NOTE: Parallel staining is required to control for variables such as disparity in the lots of detection reagents or instrument function. New lots of primary antibody and detection system reagents must be compared to the previous lot using at least one known positive control and one known negative control cellular samples or tissue. This comparison should be made on slides cut from the same control block.

Evidence of Compliance:

✓ Records of confirmation of new reagent lots

CYP.04390 Immunocytochemistry Assay Performance **Phase I**

Laboratories confirm assay performance when conditions change that may affect performance.

NOTE: A change in antibody clone requires full revalidation/verification of the assay (equivalent to initial analytic validation/verification - see CYP.04370).

Laboratories must confirm assay performance with at least two known positive and two known negative cases when an existing validated/verified assay has changed in any of the following ways: antibody dilution, antibody vendor (same clone), or the incubation or retrieval times (same method).

A more extensive study to confirm acceptable assay performance in accordance with published guidelines must be performed when any of the following have changed: fixative type, antigen retrieval protocol (eg, change in pH, different buffer, different heat platform), antigen detection system, cytologic preparation/tissue processing or testing equipment, environmental conditions of testing (eg, laboratory relocation), or laboratory water supply. This study must include a representative sampling of the assays affected by the change and an appropriate number of positive and negative cases per assay, sufficient to confirm acceptable assay performance. The laboratory director is responsible for determining the extent of the study. The rationale for the assays selected and number of positive and negative cases checked per assay must be recorded.

For specific validation/verification requirements for tests that provide independent predictive information (eg, HER2 and ER testing in breast carcinoma), refer to the subsection "Predictive Markers" in the Anatomic Pathology Checklist.

REFERENCES
1) Goldsmith JD, Troxell M, Roy-Chowdhuri S, et al. Principles of analytic validation of immunohistochemical assays: guideline update. *Arch Pathol Lab Med.* 2024. https://doi.org/10.5858/arpa.2023-0483-CP

CYP.04410 Slide Quality **Phase II**

The immunocytochemical stains produced are of acceptable technical quality.

NOTE: The inspector must examine examples of the immunochemical preparations offered by the laboratory. A reasonable sample might include 5-10 diagnostic antibody panels.

REFERENCES
1) Shellhorn N. IHC troubleshooting tips. *Advance/Lab*. 2000;9(1):33-37

PREDICTIVE MARKERS

The term predictive marker as used in this section refers to immunocytochemical biomarkers used independent of histologic and cytopathologic findings to identify individuals who are more likely to experience a favorable or unfavorable effect from a specific (targeted) therapy compared to individuals with the same diagnosis lacking the biomarker. Rather than confirming a specific diagnosis (such as B-cell lymphoma or gastrointestinal stromal tumor), these biomarkers predict responsiveness to a specific treatment among cases of the same diagnosis. For example, this section applies to estrogen receptor testing used to determine eligibility for

hormonal treatment of breast carcinoma but does not apply to estrogen receptor testing used solely to assist in determining the primary site of origin of a metastatic neoplasm.

The current CAP guidelines (https://www.cap.org/protocols-and-guidelines/current-cap-guidelines) relating to predictive marker testing (eg, ASCO/CAP HER2 and ER testing in breast cancer) may be found at cap.org in the Protocols and Guidelines section. The guidelines are periodically updated based on new evidence. Laboratories should review updated predictive marker guidelines and promptly implement changes for items relating to requirements in the checklists (eg, validation, fixation, scoring criteria).

Inspector Instructions:

	• Predictive markers policies and procedures • Sampling of patient reports for completeness, including ASCO/CAP scoring when applicable • Records of annual benchmark comparison for breast predictive markers, if applicable to the patient population tested • Records of annual analyte-specific quality assessment, as applicable • Sampling of predictive marker assay validation, verification, and revalidation/verification studies
	• What is your laboratory's course of action when negative HER2 and/or negative ER by immunocytochemical results are obtained and the fixation was not appropriate? • How did you validate/verify the most recently added predictive marker on your test menu?

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CYP.04510 Report Elements **Phase II**

For immunocytochemical tests that provide independent predictive information, the patient report includes information on specimen processing, the antibody clone, and the scoring method used.

NOTE: The laboratory processing the cytology specimen must record the cold ischemia time (if applicable) and the length of time in fixative. If the cytopathology laboratory refers immunocytochemistry or ISH studies, this information must be provided to the laboratory(ies) performing these studies.

For immunocytochemical studies used to provide predictive information independent of diagnosis or other cytopathologic findings (eg, estrogen receptors and HER2 in breast carcinoma, PD-L1 and lung adenocarcinoma predictive immunostains), the laboratory must include the following information in the patient report:

1. *The type of specimen fixation and processing (eg, formalin-fixed paraffin-embedded sections, air-dried imprints, etc.)*
2. *The antibody clone and general form of detection system used (eg, LSAB, polymer, proprietary kit, vendor name, etc.; information on the type of equipment used is not necessary)*
3. *Criteria used to determine a positive vs. negative result, and/or scoring system (eg, percent of stained cells, staining pattern)*
4. *Laboratory interpretation of predictive marker testing is reported according to the manufacturer's instructions, or when available, following the structure, format, and criteria set forth in the current CAP guidelines relating to predictive marker testing (eg, ASCO/CAP HER2 and ER testing in breast cancer and CAP/ASCP/ASCO HER2 in gastroesophageal carcinoma)*
5. *Limitations relating to suboptimal preanalytical factors that may impact results, such as prolonged cold ischemia time, unknown ischemia time, or over- or under-fixation.*