

ASCO | Guidelines

Clinical Notice

American Society of Clinical Oncology-College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology-College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer

Discussion

Cold Ischemic Time

In the HER2 Testing Guideline, it was recommended that cold ischemia time (time between removal from patient and fixation of specimen) be as short as possible. Since that time, one paper has reported the adverse consequences of prolonged cold ischemia time on HER2 as well as ER testing. Since publication of the HER2 Testing Guideline, Khoury et. al.¹ showed that HER2 FISH (fluorescence in situ hybridization) testing was particularly vulnerable, since prolonged cold ischemia time results in preferential loss of HER2 probe signals that may lead to false negative results. We therefore recommend that the cold ischemia time be kept to one hour or less as the ER and PgR IHC Testing Guideline recommendation states. The time of removal of the tissue from the patient and the time the tissue went in fixative must be documented either on the accession slip or in the report or in both.

Handling of Specimens Obtained Remotely

There are no recommendations about remote specimens specifically in the ASCO/CAP HER2 Testing Guideline. We recommend that the ASCO/CAP ER and PgR IHC Testing Guideline recommendation be followed for HER2 testing as well because the recommendations in the ER/PgR document are more specific and promote optimal cold ischemic time and fixation. The ER and PgR IHC Testing Guideline recommends that remote specimens be promptly bisected through the tumor and then placed in neutral buffered formalin and that the time of tumor removal and time in fixative both be recorded on the accession slip. This recommendation applies to any specimen whose transit time from a site will cause the specimen to exceed the recommended 1 hour cold ischemic time prior to gross examination by a pathologist and immersion in fixative.

Fixation Time in Neutral Buffered Formalin

Data about fixation was misinterpreted in the original ASCO/CAP HER2 Testing Guideline. The statement that smaller samples can be fixed less than 6 hours is not supported by the literature.^{2,3,4} We recommend that samples for HER2 testing be fixed a minimum of 6 hours regardless of sample size. HER2 FISH can be used on samples fixed longer than 48 hours, as discussed in the original guideline document. However, there are no published data about the behavior of HER2 IHC fixed for 48-72 hours except a single publication examining specimens that are strongly HER2 IHC positive;⁵ therefore, the current recommendation for fixation intervals for HER2 IHC remains as 6-48 hours of fixation. The data about the stability of ER and PgR at intervals of 48-72 hours suggest that changing this interval for HER2 testing will not result in adverse testing results.^{2,4} Based on current evidence, we did not change the recommendation because of the lack of specific published studies for HER2 IHC that included specimens with low levels of HER2 expression that would be more vulnerable to fixation time changes.

Optimal Sample for Testing

The ASCO/CAP HER2 Testing Guideline recommends that resection samples be preferentially used for HER2 testing rather than core needle biopsies because of the likelihood of artifacts which might confound interpretation. Core needle biopsies are preferentially recommended for ER and PgR testing in the ASCO/CAP ER and PgR Testing Guideline because of higher likelihood that the samples will be quickly fixed. No change in this recommendation is suggested at this time. Pathologists should use discretion in choosing the best sample for testing in each patient.

References:

1. Khoury T, Sait S, Hwang H, Chandrasekhar R, Wilding G, Tan D, Kulkarni S Delay to formalin fixation effect on breast biomarkers. *Mod Pathol.* 2009 Nov;22(11):1457-67. Epub 2009 Sep 4. PubMed PMID: 19734848.
2. Yaziji H, Taylor CR, Goldstein NS, Dabbs DJ, Hammond EH, Hewlett B, Floyd AD, Barry TS, Martin AW, Badve S, Baehner F, Cartun RW, Eisen RN, Swanson PE, Hewitt SM, Vyberg M, Hicks DG; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. *Appl Immunohistochem Mol Morphol.* 2008 Dec;16(6):513-20. PubMed PMID: 18931614.
3. Goldstein NS, Ferkowicz M, Odish E, Mani A, Hastah F. Minimum formalin fixation time for consistent estrogen receptor immunohistochemical staining of invasive breast carcinoma. *Am J Clin Pathol.* 2003 Jul;120(1):86-92. PubMed PMID: 12866377.
4. Arber DA. Effect of prolonged formalin fixation on the immunohistochemical reactivity of breast markers. *Appl Immunohistochem Mol Morphol.* 2002 Jun;10(2):183-6. PubMed PMID: 12051639.
5. Ibarra JA, Rogers LW, Fixation time does not affect expression of HER2/neu: a pilot study. *Am J Clin Pathol.* 2010 Oct;134(4):594-6. PubMed PMID: 20855640

Reconciliation Table

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American Society of Clinical Oncology-College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer

Element to Reconcile	2007 HER2 Testing Guideline Recommendation	2010 ER and PgR IHC Testing Guideline Recommendation	Reconciled Recommendation for HER2 Testing
Cold ischemic time	Time from tissue acquisition to fixation should be as short as possible; no specification of time or requirement to document	Recommends the interval be \leq one hour and requires that the time between tissue removal and initiation of fixation must be recorded to document that tissue is handed from the surgical field and placed in fixative as quickly as possible.	Follow ER and PgR ¹ Testing recommendation
Handling of specimens obtained remotely	No recommendation	Recommends that specimens be bisected through the tumor on removal and that time of removal, fixative type, and time placed in fixative must be recorded	Follow ER and PgR ¹ Testing recommendation
Fixation time in neutral buffered formalin	6 to 48 hours in neutral buffered formalin; less fixation time permissible for needle biopsy specimens	6 to 72 hours in neutral buffered formalin for all specimens	No changes in the recommendation listed in the 2007 HER2 guideline.
Optimal sample for testing	Resection specimens preferentially recommended for testing because of possible artifacts on core biopsy	Core needle biopsy specimens preferentially recommended for testing to avoid prolonged interval before fixation	No change in recommendation. Pathologist to use discretion in selecting sample for testing