

Inspector Instructions:

 READ	<ul style="list-style-type: none"> Sampling of ICC policies and procedures Sampling of new antibody validation/verification records Sampling of new reagents/shipment confirmation of acceptability records Sampling of antibody QC records Sampling of buffer pH records Sampling of batch control records
 OBSERVE	<ul style="list-style-type: none"> Sampling of slides (quality)
 ASK	<ul style="list-style-type: none"> How does your laboratory validate/verify new antibodies? How does your laboratory confirm the acceptability of new reagent lots? How does your laboratory distinguish non-specific false-positive staining from endogenous biotin?

CYP.04310 Specimen Modification

Phase II



If the laboratory performs immunochemical staining on specimens other than formalin-fixed, paraffin-embedded cellular material, the laboratory defines appropriate modifications, if any, for other specimen types.

NOTE: Such specimens include air-dried touch imprints, air-dried and/or alcohol fixed smears, cytocentrifuge or other liquid-based preparations, and cellular materials fixed in alcohol blends or other fixatives.

REFERENCES

- Perkins SL, Kjeldsberg CR. Immunophenotyping of lymphomas and leukemias in paraffin-embedded tissues. *Am J Clin Pathol* 1993;99(4):362-373
- Clinical and Laboratory Standards Institute (CLSI). *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition*. CLSI document I/LA28. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.

CYP.04320 Buffer pH

Phase II

The pH of the buffers used in immunocytochemistry is routinely monitored.

NOTE: pH must be tested when a new batch is prepared or received.

Evidence of Compliance:

- ✓ Records of buffer pH within defined limits

****REVISED** 12/26/2024**

CYP.04330 QC - Antibodies

Phase II



Positive controls are used for each antibody.

NOTE: Positive controls assess the performance of the immunocytochemistry (ICC) assay (including impact of fixation and antigen retrieval) and can assess the sensitivity of the assay. They should, whenever possible, be subjected to the same processing, antigen retrieval, and immunostaining protocol as patient specimens.

Results of controls must be recorded, either in internal laboratory records, or in the patient report. A statement in the report such as, "All controls show appropriate reactivity" is sufficient.

The ideal control is of the same specimen type as the patient specimen and is processed and fixed in the same manner as the patient specimen (eg, air-dried touch imprints, air-dried and/or alcohol fixed smears, cyt centrifuge or other liquid-based preparations, and cellular materials fixed in alcohol blends or other fixatives). However, for most laboratories, it is not practical to maintain separate positive control samples to cover every possible combination of fixation, processing and specimen type. Thus, it is reasonable for a laboratory to maintain a bank of formalin-fixed tissue samples as its positive controls; these controls can be used for patient specimens that are of different type, or fixed/processed differently, providing that the laboratory can show that these patient specimens exhibit equivalent immunoreactivity. This can be accomplished by parallel testing a small panel of common markers to show that specimens of different type, or processed in a different way (eg, alcohol-fixed cytology specimens) have equivalent immunoreactivity to routinely processed, formalin-fixed tissue.

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

A positive control included on the same slide as the patient specimen is optimal practice because it helps identify failure to apply primary antibody or other critical reagents to the patient 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 ICC 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 ICC assay. Controls that assess the ICC 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 ICC expression in human tumors.

Evidence of Compliance:

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

REFERENCES

- 1) 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)].
- 2) 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.
- 3) 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.
- 4) 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.
- 5) Clinical and Laboratory Standards Institute (CLSI). *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition*. CLSI document I/L28. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
- 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.