Each pathologist interpreting immunocytochemistry predictive markers participates in an annual analyte-specific quality assessment for each of the following predictive markers, as applicable:

- **Breast HER2**
- **Breast ER**
- **Gastric HER2**
- **Lung highly sensitive ALK**
- **Lung PD-L1 tumor proportion score (TPS)**

NOTE: This requirement applies to all pathologists in the laboratory that interpret one or more of these markers, whether in laboratories that perform both staining and interpretation or interpretation only. An Individual pathologist need participate only once for each predictive marker used by that pathologist in patient care evaluation, regardless of the number of locations where the pathologist performs interpretations. This requirement can be met by the use of laboratory-developed programs for sharing stained cytology slides or images thereof.

The quality assessment for each predictive marker must include a comparison of each pathologist's interpretation against the intended results. The laboratory director must define criteria for acceptable results and ensure follow up on each unacceptable result.

Evidence of Compliance:

✓ Records of annual assessment of each pathologist for predictive marker interpretation (performed on site or at another laboratory), where applicable

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

CYP.04530 Validation/Verification - Predictive Marker Testing **Phase II**

Predictive marker testing by immunocytochemistry is validated/verified and records of validation/verification are retained.

*NOTE: For validation of **laboratory-developed or modified FDA-cleared/approved predictive assays**, validation must be performed on a minimum of 20 cases (10 positive and 10 negative samples).*

*For verification of **unmodified FDA-cleared/approved predictive assays**, the laboratory 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 cellular samples or tissues.*

If the laboratory director determines that fewer validation/verification cases are sufficient for a specific marker (eg, a rare antigen or tissue), the rationale for that decision must be recorded. Positive cases in the validation/verification set should span the expected range of clinical results (expression levels). Only definitively positive and negative cases may be used for validation/verification.

The validation/verification data must clearly show the degree of concordance between assays or methods. Minimum acceptable concordance levels are 90% for positive and negative results.

The characteristics of the cases used for validation/verification should be similar to those seen in the laboratory's patient population (ie, core biopsy vs. open biopsy, primary vs. metastatic tumor, etc.).

Samples used for validation/verification must be handled in conformance with the guidelines in this checklist. Laboratories should use cellular samples or tissues that have been processed using the same fixative and methods as cases that will be tested clinically.

If significant changes are made to the testing methods (eg, antibody clone, antigen retrieval protocol or detection system, or pretreatment protocol), revalidation/verification is required.

This requirement is applicable to both new and existing assays. If review of the initial validation/ verification does not meet the current standard, it must be supplemented and brought into compliance. If no records exist from the initial validation/verification, the assay must be fully revalidated/verified.

Evidence of Compliance:

✓ Records of validation/verification data including criteria for concordance

REFERENCES

1) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.
2) Fitzgibbons PL, Murphy DA, Hammond ME, Allred DC, Valenstein P. Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. *Arch Pathol Lab Med* 2010; 134:930-935.
3) Wolff AC, Hammond ME, Hicks DG, *et al.* Recommendations for human epidermal growth factor receptor 2 testing in breast cancer; American Society of Clinical Oncology/College of American Pathologists. *Arch Pathol Lab Med* 2014;138(2):241-256
4) 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

CYP.04540 Estrogen Receptor and HER2 Testing in Breast Cancer Samples — Phase I

At least one tumor sample from all patients with invasive breast cancer (newly diagnosed, recurrent, or metastatic disease) is tested for estrogen receptors and HER2 (by IHC or ISH) if tissue is available.

REFERENCES

1) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.

CYP.04550 Fixation - HER2 and ER Breast Cancer Predictive Marker Testing — Phase I

If the laboratory assesses HER2 protein over-expression by immunocytochemistry, or estrogen receptor expression by immunocytochemistry for breast cancer predictive marker testing, the laboratory monitors cold ischemia time (one hour or less), if applicable, and appropriate specimen fixation time.

NOTE: The CAP strongly recommends that specimens subject to these tests be fixed in 10% neutral buffered formalin for at least six hours and up to 72 hours at room temperature. Specimens must be fully submerged in the optimal volume of formalin to achieve a formalin to specimen volume of 10:1 or higher, or if not feasible (eg, large specimens) at least 4:1. For cases with negative HER2 results by immunocytochemistry that were fixed outside these limits, confirmatory analysis by in situ hybridization is strongly recommended.

Both the time of removal of the tissue and the time of immersion of the tissue in fixative must be recorded and communicated from the submitting service to the processing laboratory.

Communication to clinical services of the need for appropriate information on cold ischemia time, fixative, and fixation time may be through memoranda, website, phone, face-to-face meetings, or other means. Information about fixative, fixation time, and cold ischemia time (if applicable) for each specimen must be recorded as part of the permanent specimen record in the pathology report. The laboratory must monitor for compliance and take corrective action as needed.

If specimens are fixed in a solution other than 10% neutral buffered formalin, the laboratory must perform a validation study showing that HER2 and ER results are concordant with results from formalin-fixed tissues.

Laboratories testing specimens obtained from another institution must have a policy that addresses cold ischemia time (if applicable) and time of fixation. Information on time of fixation may be obtained by appropriate questions on the laboratory's requisition form. If specimens have undergone any deviation from processing that may interfere with result interpretation, this must be annotated on the final report.