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.

When possible, biorepositories should use tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically. If IHC is regularly done on specimens that are not fixed or processed in the same manner as the tissues used for validation/verification (eg, alcohol fixed cell blocks, cytologic smears, formalin post fixed tissue, or decalcified tissue), the biorepository should test a sufficient number of such tissues to ensure that assays consistently achieve expected results. The biorepository director is responsible for determining the number of positive and negative cases and the number of markers to test.

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. *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.
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.
4) Uhlen M, Bandrowski A, Carr S, et al. A proposal for validation of antibodies. *Nat Methods*. 2016; 13(10):838-7.
5) 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

BAP.05361 IHC Assay Performance — Phase I

Biorepositories 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 BAP.05360).

Biorepositories 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, tissue processing or testing equipment, environmental conditions of testing (eg, biorepository relocation), or biorepository 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 biorepository 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.

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

BAP.05363 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 important 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 an appropriate panel of control tissues. This comparison must be made on slides cut from the same control block.

Evidence of Compliance:

✓ Records of confirmation of new reagent lots