antibody with the results of testing the same cellular samples or tissue in another laboratory with a validated/verified assay; or 4) comparing the results using the new antibody with previously validated/verified non-immunocytochemistry tests or testing previously graded tissue challenges from a formal proficiency testing program.

For an initial validation/verification, laboratories should achieve at least 90% overall concordance between the new test and the comparator test or expected results.

*For validation of **laboratory-developed or modified FDA-cleared/approved nonpredictive assays**, the validation must be performed on a minimum of 10 positive and 10 negative cellular samples or tissues.*

*For verification of **unmodified FDA-cleared/approved nonpredictive assays**, the laboratory must follow the instructions provided by the manufacturer. If the instructions do not list a minimum number of samples for assay verification, the verification must be performed on a minimum of 10 positive and 10 negative cellular samples or tissues.*

If the laboratory director determines that fewer validation/verification cases are sufficient for a specific marker (eg, a rare antigen or cell/tissue), the rationale for that decision needs to be recorded. Positive cases in the validation/verification set should span the expected range of clinical results (expression level), especially for those markers that are reported quantitatively.

For p16/Ki67 dual stain testing performed on gynecologic cytopathology specimens using FDA-cleared/approved kits, the laboratory must verify that test performance is consistent with the manufacturer's validation data.

When possible, laboratories should use cellular samples or tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically. If immunocytochemistry is regularly done on specimens that are not fixed or processed in the same manner as the cellular samples or tissues used for validation/verification (eg, air-dried touch imprints, air-dried and/or alcohol fixed smears, cytocentrifuge or other liquid-based preparations, and cellular materials fixed in alcohol blends or other fixatives), the laboratory should test a sufficient number of such cellular samples or tissues to ensure that assays consistently achieve expected results with the alternative fixative/processing conditions. The laboratory director is responsible for determining the number of positive and negative cases and the number of markers to test.

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

Evidence of Compliance:

✓ Records of validation/verification, if applicable

REFERENCES

1) Hsi ED. A practical approach for evaluating new antibodies in the clinical immunohistochemistry laboratory. *Arch Pathol Lab Med.* 2001;125:289-294
2) Clinical and Laboratory Standards Institute (CLSI). *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition.* CLSI document I/L28. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24): [42CFR493.1256(e)(2)].
4) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2023(Dec 28): [42CFR493.1273(a)].
5) Uhlen M, Bandrowski A, Carr S, et al. A proposal for validation of antibodies. *Nat Methods.* 2016; 13(10):838-7.
6) 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
7) Allen M, Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med.* 2016;140(9):893-898.

CYP.04380 New Reagent Lot Confirmation of Acceptability **Phase II**

The performance of new lots of antibody and detection system reagents is compared with old lots before or concurrently with being placed into service.

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