A negative tissue control must be processed for each antibody in a given run. Any of the following can serve as a negative 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 donor test slides, if these slides contain tissue elements that should not react with the antibody.*
3. *A separate negative tissue control slide.*

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

Multitissue blocks or tissue microarray (TMA) 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 donor 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 biorepository. 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:

- ✓ Donor reports or worksheet with control results **AND**
- ✓ Immunohistochemical-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) Clinical and Laboratory Standards Institute. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition*. CLSI document I/LA28-A2. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 2011.
8) Torlakovic EE, Francis G, Garratt J, et al. International Ad Hoc Expert Panel. Standardization of negative controls in diagnostic immunohistochemistry: recommendations from the international ad hoc expert panel. *Appl Immunohistochem Mol Morphol.* 2014;22(4):241-52.

BAP.05354 Endogenous Biotin — Phase I

If the biorepository 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) Clinical and Laboratory Standards Institute. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline.* 2nd ed. CLSI Document I/LA28-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
4) Allen M. Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med*. 2016;140(9):893-898.

BAP.05357 Control Slide Review **Phase II**

The biorepository director or designee reviews all control slides each day specimens are stained.

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

The control slides must be readily available upon request. The location of the slides should be stated in the procedure manual.

Evidence of Compliance:
✓ Records of worksheets with control results

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

***REVISED** 12/26/2024*
BAP.05360 Validation/Verification - IHC Antibody Testing **Phase II**

The biorepository has records of validation/verification of new antibodies, including introduction of a new clone, prior to sample characterization.

NOTE: The performance characteristics of each assay must be appropriately validated/verified before being made available as characterization data for the specimen type. The initial goal is to establish the optimal antibody titration, detection system, and antigen retrieval protocol. Once optimized, a panel of tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation/verification is at the discretion of the biorepository 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 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 tissue in another laboratory with a validated/verified assay; or 4) comparing the results using the new antibody with previously validated/verified non-IHC tests or testing previously graded tissue challenges from a formal proficiency testing program.

For an initial validation/verification, biorepositories should receive 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 assays**, the validation must be performed on a minimum of 10 positive and 10 negative tissues.*

*For verification of **unmodified FDA-cleared/approved assays**, the biorepository 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 tissues.*

If the biorepository director determines that fewer validation/verification cases are sufficient for a specific marker (eg, a rare antigen or 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.