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

ANP.22979 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 Pathologist Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA

ANP.22983 Fixation - HER2 and ER Breast Cancer Predictive Marker Testing — Phase I

If the laboratory assesses HER2 protein over-expression by immunohistochemistry, HER2 (ERBB2) gene amplification by in situ hybridization, or estrogen receptor expression by immunohistochemistry for breast cancer predictive marker testing, the laboratory monitors cold ischemia time (one hour or less) 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 IHC that were fixed outside these limits, confirmatory analysis by in-situ hybridization is strongly recommended.

Laboratories must communicate the following fixation guidelines to clinical services:

1. *Rapid immersion of specimens in fixative is critical, and must occur within one hour of the biopsy or resection*
2. *If delivery of a resection specimen to the pathology department is delayed (eg, specimens from remote sites), the tumor must be bisected prior to immersion in fixative. In such cases, it is important that the surgeon ensure that the identity of the resection margins is retained in the bisected specimen; alternatively, the margins may be separately submitted.*

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 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 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, such as the use of specimens that previously were used for frozen section diagnosis, this must be annotated on the final report.

Evidence of Compliance:

- ✓ Records of communication of cold ischemia and fixation guidelines to clinical services **AND**
- ✓ Records of action taken when cold ischemia and fixation times are consistently outside of required parameters or are not available to the laboratory

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 Pathologist Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.
2) Compton CC, Robb JA, Anderson MW, et al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Pathol Lab Med.* 2019;143(11)1346-63.
3) Allison KH, Hammond EH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update *Arch Pathol Lab Med.* 2020; 144(5):545-63.

ANP.22985 Predictive Marker Testing - Decalcified Specimens **Phase I**

If the laboratory performs in situ hybridization (ISH) and/or immunohistochemistry for predictive markers on decalcified specimens, the assay was validated for decalcified specimens or the results include a disclaimer noting that these assays have not been validated on decalcified specimens.

NOTE: Decalcification may adversely affect patient results. If the assay has not been validated for decalcified specimens, a disclaimer must be included in the patient report, such as, "This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens."

Use of decalcification solutions with strong acids is not recommended.

REFERENCES

1) Darvishian F et al. Impact of decalcification on receptor status in breast cancer. *The Breast Journal* 2011. 17:689-91.
2) Hanna W et al. Testing for HER2 in breast cancer: current pathology challenges faced in Canada. *Curr Oncol* 2012. 19:315-323.
3) Gertych A et al. Effects of tissue decalcification on the quantification of breast cancer biomarkers by digital image analysis. *Diag Pathol* 2014. 9:213.

DIGITAL IMAGE ANALYSIS

This section applies to laboratories using digital image analysis to evaluate specific features in a tissue section image following enhancement and processing of that image, including but not limited to, IHC (eg, HER2 and ER), morphometric analysis, and ISH. This checklist section does not apply to laboratories that are imaging slides for manual scoring or review by an individual.

If predictive marker testing is performed, additional requirements in the Predictive Markers section also apply.

VALIDATION AND CALIBRATION

Inspector Instructions:

READ	• Sampling of validation and calibration policies and procedures • Sampling of validation/calibration records
ASK	• What is your course of action if calibration is unacceptable?

ANP.23004 Preanalytic Testing Phase Validation **Phase II**