

NOTE: Biotin is a coenzyme present in mitochondria, and cells that have abundant mitochondria such as hepatocytes, kidney tubules and many tumors (particularly carcinomas) are rich in endogenous biotin. Biotin-rich intranuclear inclusions are also seen in gestational endometrium and in some tumors that form morules. If steps are not included in the immunostaining method to block endogenous biotin before applying the ABC detection complex, nonspecific false-positive staining may occur, particularly when using heat-induced epitope retrieval (which markedly increases the detectability of endogenous biotin). This artifact is often localized to tumor cells and may be easily misinterpreted as true immunoreactivity.

Blocking endogenous biotin involves incubating the slides with a solution of free avidin (which binds to endogenous biotin), followed by incubation with a biotin solution (which saturates any empty biotin-binding sites remaining on the avidin). Biotin-blocking steps should be performed immediately after epitope retrieval and before incubation with primary antibody.

REFERENCES

- 1) Miller RT, Kubier P. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of egg whites. *Appl Immunohistochem* 1997; 5: 63-66
- 2) Miller RT, Kubier P, Reynolds B, Henry T. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of skim milk as an economical and effective substitute for commercial biotin solutions. *Appl Immunohistochem & Molec Morphol* 1999;7:63-65
- 3) 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.04360 Control Slide Review

Phase II

The laboratory director or designee reviews batch control slides for acceptability before reporting patient/client results.

NOTE: Records of this daily review must be retained and clearly show that positive and negative controls for all antibodies stain appropriately. Batch control records must be retained for two years.

Immunochemical tests using polymer-based detection systems (biotin-free) are sufficiently free of background reactivity to obviate the need for a negative reagent control and such controls may be omitted at the discretion of the laboratory director following appropriate validation.

The batch control slides must be readily available to pathologists who are signing out cases. The location of the slides should be stated in the procedure manual.

Evidence of Compliance:

- ✓ Records of control slide review

REFERENCES

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

****REVISED** 12/26/2024**

CYP.04370 Validation/Verification - Non-Predictive Antibody Marker Testing

Phase II



The laboratory has records of validation/verification of new antibodies, including introduction of a new clone, prior to use for patient diagnosis or treatment.

NOTE: The performance characteristics of each assay must be appropriately validated/verified before being placed into clinical use. The initial goal is to establish the optimal antibody titration, detection system, and antigen retrieval protocol. Once optimized, a panel of cellular samples or tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation/verification is at the discretion of the laboratory director and will vary with the antibody.

Means of validation/verification may include, but are not limited to: 1) correlating the results using the new antibody with the morphology and expected results; 2) comparing the results using the new antibody with the results of prior testing of the same cellular samples or tissues with a validated/verified assay in the same laboratory; 3) comparing the results using the new

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

