of common markers to show that specimens of different type, or processed in a different way (eg, alcohol-fixed cytology specimens, decalcified tissue) have equivalent immunoreactivity to routinely processed, formalin-fixed tissue.

A separate tissue section may be used as a positive control, but test sections often contain normal elements that express the antigen of interest (internal controls). Internal positive controls are acceptable for these antigens, but the biorepository manual must clearly state the manner in which internal positive controls are used.

A positive control included on the same slide as the donor tissue is optimal practice because it helps identify failure to apply primary antibody or other critical reagents to the donor test slide; however, one separate positive control per staining run for each antibody in the run (ie, batch control) may be sufficient provided that the control slide is closely scrutinized by a qualified reviewer.

Ideally, positive controls have low levels of antigen expression, as is often seen in neoplasms. Different expression level controls are suggested if related to companion diagnostic clinical decision points (ie, HER2; 0, 1+, 2+, 3+). Exclusive use of normal tissues that have high levels of antigen expression may result in failure to identify assays of insufficient sensitivity, leading to false-negative results.

Synthetic materials (eg, microbeads) and cell lines containing IHC analytes of interest may be run as controls in addition to positive tissue controls. Synthetic controls and cell lines should contain the target epitope of the IHC assay. Controls that assess the IHC protocol should be sensitive to the antigen retrieval step.

Synthetic and cell line-based controls can be particularly useful to assess assay performance at low expression levels, such as detecting low levels of expression in breast cancer. Synthetic and cell line-based controls are not ideal for optimizing digital pathology algorithms, which are optimally tuned to IHC expression in human tumors.

Evidence of Compliance:

- ✓ Donor reports or worksheet with control results **AND**
- ✓ Immunohistochemical-stained slides with positive controls

REFERENCES

1) 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.
2) Allen M. Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med*. 2016;140(9):893-898.
3) Cheung CC, D'Arrigo C, Dietel M, et al; From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path). Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4: Tissue Tools for Quality Assurance in Immunohistochemistry. *Appl Immunohistochem Mol Morphol.* 2017;25(4):227-230.
4) Cheung CC, Taylor CR, Torlakovic EE. An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls. *Appl Immunohistochem Mol Morphol.* 2017;25(5):308-312.
5) Torlakovic EE, Nielsen S, Francis G, et al. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. *Appl Immunohistochem Mol Morphol.* 2015;23(1):1-18.
6) ISO 20166-4:2020 Molecular in vitro diagnostic examinations. Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue. Part 4: in situ detection techniques. International Organization for Standardization. 2020.

*****NEW** 12/26/2024***

BAP.05350 Synthetic and Commercial Control Range Establishment or Verification Phase II

If synthetic or commercial controls are used for quantitative testing, the biorepository establishes or verifies an acceptable control range for each lot of synthetic or commercial control material.

NOTE: The biorepository must verify control ranges supplied by the manufacturer if provided and establish an acceptable range by repetitive analysis if control ranges are not provided by the manufacturer.

Control values supplied by the manufacturer may be used without verification for qualitative (eg, positive or negative) testing.

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.