**Evidence of Compliance:**

- ✓ Records for control range establishment or verification of each lot, as applicable

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods. Approved Guideline.* 3rd ed. CLSI document EP05-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2014.
2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures, Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA; 2016.

## BAP.05351 QC - Antibodies — Phase II

**Appropriate negative controls are used.**

*NOTE: Negative controls must assess the presence of nonspecific staining in donor tissue as well as the specificity of each antibody with the exception listed below. Results of controls must be recorded, either in internal biorepository records, or in the donor report. A statement in the report such as, "All controls show appropriate reactivity" is sufficient.*

*For biorepositories using older biotin-based detection systems, it is important to use a negative reagent control to assess nonspecific or aberrant staining in donor tissue related to the antigen retrieval conditions and/or detection system used. A separate section of donor tissue is processed using the same reagent and epitope retrieval protocol as the donor test slide, except that the primary antibody is omitted, and replaced by any one of the following:*

- *An unrelated antibody of the same isotype as the primary antibody (for monoclonal primary antibodies)*
- *An unrelated antibody from the same animal species as the primary antibody (for polyclonal primary antibodies)*
- *The negative control reagent included in the staining kit*
- *The diluent/buffer solution in which the primary antibody is diluted*

*In general, a separate negative reagent control should be run for each block of donor tissue being immunostained; however, for cases in which there is simultaneous staining of multiple blocks from the same specimen with the same antibody (eg, cytokeratin staining of multiple axillary sentinel lymph nodes), performing a single negative control on one of the blocks may be sufficient provided that all such blocks are fixed and processed identically. This exception does not apply to stains on different types of tissues or those using different antigen retrieval protocols or antibody detection systems. The biorepository director must determine which cases will have only one negative reagent control, and this must be specified in the department's procedure manual.*

*The negative reagent control would ideally control for each reagent protocol and antibody retrieval condition; however, large antibody panels often employ multiple antigen retrieval procedures. In such cases, a reasonable minimum control would be to perform the negative reagent control using the most aggressive retrieval procedure in the particular antibody panel. Aggressiveness of antigen retrieval (in decreasing order) is as follows: pressure cooker; enzyme digestion; boiling; microwave; steamer; water bath. High pH retrieval should be considered more aggressive than comparable retrieval in citrate buffer at pH 6.0.*

*Immunohistochemical 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 biorepository director, following appropriate validation.*

*It is also important to assess the specificity of each antibody by a negative tissue control, which must show no staining of tissues known to lack the antigen. The negative tissue control is processed using the same fixation, epitope retrieval and immunostaining protocols as the donor 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, 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 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