathology report that states "IHC confirms the diagnosis" will not be covered as reasonable and necessary.~~

Special Stains and/or IHC for Lung Cancer

The diagnostic challenge of a lung biopsy can often prompt the need for additional stains to define the neoplasm. Two important considerations need to be considered in this regard:

- The diagnosis of squamous cell cancer can often be made without the use of any special stains, and
- The diagnosis of non-small cell carcinoma often requires additional stains but it is essential that tumor tissue be carefully triaged to allow the patient's sample to be tested for molecular markers (EGFR, ALK, and others) when clinically indicated.

Experts in pulmonary pathology recommend starting the evaluation of non-small cell carcinomas with a combination of TTF-1 and p40 or p63 IHCs. Often these two stains are all that are needed to come to a reasonable diagnosis and retain enough tumor

sample to complete molecular studies. In rare patients, a few additional IHCs or mucin stains may be needed.

Ki-67/MIB-1

Ki-67 and MIB-1 monoclonal antibodies are directed against different epitopes of the same proliferation-related antigen. These stains are used to determine the proliferative rate of a tumor. Ki-67 antigen or protein (hereafter Ki-67) is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). By measuring the amount of tumor cells expressing Ki-67, an estimate of DNA synthesis can be determined which has been found comparable to a mitotic count performed on a standard H&E slide. Furthermore, Ki-67/MIB-1 antibodies have suffered from a lack of international standardization which has limited their clinical usefulness.[23-24] This is noted above in the discussion of breast cancers.

~~Classification of lung neuroendocrine (NE) tumors is a step-wise process with four tumor categories being identified by morphology, namely:~~

- ~~Typical carcinoid (TC),~~
- ~~Atypical carcinoid (AC),~~
- ~~Large cell NE carcinoma, and~~
- ~~Small cell lung carcinoma (SCLC).~~

~~Ki-67 has potential usefulness in a narrow range of pathologic lung cases. Namely, it allows better classification of atypical and typical lung carcinoid tumors, and in pulmonary neuroendocrine tumors with extensive crush artifact. (As noted above, Ki-67 may be useful in the classification of some gut neuroendocrine tumors.)~~

Ki67 has been shown to be useful in the management and grading of neuroendocrine tumors of the gastrointestinal tract and pancreas. The North American Neuroendocrine Tumor Society (NANETS) in its consensus 2020 guidelines for the management and treatment of neuroendocrine tumors states these tumors should be graded according to the World Health Organization (WHO) Classification of Digestive system Tumors. Grading recommends "Ki67 and/or mitotic rate should be obtained. When both mitotic rate and Ki67 are obtained and grade is discrepant the higher grade determined by mitotic rate or Ki67 is assigned with Grade 1 (G1) tumors showing <2 mitoses/10 HPF or <3% Ki67, Grade 2 (G2) tumors showing 2-20 mitoses/10 HPF or 3-20% Ki67 and Grade 3 (G3) tumors showing >20 mitoses/10 HPF or Ki67 > 20%"[25]

When referring to Thoracic (lung) neuroendocrine tumors the NANETS society in the same consensus 2020 guidelines quoted above states "mitotic rate should be obtained. Use of the World Health Organization (WHO) and International Association for the Study of Lung Cancer grading system is recommended. Mitotic rate in mitoses /10 HPF is recommended. Ki-67 may be considered. Ki-67 (when necessary) is recommended along with mitotic rate to classify Grade 3 (G3) neuroendocrine lung tumors where mitotic rate >10 mitoses/10 HPF and Ki67 >20% classifies these as poorly differentiated neuroendocrine tumors".

Ki67 can be used as an aid in the distinction of low grade versus high grade neuroendocrine tumors where the biopsy or cytology specimen is limited or suffers from significant artefact.[25]

Ki-67 by IHC has clinical utility in the workup of lymphomas. Ki-67 has several established applications including:

- Final confirmation for the diagnosis of any low-grade lymphoma. A number of publications show a worse prognosis for follicular lymphomas which appear to be grade 1 or 2 but demonstrate high Ki-67 labeling. Similarly, small lymphocytic lymphomas/CLL with a high proliferative rate ("prolymphocytic progression") may be best detected with Ki-67.
- Distinguishing higher versus lower grade mantle cell lymphoma. A small percentage of cases behave as low grade rather than intermediate grade, and Ki-67 is the most accurate means to detect this subgroup. In addition, distinguishing the highly aggressive blastoid variant is aided by Ki-67 IHC testing.
- Recognizing Burkitt and Burkitt-like grouping as distinct from diffuse large B-cell type. One of the most important qualifying criteria is Ki-67 labeling at greater than 90%.
- Plasma cell myeloma proliferative rate has long been established as one of the most accurate prognostic markers.

IHC for ~~Chemosensitivity and Resistance~~ Predictive Marker Tumor Profiling

ER, PR, and Her2 hormonal receptor status have demonstrated clinical utility in invasive breast cancer, as well as ER, and PR when appropriate, for in-situ breast cancer. ER and PR are performed by IHC specifically for tamoxifen therapy. Her2 testing has proven clinical utility in esophago-gastric and gastric cancers to determine response to trastuzumab. ER, PR and Her2 testing for the purpose of identifying patients likely to respond to hormonal therapy, biologics or chemotherapy is a covered Medicare service when medically necessary for breast and gastric adenocarcinoma.

Similarly, the efficacy of imatinib, a CD117 inhibitor, is determined by the mutation status of CD117 expression (c-KIT mutation). CD117 by IHC has a proven clinical benefit in gastrointestinal stromal tumors (GIST), some advanced dermatofibrosarcoma protuberans (DFSP), some lymphoblastic and myeloid leukemias, and mast cell tumors, and is a covered Medicare service when medically necessary. **All predictive tumor profiles must have peer reviewed analytical and clinical validity**

However, IHC testing as above is distinctly different from chemotherapy sensitivity and/or resistance testing profiles offered by some labs to assist physicians in their selection of specific chemotherapeutic agents based on IHC antigen or protein expression in individual tumors. The goal stated by these profiles is to select a drug or combination of drugs from a panel of drugs to which a tumor has greater expression, and to avoid drugs to which the tumor has less expression.

Neither the ASCO nor the NCCN has endorsed chemosensitivity tumor profile testing by IHC. ASCO has stated, "the use of CSRA's (chemosensitivity and resistance assays) to select chemotherapeutic agents for individual patients is not recommended outside of the clinical trial setting." While the NCCN's Guidelines for Ovarian Cancer (V3.2014)

states "chemosensitivity/resistance and/or other biomarker assays are being used in some NCCN member institutions for decisions related to future chemotherapy in situations where there are multiple equivalent chemotherapy options available.[26] The current level of evidence is not sufficient (Category 3) to supplant standard of care chemotherapy." The NCCN panel also stated that in vitro chemosensitivity testing to choose a chemotherapy regimen for recurrent disease should not be recommended due to lack of demonstrated efficacy. ~~Such IHC panels include but are not limited to the following biomarkers for specific drugs:~~

- ~~ALK for crixotinib, penetrexed~~
- ~~Androgen receptor (AR) for goserelin, leuprolide, gonadorelin, flutamide, bicalutamide, abiraterone;~~
- ~~Androgen receptor for bicalutamide, flutamide, abiraterone and enzalutamide;~~
- ~~AREG for cetuximab, panitumumab~~
- ~~BRAF for venurafenib and dabrafenib~~
- ~~BRCA1 for cisplatin, carboplatin~~
- ~~cKIT for sorafenib, sunitinib, imatinib~~
- ~~cMET for erlotinib, gefitinib~~
- ~~EGFR for gefitinib, panitumumab, erlotinib, cetuximab, FOLFIRIEGFRVIII~~
- ~~EGFRvIII, GNA11, GNAQ, IDH2 – for clinical trials~~
- ~~ER and PR for tamoxifen, gefitinib, toremifene, fulvestrant, letrozole, anastrozole, exemestrane, megestrol acetate, erlotinib, panitumumab, medroxyprogesterone;~~
- ~~ERCC1 for oxaliplatin, cisplatin, carboplatin, CAPOX, FOLFOX~~
- ~~EREG for cetuximab, panitumumab~~
- ~~Her2 (ErbB2), PGP and TOP2A (topoisomerase IIA) for doxorubincin, liposomal-doxorubicin, epirubicin;~~
- ~~Her2 or labatinib; epirubicin, pertuzumab, trastuzumab, liposomal doxorubicin, doxorubicin,~~
- ~~KRAS for panitumumab, cetruximab, gefitinib, erlotinib, sorafenib~~
- ~~MGMT for temozolomide and dacarbazine~~
- ~~MRP1 for vinorelbine, vincrisxtine, doxorfubicin, epirubicin, vinblastine, methotrexate~~
- ~~NRAS for cetuximab, panitumumab~~
- ~~PDGFRA for imatinib~~
- ~~PGP (aka MDR1 and ABCB1) for doxorubicin, vincristine, vinblastine, eptoposide, liposomal doxorubicin, paclitaxel , docetaxel, vinorelbine, epirubicin;~~
- ~~PIK3CA for lapatinib, panitumumab, trastuzumab, cetuximab, temsirolimus~~
- ~~PTEN for getitinib, cetuximab, erlotinib, trastuszumab, panitumumab, everolimus, temsirolimus~~
- ~~RET for vandetanib~~
- ~~ROS1 for crizotinib~~
- ~~RRM1 for gemcitabine;~~
- ~~SPARC (monoclonal and polyclonal) for nab-paclitaxel;~~
- ~~TLE3, TUBB3 for docetaxel, paclitaxel;~~
- ~~TOPO1 for irinotecan, topotecan, FOLFIRI;~~
- ~~TS (thymidylate synthase or TYMS) for fluorouracil, capecitabine and pemetrexed~~

Chemosensitivity profile tumor panels, regardless of whether it is performed by IHC or chromogenic in-situ hybridization (CISH), is not reasonable and necessary for the reasons cited above and is not a Medicare covered service.

Note, some of these markers are legitimate biomarkers for specified drugs when performed by mutation analysis or FISH testing.

IHC for Cervical/Gyn/Bladder/Kidney Tumors

A variety of IHC stains have found limited use in cervical, gynecologic, and urologic tumor settings. In unusual cases of cervical dysplasia, markers or surrogate markers for HPV may be useful where the diagnosis on conventional H&E stain cannot be made with certainty. These markers are clearly not reasonable and necessary on all biopsies. Claims data indicate combinations of gram stain, PAS, Ki-67, p16 and ProExC stains on all cervical biopsies from select pathology practices, and combinations of p53, Ki-67, CD20 and CD44 on bladder biopsies from select pathology practices.[26]

Similarly, it is rare to need stains to prove that an endometrial or ovarian cancer is a serious cancer or that a kidney neoplasm is an oncocytoma or an eosinophilic or chromophobic renal cell cancer. The use of IHC stains in these circumstances requires adequate documentation in the pathology report, such as “Because the differential histologic diagnosis is between an endometrioid carcinoma and a serous carcinoma, I performed an xxx stain. The controls worked appropriately, and the results were positive indicating the tumor is a yyy.”

IHC for Skin & Cutaneous/Soft Tissue/Central Nervous System (CNS) & Peripheral Nervous System (PNS) Lesions

It is well recognized that most skin lesions are diagnosed with routine H&E slides. That is the case for most melanomas and other pigmented lesions as well. A minority of skin lesions require immunostains (e.g., atypical fibroxanthomas, Merkel cell lesions, lymphomas). Most common skin lesions (e.g., seborrheic keratosis) do not require IHC stains. Use of IHC morphometric codes for skin lesions is incorrect coding.

Similarly, most soft tissue lesions do not require IHC stains or other “special” stains. Soft tissue masses may require stains (e.g., smooth muscle differentiation in a malignant mass) but the most do not.

Many CNS and peripheral nervous system lesions are readily diagnosed with routine stains. It is unusual for a meningioma to require an IHC. The primary role of IHC for CNS and PNS lesions is to differentiate primary from metastatic lesions.

IHC for Bone Marrow Samples

Most bone marrow samples are diagnosed with the use of Wright’s stained smears and the use of H&E stained slides with an iron stain supplementing the battery. The use of IHC stains may assist in the interpretation of cases where flow cytometry (FC) does not fit with the routine slide interpretation, when FC was not obtained or for the evaluation of cell types that are not detected or significantly underrepresented in FC studies, such as large lymphocytes, plasma cells and Reed-Sternberg cells. IHC stains are generally not needed to confirm the results of FC and cytogenetic studies. When medically indicated,

justification for the use of both methods must be stated in the pathology report and billed accordingly.

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