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.

It is also important to assess the specificity of each antibody by a negative cellular/tissue control, which must show no staining of cells/tissues known to lack the antigen. The negative control is processed using the same fixation, epitope retrieval and immunostaining protocols as the patient tissue. Unexpected positive staining of such tissues indicates that the test has lost specificity, perhaps because of improper antibody concentration or excessive antigen retrieval. Intrinsic properties of the test tissue may also be the cause of "non-specific" staining. For example, cells/ tissues with high endogenous biotin activity such as liver or renal tubules may simulate positive staining when using a detection method based on biotin labeling.

A negative cellular sample or tissue control must be processed for each antibody in a given run. Any of the following can serve as a negative cellular/tissue control:

1. *Multitissue blocks. These can provide simultaneous positive and negative tissue controls, and are considered "good practice" (see below).*
2. *The positive control slide or patient test slides, if these slides contain cellular or tissue elements that should not react with the antibody.*
3. *A separate negative cytologic preparation or tissue control slide.*

The type of negative cellular/tissue control used (ie, separate sections, internal controls or multitissue blocks) must be specified in the laboratory manual.

Multitissue blocks or tissue microarrays (TMAs) can have a major role in maintaining quality. When used as a combined positive and negative tissue control as mentioned above, they can serve as a permanent record of the sensitivity and specificity of every stain, particularly when mounted on the same slide as the patient tissue. When the components are chosen appropriately, multitissue blocks may be used for many different primary antibodies, decreasing the number of different control blocks needed by the laboratory. Multitissue blocks are also ideal for determining optimal titers of primary antibodies since they allow simultaneous evaluation of many different pieces of tissue. Finally, they are a useful and efficient means to screen new antibodies for sensitivity and specificity or new lots of antibody for consistency, which should be done before putting any antibody into diagnostic use.

Evidence of Compliance:

- ✓ Patient reports or worksheet with control results **AND**
- ✓ Immunochemical-stained slides with appropriate negative controls

REFERENCES

1) Leong AS-Y, Cooper K, Leong FJW-M. Manual of Diagnostic Antibodies for Immunohistology. 2nd ed. London: Greenwich Medical Media; 2003
2) Dabbs DJ, ed. Diagnostic Immunohistochemistry: Theranostic and Genomic Applications. Philadelphia: Saunders/Elsevier; 2010
3) Burry RW. Specificity controls for immunocytochemical methods. *J Histochem Cytochem* 2000;48:163-166
4) Weirauch M. Multitissue control block for immunohistochemistry. *Lab Med.* 1999;30:448-449
5) Miller RT. Multitumor "sandwich" blocks in immunohistochemistry. Simplified method and preparation and practical uses. *Appl Immunohistochem* 1993;1: 156-159
6) Chan JKC, Wong CSC, Ku WT, Kwan MY. Reflections on the use of controls in immunohistochemistry and proposal for application of a multitissue spring-roll control block. *Ann Diagn Pathol* 2000;4: 329-336
7) 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)].
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9) 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.04350 Endogenous Biotin **Phase I**

If the laboratory uses an avidin-biotin complex (ABC) detection system (or a related system such as streptavidin-biotin or neutravidin-biotin), nonspecific false-positive staining from endogenous biotin is addressed.

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