

Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update

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Purpose. To update the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guideline recommendations for human epidermal growth factor receptor 2 (HER2) testing in breast cancer to improve the accuracy of HER2 testing and its utility as a predictive marker in invasive breast cancer.

Methods. ASCO/CAP convened an Update Committee that included coauthors of the 2007 guideline to conduct a systematic literature review and update recommendations for optimal HER2 testing.

Results. The Update Committee identified criteria and areas requiring clarification to improve the accuracy of HER2 testing by immunohistochemistry (IHC) or in situ

hybridization (ISH). The guideline was reviewed and approved by both organizations.

Recommendations. The Update Committee recommends that HER2 status (HER2 negative or positive) be determined in all patients with invasive (early stage or recurrence) breast cancer on the basis of one or more HER2 test results (negative, equivocal, or positive). Testing criteria define HER2-positive status when (on observing within an area of tumor that amounts to >10% of contiguous and homogeneous tumor cells) there is evidence of protein overexpression (IHC) or gene amplification (HER2 copy number or HER2/CEP17 ratio by ISH based on counting at least 20 cells within the area). If results are equivocal (revised criteria), reflex testing should be performed using an alternative assay (IHC or ISH). Repeat testing should be considered if results seem discordant with other histopathologic findings. Laboratories should demonstrate high concordance with a validated HER2 test on a sufficiently large and representative set of specimens. Testing must be performed in a laboratory accredited by CAP or another accrediting entity. The Update Committee urges providers and health systems to cooperate to ensure the highest quality testing.

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Editor's Note: This article summarizes the Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update and provides the updated recommendations with brief discussions of the relevant literature for each. Additional information, including extensive Data Supplements, used by the Update Committee to formulate these recommendations is available at www.asco.org/guidelines/her2.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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The Bottom Line

ASCO Guideline Update

Recommendations for HER2 Testing in Breast Cancer: ASCO/CAP Guideline Update

Intervention

- Recommendations for HER2 testing in breast cancer

Target Audience

- Medical oncologists, pathologists, and surgeons

Key Recommendations for Oncologists

- Must request HER2 testing on every primary invasive breast cancer (and on metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.
- Should recommend HER2-targeted therapy if **HER2 test result is positive**, if there is no apparent histopathologic discordance with HER2 testing (Tables 1 and 2), and if clinically appropriate. If the pathologist or oncologist observes an apparent histopathologic discordance after HER2 testing, the need for additional HER2 testing should be discussed.
- Must delay decision to recommend HER2-targeted therapy if **initial HER2 test result is equivocal**. Reflex testing should be performed on the same specimen using the alternative test if initial HER2 test result is equivocal or on an alternative specimen (Tables 1 and 2).
- Must not recommend HER2-targeted therapy if **HER2 test result is negative** and if there is no apparent histopathologic discordance with HER2 testing (Tables 1 and 2). If the pathologist or oncologist observes an apparent histopathologic discordance after HER2 testing, the need for additional HER2 testing should be discussed.
- Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results equivocal). The oncologist should confer with the pathologist regarding the need for additional HER2 testing on the same or another tumor specimen.
- If the HER2 test result is ultimately deemed to be equivocal, even after reflex testing with an alternative assay (ie, if neither test is unequivocally positive), the oncologist may consider HER2-targeted therapy. The oncologist should also consider the feasibility of testing another tumor specimen to attempt to definitely establish the tumor HER2 status and guide therapeutic decisions. A clinical decision to ultimately consider HER2-targeted therapy in such cases should be individualized on the basis of patient status (comorbidities, prognosis, and so on) and patient preferences after discussing available clinical evidence.

Key Recommendations for Pathologists

- Must ensure that at least one tumor sample from all patients with breast cancer (early-stage or metastatic disease) is tested for either HER2 protein expression (IHC assay) or HER2 gene expression (ISH assay) using a validated HER2 test.
- In the United States, the ASCO/CAP Guideline Update Committee preferentially recommends the use of an assay that has received FDA approval, although a CLIA-certified laboratory may choose instead to use a laboratory-developed test (LDT). In this case, the analytic performance of the LDT must be prospectively validated in the same clinical laboratory that will perform it, and the test must have documented analytic validity (CAP guidance document). Bright-field ISH assays must be initially validated by comparing them with an FDA-approved FISH assay.
- Must report a **HER2 test result as positive** if: (a) IHC 3+ positive or (b) ISH positive using either a single-probe ISH or dual-probe ISH (Table 1; Figs 1 to 3). This assumes that there is no apparent histopathologic discordance observed by the pathologist (Table 2).
- Must report a **HER2 test result as equivocal** and order reflex test on the same specimen (unless the pathologist has concerns about the specimen) using the alternative test if: (a) IHC 2+ equivocal or (b) ISH equivocal using single-probe ISH or dual-probe ISH (Table 1; Figs 1 to 3). This assumes that there is no apparent histopathologic discordance observed by the pathologist (Table 2). Note that there are some rare breast cancers (eg, gland-forming tumors, micropapillary carcinomas) that show IHC 1+ staining that is intense but incomplete (basolateral or U shaped) and that are found to be HER2 amplified. The pathologist should consider also reporting these specimens equivocal and request reflex testing using the alternative test.
- Must report a **HER2 test result as negative** if a single test (or all tests) performed on a tumor specimen show: (a) IHC 1+ negative or IHC 0 negative or (b) ISH negative using single-probe ISH or dual-probe ISH (Table 1; Figs 1 to 3). This assumes that there is no apparent histopathologic discordance observed by the pathologist (Table 2).
- Must report a **HER2 test result as indeterminate** if technical issues prevent one or both tests (IHC and ISH) performed on a tumor specimen from being reported as positive, negative, or equivocal. This may occur if specimen handling was inadequate, if artifacts (crush or edge artifacts) make interpretation difficult, or if the analytic testing failed. Another specimen should be requested for testing, if possible, and a comment should be included in the pathology report documenting intended action.
- Must ensure that interpretation and reporting guidelines for HER2 testing are followed (Table 1; Data Supplements 7, 8, 9, and 10).
- Should interpret bright-field ISH on the basis of a comparison between patterns in normal breast and tumor cells, because artifactual patterns may be seen that are difficult to interpret. If tumor cell pattern is neither normal nor clearly amplified, test should be submitted for expert opinion.
- Should ensure that any specimen used for HER2 testing (cytologic specimens, needle biopsies, or resection specimens) begins the fixation process quickly (time to fixative within 1 hour) and is fixed in 10% neutral buffered formalin for 6 to 72 hours and that routine processing, as well as staining or probing, is performed according to standardized analytically validated protocols.
- Should ensure that the laboratory conforms to standards set for CAP accreditation or an equivalent accreditation authority, including initial test validation, ongoing internal quality assurance, ongoing external proficiency testing, and routine periodic performance monitoring.
- If an apparent histopathologic discordance is observed in any HER2 testing situation (Table 2), the pathologist should consider ordering additional HER2 testing and conferring with the oncologist, and should document the decision-making process and results in the pathology report. As part of the HER2 testing process, the pathologist may pursue additional HER2 testing without conferring with the oncologist.
- Although categories of HER2 status by IHC or ISH can be created that are not covered by these definitions, in practice they are uncommon and if encountered should be considered IHC equivocal or ISH equivocal.

The Bottom Line (Continued)

Methods

- Systematic review and analysis of the medical literature were conducted by the 2013 Update Committee.

Additional Information

- The revised recommendations and a brief summary of the literature and analysis are provided in this article. Data Supplements including clinical tools and resources can be found at <http://www.asco.org/guidelines/her2> and at <http://www.cap.org>. Patient information is available at <http://www.cancer.net>. ASCO and CAP believe that cancer clinical trials are vital to inform medical decisions and improve cancer care, and that all patients should have the opportunity to participate.

expression reverse-transcriptase polymerase chain reaction, have been introduced into practice, and the Update Committee felt these required evidence-based review. The Update Committee wishes to re-emphasize that it is important that any new test methodology, for the same clinical use, be compared with a reference test that assays for the same analyte and for which there are high levels of evidence that use of the test leads to clinical benefit for the patient (ie, clinical utility). It is the opinion of the Update Committee that there is insufficient evidence to support use of mRNA or DNA microarray assays to determine *HER2* status in unselected patients (Data Supplement 2A).

Further experience with established *HER2* assays also led to the identification of unusual *HER2* genotypic abnormalities, like aneusomy of chromosome 17 (polysomy and monosomy), colocalization of *HER2* and CEP17 signals that affect *HER2*/CEP17 ratio in dual-signal in situ hybridization (ISH) assays, and genomic heterogeneity. Limited retrospective data on the clinical significance of these abnormalities in completed prospective trials also guided the discussions that were part of this guideline update.⁶⁻²² Some of these issues are discussed in Data Supplements 2B and 2C and in a separate review article by Hanna et al.²³

During the deliberations, the Update Committee was concerned about false-negative and false-positive *HER2* assessments. For example, a false-negative test result could lead to denial of trastuzumab treatment for a patient who could benefit from it. False-positive results could lead to the administration of potentially toxic, costly, and ineffective adjuvant *HER2*-targeted therapy for 1 year.²⁴⁻²⁷ The Update Committee considered mandatory testing of all *HER2*-negative tests (Data Supplement 2D) and addressed also a narrower set of scenarios that may on occasion be observed with dual-signal ISH assays (Data Supplement 2E; Interpretation Criteria If Using a Dual-Signal *HER2* Assay and Average *HER2* Copy Number <6 Signals Per Cell).

Trastuzumab had previously been shown to improve progression-free survival and overall survival when combined with chemotherapy in the metastatic setting.²⁸ Since 2005, several of the first-generation adjuvant trials have been updated and have confirmed the disease-free and overall survival benefit offered by 1 year of trastuzumab administered with or after adjuvant chemotherapy.²⁹⁻³¹ Prospective randomized trials, first reported in abstract form in late 2012, seem to suggest that 12 months is the optimal duration of adjuvant trastuzumab therapy.

Other *HER2*-targeted drugs (eg, the kinase inhibitor lapatinib,³² the antibody pertuzumab,³³ and the antibody-drug conjugate ado-trastuzumab emtansine [T-DM1]³⁴) have been approved for the treatment of *HER2*-positive metastatic breast cancer. At the same time, data show that lapatinib (when added to paclitaxel)³⁵ and pertuzumab (as a

single agent)³⁶ offer no clinical benefit in patients with *HER2*-negative metastatic disease. These new *HER2*-targeted drugs are now being tested in the adjuvant setting, including in studies evaluating their adjuvant role alone or in dual-antibody regimens without concomitant or sequential chemotherapy. Compared with regimens already in use, the newer agents are as or more expensive, and they may be associated with other dose-limiting toxicities, such as skin and GI tract toxicities with lapatinib and liver toxicities with ado-trastuzumab emtansine.³⁷

Therefore, the need for an updated ASCO/CAP guideline on accurate *HER2* testing to ensure that the right patient receives the right treatment is now more critical than ever.^{22,24-27,38} Since the publication of the 2007 *HER2* testing guideline, CAP has observed a remarkable uptake of proficiency testing (Fig 4),⁵ with nearly 1,500 laboratories currently participating. CAP has also observed fewer laboratories experiencing deficiencies on laboratory inspection. Indirect evidence suggests that the performance of laboratories that conduct *HER2* testing in the United States and elsewhere is improving.³⁹⁻⁴² Available evidence and experience since 2007 reinforce the importance of robust validation of new assays by laboratories before clinical implementation, as well as their ongoing monitoring, and the value of various external quality assurance schemes adopted in many countries.

METHODS

The *HER2* testing Update Committee (Appendix Table A1, online only at www.asco.org/guidelines/her2) met 3 times via Webinars coordinated by its Steering Committee to review the data published from January 2006 to January 2013 and to revise the recommendations. Additional data were gathered from in-press publications and personal correspondence with researchers to address the issue of mandatory testing if a test result is 0 or 1+. Draft manuscripts were circulated by e-mail, and the Update Committee approved the final manuscript. This guideline was reviewed by external reviewers and approved by the ASCO Clinical Practice Guideline Committee and relevant CAP entities.

Literature Search Strategy

The MEDLINE and the Cochrane Collaboration Library electronic databases were searched with the date parameters of January 2006 through January 2013 for articles in English. The MEDLINE search terms are included in Data Supplement 3, and a summary of the literature search results is provided in Data Supplement 4.

Inclusion and Exclusion Criteria

Articles were selected for inclusion in the systematic review of the evidence if they met the following criteria: (1) the study compared, prospectively or retrospectively, fluorescent ISH (FISH) and immunohistochemistry (IHC) results or other tests; described technical comparisons across various assay platforms; examined potential testing algorithms for *HER2* testing; or examined the

correlation of HER2 status in primary versus metastatic tumors from the same patients; (2) the study population consisted of patients with a diagnosis of invasive breast cancer; or (3) the primary outcomes included the negative predictive value (NPV) or positive predictive value (PPV) of ISH and IHC assays used to determine HER2 status, alone and in combination; negative and positive concordance across platforms; and accuracy in determining HER2 status and benefit from anti-HER2 therapy and in determining sensitivity and specificity of individual tests. Consideration was given to studies that directly compared results across assay platforms.

Studies were not limited to randomized controlled trials but also included other study types, including cohort designs, case series, evaluation studies, and comparative studies. The Update Committee also reviewed other testing guidelines and proficiency strategies of various US and international organizations, including unpublished data. Letters, commentaries, and editorials were reviewed for any new information. Case reports were excluded. The clinical questions addressed in the update are available in Data Supplement 5.

This information was used to help the Update Committee develop new algorithms (for pathologists and oncologists) for testing, specify testing requirements and exclusions, and facilitate the necessary quality assurance monitoring that will make HER2 testing less variable and ensure more analytic consistency between laboratories. The term ratio, as used in the guideline recommendations and algorithms, always applies to the *HER2*/CEP17 ratio, which means the ratio of *HER2* signals per cell (numerator) over CEP17 signals per cell (denominator).

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Guideline and Conflicts of Interest

The Update Committee was assembled in accordance with CAP and ASCO Conflicts of Interest Management Procedures for Clinical Practice Guidelines (ASCO procedures are summarized at <http://www.asco.org/guidelinescoi>). Members of the Update Committee completed the ASCO disclosure form, which requires disclosure of financial and other interests that are relevant to the subject matter of the guideline, including relationships with commercial entities that are reasonably likely to experience direct regulatory or commercial impact as the result of promulgation of the guideline. Categories for disclosure include employment relationships, consulting arrangements, stock ownership, honoraria, research funding, and expert testimony. In accordance with the procedures, the majority of the members of the Update Committee did not disclose any such relationships.

RECOMMENDATIONS

CLINICAL QUESTION 1

What is the optimal testing algorithm for the assessment of HER2 status?

Literature Update and Discussion

The Update Committee found more than 70 new publications that informed a revision of the testing algorithms contained in the original 2007 guideline. At the time of the original guideline, significant concern existed about false-positive HER2 test results. Guideline recommendations emphasized those changes that would mitigate false positives, particularly relating to issues of specimen fixation and pathologist interpretation.^{39,43–47} Preliminary data from an ongoing prospective study seem to suggest that the frequency of false-positive test results may have diminished, in that the concordance between local testing in laboratories throughout the United States and confirmatory central HER2 testing at the Mayo Clinic (Rochester, MN) for the ALTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization HER2 Adjuvant Trial) trial showed that less than 6% of patients initially considered eligible were not subsequently centrally confirmed as being HER2 positive.⁴⁸

On the other end of the spectrum, clinical experience and recent literature have indicated that false-negative HER2 test results must also be considered. The Update Committee was sensitive to the concerns that surfaced after the publication of the 2007 guideline about the very small number of patients potentially affected by the recommendation to consider as HER2 positive only those tumors with more than 30% of cells (or >10% to ≤30% if *HER2* amplified by FISH) with diffuse and intense circumferential staining.⁴⁹ Therefore, the

Update Committee decided to revert to the previously used IHC criterion of more than 10% cells staining for HER2, which had been used as an entry criterion for eligibility for the first generation of prospective randomized trials of adjuvant trastuzumab.^{18,22,49–53} The rationale for this recommendation by the Update Committee is detailed in Data Supplement 1. Aside from the very small number of patients affected (as few as 0.15% of all newly diagnosed patients, as previously discussed),⁵ the Update Committee was also of the opinion that improvements in analytic performance of HER2 testing in clinical practice since 2007 have further reduced the already small number of patients potentially at risk of receiving a false-negative test result.

Testing is now recommended for primary, recurrent, and metastatic tumors.^{19,35,45,54–63,64} Tissue from the primary tumor can be obtained through a core needle biopsy, as well as from an incisional and excisional surgical procedure.⁶⁵ Metastases can be biopsied from chest wall, regional lymph nodes, or distant organs.^{66–74} It is essential to ensure that time to fixation (cold ischemic time) and time in fixative (which has increased from 6 to 48 hours to 6 to 72 hours in this update on the basis of available data and to conform with the ASCO/CAP estrogen receptor [ER]/progesterone receptor [PgR] testing guideline^{75,76}) are recorded and considered in defining the test result. More detail about preanalytic issues is available in Data Supplement 6.

In summary, if available, perform the first test in the core biopsy specimen in a patient with newly diagnosed breast cancer. If the test result is clearly positive or clearly negative as defined in Table 1, no retesting is needed. If the test is negative and there is apparent histopathologic discordance (Table 2), or if specimen handling has not been in accordance with guideline recommendations, a section of the tumor from the excisional specimen should be tested. If this result is positive, no further testing is needed. However, if the test is negative and there remains significant clinical concern about the result after consultation between the pathologist and the medical oncologist, it may be appropriate to repeat the test in a different block from the patient's tumor. If all three tests are negative, no additional testing is recommended.

Data Supplement 7 is a table of IHC Interpretation Criteria, and Data Supplement 8 provides ISH Interpretation Criteria. Both of these Data Supplements expand on details provided in Table 1.

The Update Committee clarified several issues in the update on the basis of recently published literature. The recommendations in Table 1 reflect the Update Committee's interpretation of the new data on polysomy, heterogeneity in ISH, types of assays, and methods of analysis^{10–14,19–21,45,67,69,79–135} for inclusion in this update. See Data Supplement 2 for an extensive discussion of these issues.

A list of US Food and Drug Administration (FDA) approved assays is available at http://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?start_search=1&search_term=HER2&approval_date_from=&approval_date_to=07/14/2013&sort=approvaldatedesc&pagenum=10 (last checked July 14, 2013). The product package inserts for trastuzumab and pertuzumab prepared by the FDA indicate that "HER2 testing should be performed using US Food and Drug Administration-approved tests by laboratories with demonstrated proficiency."^{77,78}

HER2 Assay Exclusions

Each assay type has diagnostic pitfalls to be avoided. The Update Committee agreed that there were situations in which one assay type was preferred because of assay or sample considerations. Exclusion criteria to perform or interpret an IHC or any ISH assay for HER2 are unchanged but can be viewed in the original guideline.^{1,2} The pathologist who reviews the histologic findings should determine the optimal assay (IHC or ISH) for determination of HER2 status.

Algorithms for HER2 Testing by IHC and ISH

Algorithms for evaluation of HER2 protein expression by IHC and HER2 amplification by single-probe or dual-probe ISH are presented in Figures 1, 2, and 3.

CLINICAL QUESTION 2

What strategies can help ensure optimal performance, interpretation, and reporting of established assays?

Literature Update and Discussion

Testing analytic validation requirements.—The Update Committee reviewed new papers and reports on strategies to ensure optimal performance, interpretation, and reporting of assays.^{16,22,100,136,137} Most new HER2 assays have been submitted to the FDA for premarket approval review as class III devices in view of their use for therapy selection. Although a new HER2 assay ideally should have its clinical utility validated using specimens from prospective therapeutic trials that tested the effects of anti-HER2 therapy, the Update Committee recognizes that the rarity of these valuable specimens requires that new HER2 assays be approved on the basis of concordance studies comparing them with other established HER2 tests. Consequently, it is important that tissues selected for such concordance studies come from datasets that include a broad representation of patients with breast cancer in whom HER2-positive status will be observed in approximately 15% to 20%.

Ongoing competency assessment.—The Update Committee urges ongoing competency assessment as a part of every laboratory's internal quality assessment program. The competency of the laboratory professionals and pathologists interpreting assays must be continuously addressed as required under the Clinical Laboratory Improvements Amendments (CLIA 88). The acceptable performance standard for such competency tests remains the same as in the original guideline.

Reporting requirements.—Data Supplements 9 and 10 are tables of reporting elements for IHC and reporting elements for ISH, respectively. Some changes have been made to the reporting elements for IHC and ISH to ensure that they are in accordance with the revised recommendations. In addition, a disclaimer statement is required if the specimen handling requirements are not met.

New interpretation requirements relate to the definition of tumor samples with genomic heterogeneity as well as the examination of specimens and interpretation of results in these samples. No specific requirements were added for designation of polysomy by ISH. Laboratories should maintain documentation of their quality assurance practices and ensure that such documentation is available for inspection.

Regulatory framework.—The regulatory framework remains the same as discussed in the original guideline.

Table 1. Summary of 2007 and 2013 HER2 Test Guidelines and Recommendations

Topic	2007 Recommendation	2013 Recommendation
Specimens to be tested	All primary breast cancer specimens and metastases should have at least one HER2 test performed	All newly diagnosed patients with breast cancer must have a HER2 test performed. Patients who then develop metastatic disease must have a HER2 test performed in a metastatic site, if tissue sample is available.
Optimal algorithm for HER2 testing	Positive for HER2 is either IHC HER2 3+ (defined as uniform intense membrane staining of >30% of invasive tumor cells) or FISH amplified (ratio of <i>HER2</i> to <i>CEP17</i> of >2.2 or average <i>HER2</i> gene copy number >6 signals/nucleus for those test systems without an internal control probe) Equivocal for HER2 is defined as: IHC 2+ or FISH <i>HER2/CEP17</i> ratio of 1.8-2.2 or average <i>HER2</i> gene copy number 4-6 <i>HER2</i> signals/nucleus for test systems without an internal control probe Negative for HER2 is defined as: - IHC HER2 0: no staining - IHC HER2 1+: weak incomplete membrane staining in any proportion of tumor cells or weak, complete membrane staining in <10% of cells - FISH <i>HER2/CEP17</i> ratio of <1.8 or average <i>HER2</i> gene copy number of <4 signals/nucleus for test systems without an internal control probe Indeterminate for HER2	Must report HER2 test result as positive for HER2 if:^{a,b} - IHC 3+ based on circumferential membrane staining that is complete, intense^{c,d} - ISH positive based on: - Single-probe average <i>HER2</i> copy number ≥6.0 signals/cell^{c,e} - Dual-probe <i>HER2/CEP17</i> ratio ≥2.0^{c,e} with an average <i>HER2</i> copy number ≥4.0 signals per cell - Dual-probe <i>HER2/CEP17</i> ratio ≥2.0^{c,e} with an average <i>HER2</i> copy number <4.0 signals/cell^b - Dual-probe <i>HER2/CEP17</i> ratio <2.0^{c,e} with an average <i>HER2</i> copy number ≥6.0 signals/cell Must report a HER2 test result as equivocal and order reflex test (same specimen using the alternative test) or new test (new specimen, if available, using same or alternative test) if:^{a,b} - IHC 2+ based on circumferential membrane staining that is incomplete and/or weak/moderate^f and within >10% of the invasive tumor cells^d or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumor cells^d - ISH equivocal based on: - Single-probe ISH average <i>HER2</i> copy number ≥4.0 and <6.0 signals/cell^{e,f} - Dual-probe <i>HER2/CEP17</i> ratio <2.0 with an average <i>HER2</i> copy number ≥4.0 and <6.0 signals/cell^{e,f} Must report a HER2 test result as negative if a single test (or both tests) performed show:^{a,b} - IHC 1+ as defined by incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells^d - IHC 0 as defined by no staining observed^f or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells^d - ISH negative based on: - Single-probe average <i>HER2</i> copy number <4.0 signals/cell - Dual-probe <i>HER2/CEP17</i> ratio <2.0 with an average <i>HER2</i> copy number <4.0 signals/cell Must report a HER2 test result as indeterminate if technical issues prevent one or both tests (IHC and ISH) from being reported as positive, negative, or equivocal. Conditions may include: - Inadequate specimen handling - Artifacts (crush or edge artifacts) that make interpretation difficult - Analytic testing failure Another specimen should be requested for testing to determine HER2 status. Reason for indeterminate testing should be noted in a comment in the report. Same and report HER2 test result as indeterminate as per parameters described immediately above.
ISH rejection criteria	Test is rejected and repeated if: - Controls are not as expected - Observer cannot find and count at least two areas of invasive tumor >25% of signals are unscorable due to weak signals >10% of signals occur over cytoplasm - Nuclear resolution is poor - Autofluorescence is strong	

Table 1. Continued

Topic	2007 Recommendation	2013 Recommendation
ISH interpretation	Interpretation performed by counting at least 20 cells; a pathologist must confirm that counting involved invasive tumor criteria followed	The pathologist should scan the entire ISH slide prior to counting at least 20 cells or use IHC to define the areas of potential <i>HER2</i> amplification. If there is a second population of cells with increased <i>HER2</i> signals/cell, and this cell population consists of more than 10% of tumor cells on the slide (defined by image analysis or visual estimation of the ISH or IHC slide), a separate counting of at least 20 nonoverlapping cells must also be performed within this cell population and reported. For bright-field ISH, counting requires comparison between patterns in normal breast and tumor cells because artifactual patterns may be seen that are difficult to interpret. If tumor cell pattern is neither normal nor clearly amplified, test should be submitted for expert opinion. Should preferentially use an FDA-approved IHC, bright-field ISH, or FISH assay. ^{g,h} Same
Acceptable (IHC and ISH) tests ^g Optimal IHC testing requirements	Test is rejected and repeated or tested by FISH if: • Controls are not as expected • Artifacts involve most of sample • Sample has strong membrane staining of normal breast ducts (internal controls) Positive <i>HER2</i> result requires homogeneous, dark circumferential (chicken wire) pattern in >30% of invasive tumor. Interpreters have method to maintain consistency and competency Report must include guideline-detailed elements	Should interpret IHC test using a threshold of more than 10% of tumor cells that must show homogeneous, dark circumferential (chicken wire) pattern to call result 3+, <i>HER2</i> positive.
IHC interpretation criteria		
Reporting requirements for all assay types Optimal tissue handling requirements	Time from tissue acquisition to fixation should be as short as possible; samples for <i>HER2</i> testing are fixed in 10% neutral buffered formalin for 6–48 hours; cytology specimens must be fixed in formalin. Samples should be sliced at 5- to 10-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of neutral buffered formalin Sections should ideally not be used for <i>HER2</i> testing if cut >6 weeks earlier; this may vary with primary fixation or storage conditions Validation of test must be performed before test is offered	Same except for changes to reporting requirement and algorithms defined in this table (Data Supplements 9 and 10). Duration of fixation has been changed from 6–48 hours to 6–72 hours . Any exceptions to this process must be included in report.
Optimal tissue sectioning requirements		Same
Optimal internal validation procedure Optimal initial test validation	Initial test validation requires 25–100 samples tested by alternative validated method in the same laboratory or by validated method in another laboratory Proof of initial testing validation in which positive and negative <i>HER2</i> categories are 90% concordant with alternative validated method or same validated method for <i>HER2</i>	Same (Data Supplement 12 lists examples of various external quality assurance schemes) Laboratories performing these tests should be following all accreditation requirements, one of which is initial testing validation. The laboratory should ensure that initial validation conforms to the published 2010 ASCO/CAP recommendations for IHC testing of ER and PgR guideline validation requirements with 20 negative and 20 positive for FDA-approved assays and 40 negative and 40 positive for LDTs. This requirement does not apply to assays that were previously validated in conformance with the 2007 ASCO/CAP <i>HER2</i> testing guideline and to those who are routinely participating in external proficiency testing for <i>HER2</i> tests, such as the program offered by CAP (Data Supplement 12). Laboratories are responsible for ensuring the reliability and accuracy of their testing results, by compliance with accreditation and proficiency testing requirements for <i>HER2</i> testing assays. Specific concordance requirements are not required (Data Supplement 11).

Table 1. Continued

Topic	2007 Recommendation	2013 Recommendation
Optimal monitoring of test concordance between methods	Concordance testing must be performed prior to initiation of testing, optimally as the form of testing validation. If concordance is below 95% for any testing category, that category of test result of either FISH or IHC must be automatically flexed to alternative method before final interpretation	See text under Optimal Laboratory Accreditation.
Optimal internal QA procedures	Ongoing quality control and equipment maintenance Initial and ongoing laboratory personnel training and competency assessment Use of standardized operating procedures including routine use of control materials Revalidation of procedure if changed Ongoing competency assessment and education of pathologists	Should review and document external and internal controls with each test and each batch of tests. Same Same Same
Optimal external proficiency assessment	Participation in and successful completion of external proficiency testing program with at least two testing events (mailings) a year Satisfactory performance requires at least 90% correct responses on graded challenges for either test • Unsatisfactory performance will require laboratory to respond according to accreditation agency program requirements	Same Should perform ongoing competency assessment and document the actions taken as a part of the laboratory record. Same
Optimal laboratory accreditation	Onsite inspection every other year with annual requirement for self-inspection • Reviews laboratory validation, procedures, QA results and processes, results, and reports • Unsatisfactory performance results in suspension of laboratory testing for HER2 for that method	Same (Data Supplement 11)

NOTE. For all recommendations, evidence quality and recommendation strength are strong, except as noted. Bold font indicates changes in the updated version.

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; ER, estrogen receptor; FDA, US Food and Drug Administration; FISH, fluorescent in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; LDT, laboratory-developed test; PgR, progesterone receptor; QA, quality assurance.

^aIf a reflex test (same specimen/same tissue) ordered after an initial equivocal HER2 test result does not render a positive or negative HER2 test result, the pathologist should review histopathologic features, confer if possible with the oncologist regarding additional HER2 testing, and document it in the pathology report. The pathologist may pursue additional HER2 testing without conferring with the oncologist. This should be accomplished using: (1) the alternative test (IHC or ISH) on the same specimen, (2) either test on another block (same specimen), or (3) either test on another specimen (eg, core biopsy, surgical resection, lymph node, and/or metastatic site). Because the decision to recommend HER2-targeted therapy requires a HER2-positive test result, additional HER2 testing should be attempted in equivocal specimens to attempt to obtain a positive or negative HER2 test result and most accurately determine the HER2 status of the tumor specimen.

^bSee Data Supplement 2E for additional information on rare scenarios.

^cObserved in a homogeneous and contiguous population and within > 10% of the invasive tumor cells.

^dReadily appreciated using a low-power objective.

^eBy counting at least 20 cells within the area.

^fObserved in a homogeneous and contiguous population.

^gAlternatively, a laboratory accredited by CAP or another accrediting entity may choose to use an LDT, in which case its analytical performance must be documented in the same clinical laboratory that will use the assay, and documentation of analytical validity of the assay must be available.

^hA list of HER2 assays approved by the FDA as in vitro companion diagnostic devices to aid in the assessment of patients for whom trastuzumab treatment is being considered can be found in the Medical Devices section of the US FDA Web site (http://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?start_search=1&search_term=HER2&approval_date_to=07/14/2013&sort=approvaldatedesc&pagenum=10; last checked July 14, 2013). The product package insert for trastuzumab and pertuzumab prepared by the FDA indicates that "HER2 testing should be performed using FDA-approved tests by laboratories with demonstrated proficiency."^{7,78}

Table 2. Histopathologic Features Suggestive of Possible HER2 Test Discordance

Criteria to Consider*

New HER2 test should not be ordered if the following histopathologic findings occur and the initial HER2 test was negative:

Histologic grade 1 carcinoma of the following types:

- Infiltrating ductal or lobular carcinoma, ER and PgR positive
- Tubular (at least 90% pure)
- Mucinous (at least 90% pure)
- Cribriform (at least 90% pure)
- Adenoid cystic carcinoma (90% pure) and often triple negative

Similarly, a new HER2 test should be ordered if the following histopathologic findings occur and the initial HER2 test was positive:

Histologic grade 1 carcinoma of the following types:

- Infiltrating ductal or lobular carcinoma, ER and PgR positive
- Tubular (at least 90% pure)
- Mucinous (at least 90% pure)
- Cribriform (at least 90% pure)
- Adenoid cystic carcinoma (90% pure) and often triple negative

If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test must be ordered on the excision specimen if one of the following is observed:

- Tumor is grade 3
- Amount of invasive tumor in the core biopsy is small
- Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core
- Core biopsy result is equivocal for HER2 after testing by both ISH and IHC
- There is doubt about the specimen handling of the core biopsy (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; PgR, progesterone receptor.

*Criteria to consider if there are concerns regarding discordance with apparent histopathologic findings and possible false-negative or false-positive HER2 test result.

At the current time, the FDA exercises enforcement discretion over laboratory-developed tests (LDTs) that are generated and performed within an individual laboratory under CLIA 88. CLIA 88 provides stringent quality standards for highly complex tests, which include all predictive cancer factor assays. This legislation also requires biannual surveys of laboratories that perform highly complex tests, with defined criteria and actions required when performance is deficient. However, CLIA certification does not require that the tests performed have been shown with a high level of evidence to have clinical utility.^{138,139} Moreover, FDA approval of devices, which includes in vitro diagnostic tests such as those discussed in this guideline,

does not necessarily require demonstration that use of the assay results in improved clinical outcomes compared with not using the assay. The Update Committee expresses concern about the need for greater clarity in the regulatory environment with regard to companion diagnostic tests and LDTs for higher-risk tumor biomarker tests, such as HER2. Some of this has been discussed by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative and endorsed by the Institute of Medicine Committee in regard to omics-based tests, as well as others,¹³⁹ and the Update Committee understands the FDA is developing a risk-based framework to address concerns about test accuracy and clinical utility.

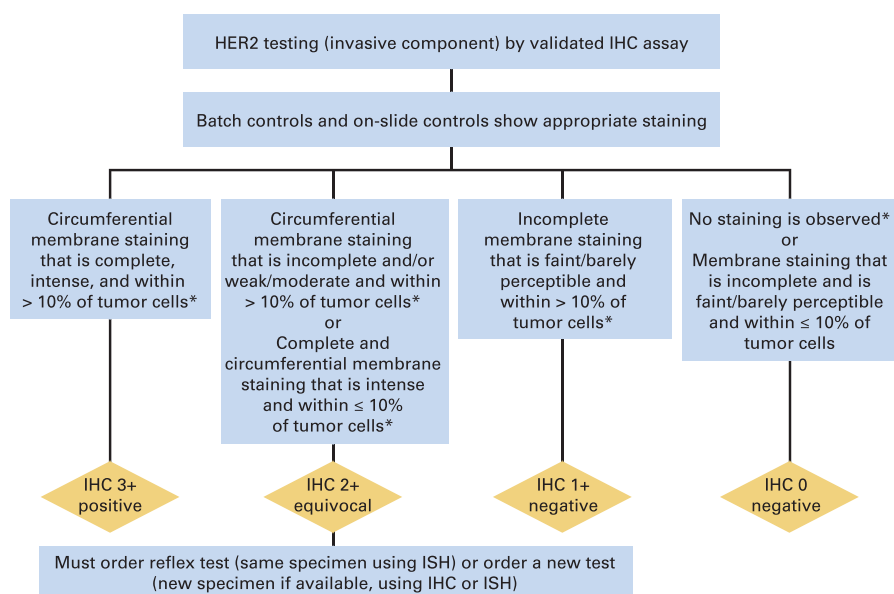
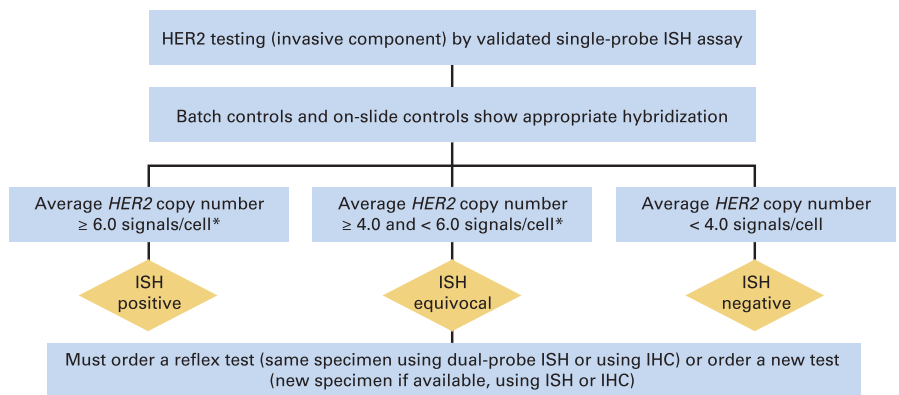


Figure 1. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) protein expression by immunohistochemistry (IHC) assay of the invasive component of a breast cancer specimen. Although categories of HER2 status by IHC can be created that are not covered by these definitions, in practice they are rare and if encountered should be considered IHC 2+ equivocal. ISH, in situ hybridization. NOTE: the final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. (*) Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.

Figure 2. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) gene amplification by in situ hybridization (ISH) assay of the invasive component of a breast cancer specimen using a single-signal (HER2 gene) assay (single-probe ISH). Amplification in a single-probe ISH assay is defined by examining the average HER2 copy number. If there is a second contiguous population of cells with increased HER2 signals per cell, and this cell population consists of more than 10% of tumor cells on the slide (defined by image analysis or visual estimation of the ISH or immunohistochemistry [IHC] slide), a separate counting of at least 20 nonoverlapping cells must also be performed within this cell population and also reported. Although categories of HER2 status by ISH can be created that are not covered by these definitions, in practice they are rare and if encountered should be considered ISH equivocal (see Data Supplement 2E). NOTE: the final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. (*) Observed in a homogeneous and contiguous population.



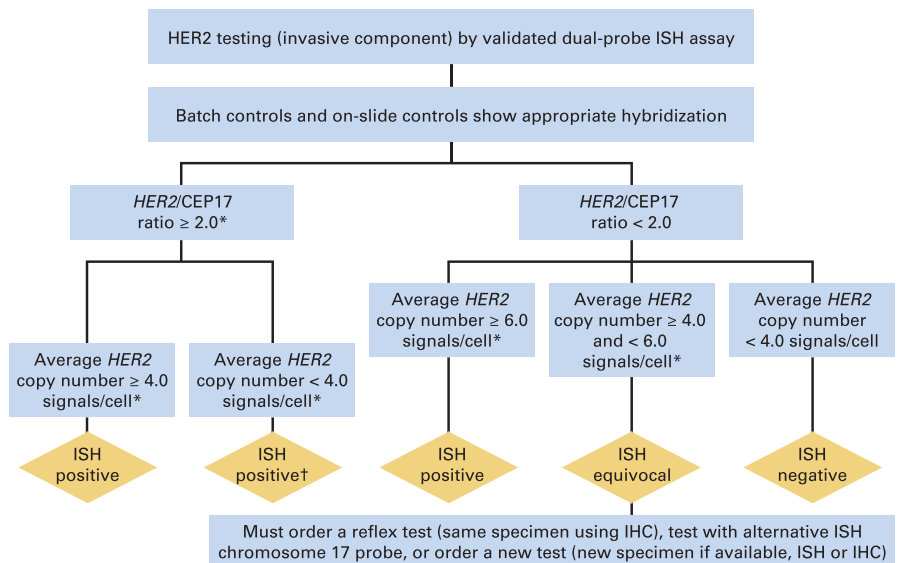
Optimal external quality assurance methods to ensure accuracy in HER2 testing and laboratory accreditation.—External proficiency testing is a mandatory requirement for CAP-accredited laboratories, beginning with the 2007 guideline. External proficiency testing challenge failure requires investigation and corrective action before the laboratory can continue to offer HER2 testing.

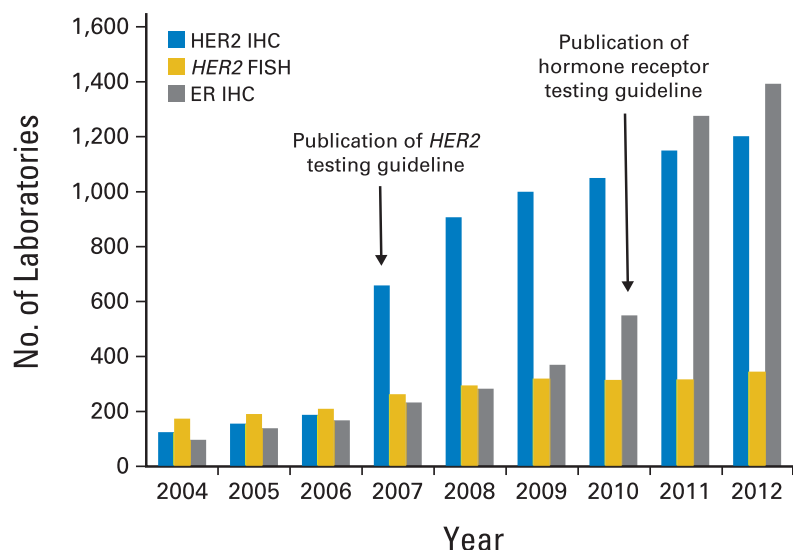
CAP modified its laboratory accreditation program to include more careful scrutiny of HER2 testing, thus creating a mandatory and expanded proficiency testing program to evaluate laboratory performance. The systematic review revealed many new papers on quality assurance, quality improvement, proficiency testing, and establishment of

concordance between local and central laboratories, both in the United States and internationally.^{40,51,136,140–146} A revised table addressing proficiency testing is contained in Data Supplement 11, which describes statistical requirements for proficiency testing. Examples of international external quality assurance schemas are included in Data Supplement 12.

The number of laboratories participating in predictive marker proficiency testing for HER2 and ER as part of the CAP laboratory improvement program since 2004 is shown in Figure 4, and the program is described at http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=accreditation (last checked July 14, 2013).

Figure 3. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) gene amplification by in situ hybridization (ISH) assay of the invasive component of a breast cancer specimen using a dual-signal (HER2 gene) assay (dual-probe ISH). Amplification in a dual-probe ISH assay is defined by examining first the HER2/CEP17 ratio followed by the average HER2 copy number (see Data Supplement 2E for more details). If there is a second contiguous population of cells with increased HER2 signals per cell, and this cell population consists of more than 10% of tumor cells on the slide (defined by image analysis or visual estimation of the ISH or immunohistochemistry [IHC] slide), a separate counting of at least 20 nonoverlapping cells must also be performed within this cell population and also reported. Although categories of HER2 status by ISH can be created that are not covered by these definitions, in practice they are rare and if encountered should be considered ISH equivocal (see Data Supplement 2E). NOTE: The final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. (*) Observed in a homogeneous and contiguous population. (†) See Data Supplement 2E for more information on these rare scenarios.





No. of laboratories											
HER2 IHC		125	156	188	659	907	1,000	1,050	1,150	1,202	
HER2 FISH		174	191	210	263	295	320	315	317	345	
ER IHC		97	139	168	233	283	370	550	1,276	1,393	

Figure 4. Number of laboratories participating in predictive marker proficiency testing for human epidermal growth factor receptor 2 (HER2) by immunohistochemistry (IHC), HER2 by fluorescent in situ hybridization (FISH), and estrogen receptor (ER) by IHC through the College of American Pathologists (CAP) Laboratory Improvement Program. Arrows indicate the years during which the HER2 and hormone receptor testing guidelines were published by the American Society of Clinical Oncology (ASCO)/CAP. The numbers of participating laboratories are shown both graphically and in tabular form. After the publication of the 2007 ASCO/CAP HER2 and the 2010 ASCO/CAP ER/progesterone receptor testing guidelines, there was a significant increase in the number of laboratories participating in CAP proficiency testing surveys in breast cancer (http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=accreditation; last checked June 14, 2013). CAP has a core goal to improve the quality of pathology and laboratory services through education and standard setting in order to enhance patient safety, and help laboratories meet or exceed regulatory requirements set by the Centers for Medicare and Medicaid Services, the Joint Commission, and many states in the United States.

Ongoing Communication, Education, and Evaluation Efforts by CAP

CAP has undertaken comprehensive efforts to educate pathologists about ways to improve laboratory performance of HER2, ER, and PgR assays. Numerous live and online educational offerings are available from CAP and other organizations. Examples in North America include the American Society of Clinical Pathology (ASCP) and United States and Canadian Academy of Pathology (USCAP). CAP provides varied live and online education focused on HER2 and ER/PgR testing elements of relevance to pathologists in meeting the original ASCO/CAP HER2 and ER/PgR guidelines and updates. In follow-up surveys, participants routinely report they made changes to their practice as a result of the educational experience. Many of these learning opportunities have a scored assessment component, allowing participants to test their knowledge as part of completing the courses, and can be used to meet the American Board of Pathology (ABP), the US pathologist certifying organization, Maintenance of Certification requirements. More information can be found at the CAP learning portal (<http://www.cap.org>) and in the original guideline. CAP has also created a listing of competencies in breast pathology, compiled by experts and available for pathologist self-assessment. After taking this self-assessment, pathologists are prompted to learning offerings that target those areas of self-reported educational deficiency. A listing of the courses is available online at <http://www.cap.org> via the learning portal.

STUDY QUALITY, LIMITATIONS OF THE LITERATURE, AND FUTURE RESEARCH

Whether in the context of trastuzumab clinical trials or of studies comparing HER2 testing platforms, interpretation of the literature in the field of HER2 testing is still complicated by a lack of standardization across trials in assay utilization

and interpretation, presence or absence of confirmatory testing, and local versus central laboratory testing, among other considerations. Although FDA-approved assays have been carefully validated, not all LDTs may have, which complicates direct comparisons across trials and platforms, and we maintain that this situation leaves open the possibility that a substantial percentage of some patients with breast cancer could be either over- or undertreated with HER2-targeted therapies.

An important gap in the literature identified by the Update Committee concerns those patients with test results reported as equivocal. The decision to treat with specific therapies like trastuzumab is by necessity dichotomous (yes or no) and will not be informed by an equivocal diagnosis with respect to HER2 status without repeat testing, if possible. However, HER2 test results are derived from a continuous variable, which can be expected to lead to some results falling into a gray area. Adding to this confusion is the fact that there is variability in the reporting definitions of the equivocal ranges for both bright-field ISH and FISH assays.

The literature is lacking evidence on response to HER2-targeted therapy in the subgroup of patients with equivocal results, and there are limited efficacy data in the subgroup tested with both high quality IHC and FISH and found to have a discordant result between these two tests. Patients with such results constitute poorly studied subsets for which there is less confidence in the scores and actual benefit from trastuzumab therapy. Because the retrospective evaluation of the benefit from trastuzumab in patients with apparent discordance between IHC and FISH who were enrolled onto the first generation of trastuzumab trials included only a small number of patients in each of the discordant subsets, patients who would have qualified for enrollment in those trials should be considered for HER2-targeted therapy.

The Update Committee's goal was to address the most common clinical situations encountered by pathologists and

oncologists in routine clinical practice. Specifically in regard to ISH assays, it is expected that additional but rare categories of HER2 status by ISH could be created that are not covered by the definitions illustrated in Figures 2 and 3. Data Supplement 2E addresses a narrower set of scenarios that may on occasion be observed with dual-signal ISH assays.

For patients with low levels of HER2 expression that do not reach the threshold for HER2-positive disease, the Update Committee encourages enrollment of such patients, if eligible, onto prospective clinical trials that aim to address the value of adjuvant HER2-targeted therapies in patients whose breast cancers show low levels of HER2 expression, like the NSABP B-47 (National Surgical Adjuvant Breast and Bowel Project B-47) trial (NCT01275677). The Update Committee also supports participation in studies evaluating other cutoffs and other technologies to optimize eligibility for HER2-targeted therapies.

PATIENT AND CLINICIAN COMMUNICATION

Patients (and family members or caregivers) should be educated about the results of pathology tests and how they are used to develop a treatment plan tailored to the biology of their cancers. Because many newly diagnosed patients are under emotional stress and/or may be unaccustomed to complex medical terminology, the use of easily understood language (at an educational level that the patient can understand) is key to clear communication. Asking patients to repeat back key pieces of information, providing written or recorded notes, and using visual aids can help ensure information is effectively communicated.

Patients should be given a copy of their pathology report and HER2 test results. The clinician should review the results with the patient, discuss any issues with the test interpretation or performance, and ask if he or she has any additional questions about the results.

Key Points for Clinicians to Discuss With Patients Regarding HER2 Status

Explain the importance of determining the biologic characteristics of breast cancer.—Patients should understand that the most common biologic tests are those for ER, PgR, and HER2 and that testing for these markers is important to select an appropriate treatment. The overall percentage of patients with HER2-positive breast cancer is between 15% and 20%. Observed numbers may vary depending on the population being tested by individual laboratories.

Explain the importance of HER2 testing.—Patients should understand that HER2 status determines whether certain drugs (eg, trastuzumab, lapatinib, pertuzumab, T-DM1) are recommended. They should also understand that the *HER2* gene is important in tumor cell growth and that tumors that have increased levels of HER2 (as measured by *HER2* gene amplification or HER2 protein overexpression) usually have a higher growth rate and a more aggressive clinical behavior.

Explain the type of tissue used for HER2 testing.—Patients should understand the type of tissue used for HER2 testing (eg, core biopsy, excisional biopsy).

Explain the types of tests used to determine HER2 status.—Patients should understand that there are different FDA-approved testing methods that detect HER2 protein overexpression or the presence of *HER2* gene amplification.

Explain the interpretation of the HER2 test results.—

Patients should understand that although most HER2 test results are definitively positive or negative, there are equivocal results that require additional testing using an alternative test or using the same or alternative test on a different portion of the same specimen (different block). Sometimes, the oncologist or pathologist may recommend additional testing using a different type of tumor specimen (eg, surgical excision *v* core biopsy), if available. Patients should be informed about which test or tests were performed and the expected turnaround time for these tests. Unfortunately, some results remain indeterminate or inconsistent with other histopathologic findings. In such cases, a final treatment decision to consider treatment with HER2-targeted therapy should be made after consultation between the pathologist and oncologist and a discussion with the patient.

Explain the importance of retesting HER2 status in new, metastatic tumors.—Patients should understand that HER2 status may occasionally be different (discordant) when comparing a previous primary tumor and a site of recurrence or in the setting of multiple simultaneous metastatic sites. In some cases, it is not possible to fully differentiate between a true biologic change, tumor heterogeneity, or variability in the performance of the assay.

Explain that HER2 testing guidelines exist.—Patients should be assured that HER2 testing guidelines were followed. Refer patients to the ASCO/CAP guideline update at www.asco.org/guideline/her2 and/or <http://www.cap.org> and to www.cancer.net for additional patient-focused information.

HEALTH DISPARITIES

Although ASCO clinical practice guidelines present recommendations on the best practices in diagnosis and disease management to provide the highest level of cancer diagnosis and care, it is important to note that some racial/ethnic minority patients have limited access to optimal medical care and/or accredited pathology laboratories. At the same time, some Medicaid or uninsured patients may have access to accredited pathology laboratories by virtue of receiving some or all of their care in an academic medical center.^{147–150}

Disparities clearly exist in the likelihood of receiving HER2 testing. In the United States, Lund et al¹⁵¹ used data from the National Cancer Institute Metropolitan Atlanta SEER Registry in conjunction with the Georgia Comprehensive Cancer Registry to examine HER2 testing among all cases of primary invasive breast cancer diagnosed among female residents during 2003 to 2004. Overall, 90.1% of women had evidence of HER2 testing. Rates of HER2 testing did not vary significantly based on socioeconomic status (based on the percent living below the federal poverty level) and were similar between black (91.3%) and white (89.8%) women. This is in agreement with other reports showing similar or greater rates of HER2 testing among black versus white women with breast cancer.¹⁵² However, in the Lund et al study, Hispanic women were significantly less likely to receive HER2 testing (79.3%), as were women diagnosed with stage IV (80.7%) or unknown stage (71.7%) disease. In addition, the mean age of women who received HER2 testing (58.8 years) was significantly younger than that of women who did not receive testing (61.3 years). Other studies have also reported that older women^{153,154} and those

with distant disease are significantly less likely to have documentation of HER2 testing. Stark et al¹⁵⁵ also reported that women with capitated insurance (versus fee-for-service insurance) were significantly more likely to be tested for HER2 status. Awareness of possible disparities in access to care should be considered in the context of this clinical practice guideline, and health care providers should strive to deliver the highest level of cancer care to these vulnerable populations.

ADDITIONAL RESOURCES

Data Supplements, including evidence tables, and clinical tools and resources can be found at www.asco.org/guidelines/her2. Information for patients is available at <http://www.cancer.net>.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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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

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• **Purpose.**—To develop a guideline to improve the accuracy of immunohistochemical (IHC) estrogen receptor (ER) and progesterone receptor (PgR) testing in breast cancer and the utility of these receptors as predictive markers.

Methods.—The American Society of Clinical Oncology and the College of American Pathologists convened an international Expert Panel that conducted a systematic review and evaluation of the literature in partnership with Cancer Care Ontario and developed recommendations for optimal IHC ER/PgR testing performance.

Results.—Up to 20% of current IHC determinations of ER and PgR testing worldwide may be inaccurate (false negative or false positive). Most of the issues with testing have occurred because of variation in preanalytic

variables, thresholds for positivity, and interpretation criteria.

Recommendations.—The Panel recommends that ER and PgR status be determined on all invasive breast cancers and breast cancer recurrences. A testing algorithm that relies on accurate, reproducible assay performance is proposed. Elements to reliably reduce assay variation are specified. It is recommended that ER and PgR assays be considered positive if there are at least 1% positive tumor nuclei in the sample on testing in the presence of expected reactivity of internal (normal epithelial elements) and external controls. The absence of benefit from endocrine therapy for women with ER-negative invasive breast cancers has been confirmed in large overviews of randomized clinical trials.

(*Arch Pathol Lab Med.* 2010;134:E1–E16)

INTRODUCTION

In 2008, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP)

decided to pursue an investigation of whether a guideline for estrogen receptor (ER) and progesterone receptor (PgR) testing would be necessary and beneficial for patients with breast cancer. The two organizations

This guideline was developed through a collaboration between American Society of Clinical Oncology and College of American Pathologists and has been published jointly by invitation and consent in both the *Journal of Clinical Oncology* and the *Archives of Pathology & Laboratory Medicine*. It has been edited in accordance with the standards established at the *Journal of Clinical Oncology*.

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Rochester, NY; Brigham and Women's Hospital, Boston, MA; National Cancer Institute, Bethesda; ST Consulting, Glen Echo; The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; Ohio State University, Columbus, OH; Baylor College of Medicine, Houston, TX; National Surgical Adjuvant Breast and Bowel Project, Pittsburgh, PA; The Delta Pathology Group, Shreveport, LA; University of Louisville, Louisville, KY; Royal Marsden Hospital, London; United Kingdom National External Quality Assessment Service, Sheffield; University of West of England, Bristol, United Kingdom; Princess Alexandra Hospital, Brisbane, Queensland, Australia; University of British Columbia, Vancouver, British Columbia; Royal University Hospital, Saskatoon, Saskatchewan, Canada; Tohoku University School of Medicine, Sendai, Japan; Radboud University, Nijmegen, the Netherlands; and European Institute of Oncology and University of Milan, Milan, Italy.

Steering Committee members: M.E.H., D.F.H., M.D., D.G.A., and A.C.W.

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Table 1. Summary of Guideline Recommendations for ER and PgR Testing by IHC in Breast Cancer Patients

Recommendation		Comments
Optimal algorithm for ER/PgR testing	Positive for ER or PgR if finding of $\geq 1\%$ of tumor cell nuclei are immunoreactive Negative for ER or PgR if finding of $< 1\%$ of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PgR (positive intrinsic controls are seen) Uninterpretable for ER or PgR if finding that no tumor nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining	These definitions depend on laboratory documentation of the following: 1. Proof of initial validation in which positive ER or PgR categories are 90% concordant and negative ER or PgR categories are 95% concordant with a clinically validated ER or PgR assay. ³ 2. Ongoing internal QA procedures, including use of external controls of variable ER and PgR activity with each run of assay, regular assay reassessment, and competency assessment of technicians and pathologists. 3. Participation in external proficiency testing according to the proficiency testing program guidelines. 4. Biennial accreditation by valid accrediting agency.
Optimal testing conditions	Large, preferably multiple core biopsies of tumor are preferred for testing if they are representative of the tumor (grade and type) at resection. Interpretation follows guideline recommendation.	Specimen should be rejected and testing repeated on a separate sample if any of the following conditions exist: 1. External controls are not as expected (scores recorded daily show variation). 2. Artifacts involve most of sample. Specimen may also be rejected and testing repeated on another sample if: 1. Slide has no staining of included normal epithelial elements and/or normal positive control on same slide. 2. Specimen has been decalcified using strong acids. 3. Specimen shows an ER-negative/PgR-positive phenotype (to rule out a false-negative ER assay or a false-positive PgR assay). 4. Sample has prolonged cold ischemia time or fixation duration < 6 hours or > 72 hours and is negative on testing in the absence of internal control elements. Positive ER or PgR requires that $\geq 1\%$ of tumor cells are immunoreactive. Both average intensity and extent of staining are reported. Image analysis is a desirable method of quantifying percentage of tumor cells that are immunoreactive. H score, Allred score, or Quick score may be provided. Negative ER or PgR requires $< 1\%$ of tumor cells with ER or PgR staining. Interpreters have method to maintain consistency and competency documented regularly.
Optimal tissue handling requirements	Accession slip and report must include guideline-detailed elements. Time from tissue acquisition to fixation should be as short as possible. Samples for ER and PgR testing are fixed in 10% NBF for 6 to 72 hours. Samples should be sliced at 5-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of NBF to allow adequate tissue penetration. If tumor comes from remote location, it should be bisected through the tumor on removal and sent to the laboratory immersed in a sufficient volume of NBF. Cold ischemia time, fixative type, and time the sample was placed in NBF must be recorded. As in the ASCO/CAP HER2 guideline, storage of slides for more than 6 weeks before analysis is not recommended. Time tissue is removed from patient, time tissue is placed in fixative, duration of fixation, and fixative type must be recorded and noted on accession slip or in report.	

had published a joint guideline on human epidermal growth factor receptor 2 (HER2) testing in 2007.^{1,2} A new Expert Panel was convened to address this issue in 2008, and a document reflecting their expert and evidence-based opinions was developed and approved by both

organizations. This version of that document is abbreviated from the original approved document, which is available online and includes introductory sections dealing with ER physiology and measurement, history of ER testing, and discussion of the current issues

Continued		
	Recommendation	Comments
Optimal internal validation procedure	Validation of any test must be done before test is offered. See separate article on testing validation (Fitzgibbons et al ³). Validation must be done using a clinically validated ER or PgR test method. Revalidation should be done whenever there is a significant change to the test system, such as a change in the primary antibody clone or introduction of new antigen retrieval or detection systems.	
Optimal internal QA procedures	Initial test validation. See separate article on testing validation (Fitzgibbons et al ³). Ongoing quality control and equipment maintenance. Initial and ongoing laboratory personnel training and competency assessment. Use of standardized operating procedures including routine use of external control materials with each batch of testing and routine evaluation of internal normal epithelial elements or the inclusion of normal breast sections on each tested slide, wherever possible. Regular, ongoing assay reassessment should be done at least semiannually (as described in Fitzgibbons et al ³). Revalidation is needed whenever there is a significant change to the test system. Ongoing competency assessment and education of pathologists.	
Optimal external proficiency assessment	Mandatory participation in external proficiency testing program with at least two testing events (mailings) per year. Satisfactory performance requires at least 90% correct responses on graded challenges for either test.	Unsatisfactory performance will require laboratory to respond according to accreditation agency program requirements.
Optimal laboratory accreditation	On-site inspection every other year with annual requirement for self-inspection.	Reviews laboratory validation, procedures, QA results and processes, and reports. Unsuccessful performance results in suspension of laboratory testing for ER or PgR.

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; IHC, immunohistochemistry; QA, quality assurance; NBF, neutral buffered formalin; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2.

related to ER and PgR testing for patients with breast cancer.

GUIDELINE QUESTIONS

The overall purpose of this guideline is to improve the accuracy of hormone receptor testing and the utility of ER and PgR as prognostic and predictive markers for assessing in situ and invasive breast carcinomas. Therefore, this guideline addresses two principal questions regarding ER and PgR testing. Findings are listed in Table 1.

1. What is the optimal testing algorithm for determining ER and PgR status?

1.1. What are the clinically validated methods that can be used in this assessment?

2. What strategies can ensure optimal performance, interpretation, and reporting of established assays?

2.1. What are the preanalytic, analytic, and post-analytic variables that must be controlled to ensure that assay results reflect tumor ER and PgR status?

2.2. What is the optimal internal quality management regimen to ensure ongoing accuracy of ER and PgR testing?

2.3. What is the regulatory framework that permits application of external controls such as proficiency testing and on-site inspection?

2.4. How can internal and external control efforts be implemented and their effects measured?

The Panel also reviewed a few special questions.

1. Should immunohistochemistry (IHC) of ER/PgR be performed in ductal carcinoma in situ (DCIS) or recurrent breast cancer specimens?

2. Does PgR expression in breast cancer correlate with or influence the choice of endocrine therapy?

PRACTICE GUIDELINES

ASCO/CAP's practice guidelines reflect expert consensus based on the best available evidence. They are intended to assist physicians and patients in clinical decision making and to identify questions and settings for further research. With the rapid flow of scientific information in oncology, new evidence may emerge between the time an updated guideline was submitted for publication and when it is read or appears in print. Guidelines are not continually updated and may not reflect the most recent evidence. Guidelines address only the topics specifically identified in the guideline and are

not applicable to interventions, diseases, or stages of diseases not specifically identified. Furthermore, guidelines cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge of the patient, to determine the best course of treatment for the patient. Accordingly, adherence to any guideline is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. ASCO/CAP guidelines describe the use of procedures and therapies in clinical practice and cannot be assumed to apply to the use of interventions in the context of clinical trials. ASCO and CAP assume no responsibility for any injury or damage to persons or property arising out of or related to any use of ASCO/CAP's guidelines or for any errors or omissions.

METHODS

Panel Composition

The ASCO Clinical Practice Guidelines Committee (CPGC) and the CAP Council on Scientific Affairs (CSA) jointly convened an Expert Panel (hereafter referred to as the Panel) consisting of experts in clinical medicine and research relevant to hormone receptor testing, including medical oncology, pathology, epidemiology, statistics, and health services research. Academic and community practitioners, a patient representative, and experts from the US National Cancer Institute (NCI) and international organizations were also part of the Panel. Representatives from the US Food and Drug Administration (FDA) and the US Centers for Medicare and Medicaid Services served as ex-officio members. The opinions of Panel members associated with official government agencies like the US National Cancer Institute represent their individual views and not necessarily those of the agency with which they are affiliated. The Panel members are listed in Appendix Table A1 (online only). Representatives of commercial laboratories and assay manufacturers (Appendix Table A2, online only) were invited as guests to attend the open portion of the 2-day meeting held at ASCO headquarters in Alexandria, VA, in December 2008. The planning, deliberations, and manuscript drafting were led by a six-member steering committee composed of two ASCO representatives (Drs Hayes and Wolff), two CAP representatives (Drs Hammond and Schwartz), and two additional experts in testing and evaluation of ER (Drs Allred and Dowsett).

Literature Review and Analysis

ASCO/Cancer Care Ontario (CCO) Systematic Review.—ASCO and CAP commissioned a systematic review of the literature on hormone receptor testing published since 1990. That review conducted by ASCO and CCO is being published separately (manuscript in preparation) and served as the primary source of the evidence for this guideline. Articles were selected for inclusion in the systematic review if they met the following prospective criteria. Studies comparing IHC in paraffin-embedded female breast cancer sections with another assay and comparative studies whose objectives were to improve or validate the quality of IHC studies that linked test

performance to clinical outcome were specifically sought. Systematic reviews, consensus statements, and practice guidelines from 1990 onward were included if they addressed hormone receptor testing in female breast cancer using IHC in paraffin-embedded sections or gene expression signatures for ER and PgR. A cutoff date of 1990 was chosen because this was the time that IHC began to come into common use. Additional details of the literature search strategy are provided in the Systematic Review (manuscript in preparation).

ASCO/CAP Expert Panel literature review and analysis.—The Panel reviewed all data from the systematic review, as well as additional studies obtained from personal files.

Consensus Development Based on Evidence

The entire Panel met in December 2008, and additional work on the guideline was completed through e-mail and teleconferences of the Panel. The purpose of the Panel meeting was to refine the questions addressed by the guideline, draft guideline recommendations, and distribute writing assignments. All members of the Panel participated in the preparation of the draft guideline document, which was then disseminated for review by the entire Panel. The guideline was submitted to *Journal of Clinical Oncology* and *Archives of Pathology & Laboratory Medicine* for peer review. Feedback from external reviewers was also solicited. The content of the guidelines and the manuscript were reviewed and approved by the ASCO CPGC and Board of Directors and by the CAP CSA and Board of Governors before publication.

Guideline and Conflict of Interest

The Expert Panel was assembled in accordance with ASCO's Conflict of Interest Management Procedures for Clinical Practice Guidelines ("Procedures," summarized at www.asco.org/guidelinescoi). Members of the Panel completed ASCO's disclosure form, which requires disclosure of financial and other interests that are relevant to the subject matter of the guideline, including relationships with commercial entities that are reasonably likely to experience direct regulatory or commercial impact as the result of promulgation of the guideline. Categories for disclosure include employment relationships, consulting arrangements, stock ownership, honoraria, research funding, and expert testimony. In accordance with the Procedures, the majority of the members of the Panel did not disclose any of these types of relationships. Disclosure information for each member of the Panel is published adjunct to this guideline.

Revision Dates

At biannual intervals, the Panel Co-Chairs and two Panel members designated by the Co-Chairs will determine the need for revisions to the guidelines based on an examination of current literature. If necessary, the entire Panel will be reconvened to discuss potential changes. When appropriate, the Panel will recommend revised guidelines to the ASCO CPGC, the CAP CSA, the ASCO Board, and the CAP Board for review and approval.

Definition of Terms

See Appendix (online only) for definitions of terms used throughout this document.

Summary of Outcomes Assessed

The primary outcome of interest was the correlation between hormone receptor status, as tested by various assays and methods, and benefit from endocrine therapy, as measured by prolongation of disease-free, progression-free, or overall survival or, in selected instances, response rates. Other outcomes of interest included the positive and negative predictive values, accuracy, and correlation of assays used to determine hormone receptor status, including (but not necessarily limited to) specific assay performance, technique, standardization attempted, quality assurance, proficiency testing, and individual or institutional training. Finally, improvement in assay results based on any of these interventions was examined.

Literature Search

The ASCO/CCO systematic review identified 337 studies that met the inclusion criteria.

RECOMMENDATIONS

What Is the Optimal Testing Algorithm for the Assessment of ER and PgR Status?

Summary and recommendations.—The Panel reviewed the literature on ER and PgR testing and discussed its implications for patients diagnosed with breast cancer. The purpose of both tests is to help determine likelihood of patients responding to endocrine therapy. Therefore, the optimal threshold to define clinical benefit should be based on thresholds that are clinically validated against patient outcome in patients treated with endocrine therapy compared with those who were not.

What Are the Clinically Validated Methods That Can Be Used in This Assessment?

Table 2 shows significant correlations between ER levels determined by IHC and clinical outcome in patients with less advanced disease treated with adjuvant hormonal therapy. Table 3 lists the assays that are currently considered to be clinically validated. A thorough discussion of these topics appears in the unabridged version of this guideline.

Laboratory concordance with standards.—In the case of IHC assays of ER and PgR assays, there is no gold standard assay available. The Panel agreed that a relevant standard would be any assay whose specific preanalytic and analytic components conformed exactly to assays whose results had been validated against clinical benefit from endocrine therapy (clinical validation). Currently, there are several assay formats that meet this criterion as models against which a laboratory can compare its testing. Examples include the ER and PgR methods described in the publications by Harvey et al⁶ and Mohsin et al¹⁰ and the FDA 510(k)-cleared ER/PR pharmDx assay kit (Dako, Glostrup, Denmark). ER can also be determined by evaluation of RNA message, either by individual assay or as part of a multigene expression assay, such as a multigene array or as a multigene quantitative polymerase chain reaction. For example, the 21-gene recurrence score (RS) assay includes ER and PgR as one of the genes in the signature.⁷¹ However, comparison between measures of ER/PgR protein by local IHC and of mRNA by central reverse transcription polymerase chain reaction showed a discordance rate of 9% and 12%, respectively,⁶⁰ and there are no published correlations of the individual measures of

ER and PgR mRNA from the 21-gene signature with clinical outcome. As a result of this lack of published data correlating the ER and PgR individual measures within the 21-gene RS directly with clinical outcome, the Panel concluded it was premature to recommend these individual measures for assay standardization and validation.

As discussed later, a laboratory performing ER testing should initially validate its proposed or existing assay against one of these clinically validated assays and demonstrate acceptable concordance. Details of acceptable validation methods are described in a separate publication.³ To be considered acceptable, the results of the assay must be initially 90% concordant with those of the clinically validated assay for the ER- and PgR-positive category and 95% concordant for the ER- or PgR-negative category. Table 3 lists details of clinically validated assays including reagents, thresholds, and publications.

Definition of positive and negative ER and PgR tests.—The Panel deliberated carefully about recommending a universal cut point to distinguish “positive” and “negative” ER levels by IHC. The original cut point established for the ligand-binding assays (LBAs) in the 1970s was based primarily on the odds of response in the metastatic setting to a variety of endocrine treatments being used at the time in many centers.¹⁸ Cytosol protein 10 fmol/mg was generally accepted as the optimum clinically useful cut point, and the FDA-approved kits using radiolabeled LBAs specified this value. Even then, the odds of responding for patients with ER levels less than 10 fmol/mg tissue were greater than 0, and others suggested that lower levels, such as more than 3 fmol/mg, might be appropriate.^{19,20}

When IHC assays replaced LBAs in the early to mid-1990s, relatively few clinical studies were performed to establish optimum cut points for these assays. Instead, most studies simply compared the two and assumed that the IHC level corresponding to the previously determined LBA cut point was also valid. However, some early studies demonstrated that IHC was equivalent or superior to LBA in predicting benefit from adjuvant endocrine therapy.^{6,10} Others showed significant correlations between ER levels determined by IHC and clinical outcome in patients with less advanced disease treated with adjuvant hormonal therapy (Tables 2 and 3).

Overall, the most comprehensive breast cancer studies have consistently shown that IHC is equivalent or superior to LBA in predicting response to hormonal therapy and that levels as low as 1% positive-staining carcinoma cells are associated with significant clinical response (Tables 2 and 3). Therefore, given the substantial impact of tamoxifen and other endocrine therapies on mortality reduction and their relatively low toxicity profile, the Panel recommended that the cutoff to distinguish “positive” from “negative” cases should be $\geq 1\%$ ER-positive tumor cells. The Panel recommended considering endocrine therapy in patients whose breast tumors show at least 1% ER-positive cells and withholding endocrine therapy if less than 1%. We recognize that these recommendations will result in a slight increase in the application of endocrine therapy in some practices. We also recognize that it is reasonable for oncologists to discuss the pros and cons of endocrine therapy with patients whose tumors contain low levels of ER by IHC (1% to 10% weakly positive cells) and to make an informed decision based on the balance.

Table 2. ER Expression by Original LBA and Retrospective IHC Versus Benefit From Endocrine Therapy (Selected Trials)

Reference	No. of Patients (eligibility)	Intervention (outcome)	Original Assay (cutoff)	Retrospective Assay (cutoff)	Assay Concordance	Outcome According to Biomarker	Comments
McCarthy et al ⁴	Pop A, n = 62 (early stage); Pop B, n = 72 (early stage); Pop C, n = 23 (MBC)	Endocrine Rx (Pop C)	LBA ($\geq$ 20 fmol/mg)	H222 Sp γ Pop (score 75)	Pop A = specificity, 89% and sensitivity, 95%; Pop B = specificity, 94% and sensitivity, 88%	Objective clinical response: specificity, 89%; sensitivity, 93%	Among the original reports describing IHC correlation with LBA and with response to endocrine Rx
Barnes et al ⁵	170 patients; 74% ER positive by LBA	First-line TAM in MBC (51% response rate)	LBA; 74% ER positive ($\geq$ 20 fmol/mg); response rate, 58%	IHC with ER 1D5 antibody; 31% to 69% ER positive (various IHC scoring methods); response rate, 64% to 69%	137 (81%) of 170	Responses in 72% of ER/PgR positive and 61% of ER positive/PgR negative; IHC superior for predicting duration of response	All 8 IHC scoring methods useful
Harvey et al ⁶	1,982 patients	26% received endocrine Rx and 13% received combined chemoendocrine Rx	LBA (positive if $\geq$ 3 fmol/mg)	IHC with 6F11 (Allred score $>$ 2 or 1% to 10% weakly positive cells)	71% of all tumors were ER positive by IHC (86% concordance with LBA)	Multivariate analysis of patients tested by LBA showed ER status determined by IHC better than by LBA at predicting better DFS	This study was based on samples prepared in an unconventional manner (see text for details)
Elledge et al ⁷	205 patients with blocks (original n = 349, all ER positive by LBA)	SWOG 8228, TAM 10 mg twice a day (n = 56) or 10 mg/m ² twice a day (n = 149)	LBA (positive if $\geq$ 3 fmol/mg)	IHC with ER-6F11 antibody (Allred score)	185 (90%) of 205 were IHC positive	Overall response rate of 56% if LBA positive and 60% if IHC positive; significant correlation between IHC ER and response (ER negative, 25%; intermediate, 46%; and high, 66%) and time to Rx failure (ER negative, 5 months; intermediate, 4 months; and high, 10 months)	In low ER by LBA ($<$ 50 fmol/mg), response rate of 25% if IHC negative and 63% if IHC high
Thomson et al ⁸	332 patients (premenopausal patients with stage II disease); 81% had tumor assayed for ER by LBA	Adjuvant OA v CMF chemotherapy	LBA originally done in 270 patients or 81% (negative if $<$ 20 fmol/mg with 2 categories, or negative if 0–4 fmol/mg with 4 categories)	IHC done in 236 patients (or 71%; quick score)	Spearman's rank correlation coefficient, 0.55	Significant interaction between IHC quick score and Rx with OA more beneficial for patients with positive quick score, whereas patients with quick score of 0 had significantly higher risk of death with OA	Original trial = better outcome with OA if ER $>$ 20 fmol/mg v with CMF if ER $<$ 20 fmol/mg

Continued							
Reference	No. of Patients (eligibility)	Intervention (outcome)	Original Assay (cutoff)	Retro-spective Assay (cutoff)	Assay Concordance	Outcome According to Biomarker	Comments
Regan et al ⁹	571 patients [premenopausal (IBCSG trial VIII)] and 976 patients [postmenopausal with node-negative disease (IBCSG trials VIII and IX)]	IBCSG trial VIII (none, CMF, goserelin, or CMF → goserelin); IBCSG trial IX (TAM or CMF → TAM)	55% patients had LBA (positive if ≥ 20 fmol/mg) and 45% had ELISA	IHC with 1D5 antibody (present if $> 0\%$ stained cells and positive if $\geq 10\%$ stained cells)	Concordance of 88% ($\kappa = 0.66$) in postmenopausal patients	HR similar for association between DFS and ER status (all patients) or PgR status (postmenopausal patients) as determined by the various methods	

Abbreviations: ER, estrogen receptor; LBA, ligand-binding assay; IHC, immunohistochemistry; Pop, population; MBC, metastatic breast cancer; Rx, therapy; TAM, tamoxifen; PgR, progesterone receptor; DFS, disease-free survival; SWOG, Southwest Oncology Group; CMF, cyclophosphamide, methotrexate, and fluorouracil; OA, ovarian ablation; IBCSG, International Breast Cancer Study Group; ELISA, enzyme-linked immunosorbent assay.

The percentage of stained tumor cells may provide valuable predictive and prognostic information to inform treatment strategies. Eight studies described the relationship between hormone receptor levels and patient outcomes.^{5,7,17,21–25} Overall survival,^{7,23,24} disease-free survival,²⁴ recurrence/relapse-free survival,^{22,23} 5-year survival,²¹ time to treatment failure,⁷ response to endocrine therapy,^{7,25} and time to recurrence¹⁷ were all positively associated with ER levels. Overall survival,⁷ time to treatment failure/progression,^{5,7} response to endocrine therapy,^{7,25} and time to recurrence¹⁷ were positively related to PgR levels. These studies suggest that patients with higher hormone receptor levels will have a higher probability of positive outcomes and may influence oncologists' and patients' treatment decisions.

Although some studies suggest that the predictive role of PgR may not be as important clinically as ER,^{5,13,26} other studies have shown that PgR status provides additional predictive value¹⁰ independent of ER values,^{25,27} especially among premenopausal women.^{9,22} Again, predictive validity for PgR has been demonstrated with as few as 1% of stained tumor nuclei cells in retrospective studies.^{10,25} Among patients who received adjuvant endocrine therapy, the best cutoff for both disease-free (adjusted $P = .0021$) and overall (adjusted $P = .0014$) survival was a total PgR Allred score of greater than 2, which corresponds to greater than 1% of carcinoma cells exhibiting weakly positive staining.¹⁰ For patients with metastatic breast cancer who received first-line endocrine therapy on relapse, a correla-

tion was found between PgR receptor status and response to endocrine therapy at a 1% staining threshold ($P = .044$) or response to tamoxifen therapy at 10% ($P = .021$) and 1% staining thresholds ($P = .047$). Furthermore, patients with carcinomas exhibiting $\geq 1\%$ PgR staining levels had better survival after relapse ($P = .0008$).²⁵

Reporting Results

Taking these issues into consideration, the Panel recommends that ER and PgR results be reported with three required result elements and two optional result elements (Table 1). The three required elements are as follows.

1. The percentage/proportion of tumor cells staining positively should be recorded and reported; all tumor containing areas of the tissue section on the slide should be evaluated to arrive at this percentage. The percentage can be arrived at either by estimation or by quantification, either manually by counting cells or by image analysis. Image analysis holds promise for improving inter- and intraobserver reproducibility, but controversy exists about how imaging should be implemented at this time. Standards of system performance have not yet been developed. If the sample is a cytology specimen, at least 100 cells should be counted or used to estimate the percentage of hormone receptor–positive tumor cells, particularly if the tumor specimen is limited and if the

Table 3. Well-Validated Assays for Evaluating Estrogen Receptor and Progesterone Receptor in Breast Cancer by Immunohistochemistry

Reference	Primary Antibody	Cut Point for "Positive"
Estrogen receptor		
Harvey et al, 1999 ⁶	6F11	Allred score ≥ 3 (1% to 10% weakly positive cells)
Regan et al, 2006 ⁹ ; Viale et al, 2007 ¹³ ; Viale et al, 2008 ¹⁴	1D5	1% to 9% (low) and $\geq 10\%$ (high)
Cheang et al, 2006 ¹⁵	SP1	$\geq 1\%$
Phillips et al, 2007 ¹⁶	ER.2.123 + 1D5 (cocktail)	Allred score ≥ 3 (1% to 10% weakly positive cells)
Dowsett et al, 2008 ¹⁷	6F11	H score > 1 ($\geq 1\%$)
Progesterone receptor		
Mohsin et al, 2004 ¹⁰	1294	Allred score ≥ 3 (1% to 10% weakly positive cells)
Regan et al, 2006 ⁹ ; Viale et al, 2007 ¹³ ; Viale et al, 2008 ¹⁴	1A6	1% to 9% (low) and $\geq 10\%$ (high)
Phillips et al, 2007 ¹⁶	1294	Allred score ≥ 3 (1% to 10% weakly positive cells)
Dowsett et al, 2008 ¹⁷	312	$\geq 10\%$

positive staining seems to involve only a minority of tumor cells.

2. The intensity of staining should be recorded and reported as weak, moderate, or strong; this measurement should represent an estimate of the average staining of the intensity of the positively stained tumor cells on the entire tissue section relative to the intensity of positive controls run with the same batch. Intensity is provided as a measure of assay quality over time and also allows for optional composite scoring.

3. An interpretation of the assay should be provided, using one of three mutually exclusive interpretations. The reader should provide an interpretation of the assay based on the following criteria.

- Receptor positive (either ER or PgR). The Panel recommends a cutoff of a minimum of 1% of tumor cells positive for ER/PgR for a specimen to be considered positive. There is no agreement about a range for receptor equivocal, so this term should not be used.
- Receptor negative. Tumors exhibiting less than 1% of tumor cells staining for ER or PgR of any intensity should be considered negative based on data that such patients do not receive meaningful benefit from endocrine therapy. The sample should only be considered negative in the presence of appropriately stained extrinsic and intrinsic controls. Any specimen lacking intrinsic elements (normal breast epithelium) that is negative on ER and/or PgR assay should be repeated using another tumor block or another tumor specimen and reported as uninterpretable rather than as negative.
- Receptor uninterpretable. The Panel agreed that there are no absolute assay exclusions. Nevertheless, a result should be considered uninterpretable if a sample did not conform to preanalytic specifications of the guideline, was processed using procedures that did not conform to guideline specifications or the laboratory's standard operating procedure, or the assay used to analyze the specimen was not validated and controlled as specified in the guideline. Examples of circumstances that may lead to uninterpretable results include testing of needle biopsies or cytology samples fixed in alcohol, use of fixatives other than 10% neutral buffered formalin (NBF) unless that fixative has been validated by the laboratory before offering the assay), biopsies fixed for intervals shorter than 6 hours or longer than 72 hours, samples where fixation was delayed for more than 1 hour, samples with prior decalcification using strong acids, and samples with inappropriate staining of internal assay controls (including intrinsic normal epithelial elements) or extrinsic assay controls. These conditions are not absolute because they depend on which conditions have been validated by the laboratory and which are subject to the judgment of the circumstances by the pathologist. The reason for an uninterpretable result should be specified (eg, fixation for < 6 hours), and an alternative potential sample for retesting should be suggested, if appropriate.

Two optional report elements are recommended by the Panel, but not required.

1. A cautionary statement may be added to negative ER and PgR interpretations when the histopathology of the tumor is almost always associated with ER-positive

and PgR-positive results. These include tubular, lobular, and mucinous histologic types or tumors with a Nottingham score of 1. The cautionary statement should indicate that although the patient's tumor tested as ER negative, tumors with the same histologic type or Nottingham score almost always test positive.

2. Using the percentage and intensity measurements provided, the pathologist may also provide a composite score such as the H score, Allred score, or quick score (Table 3). Because each of these is somewhat differently calculated and may lead to confusion across institutions, scoring is not required.

Appropriate populations to be tested.—The Panel developed consensus that ER and PgR status should be determined on all newly diagnosed invasive breast cancers. For patients with multiple synchronous tumors, testing should be performed on at least one of the tumors, preferably the largest. The Panel acknowledges that all newly diagnosed DCISs are also commonly being tested for ER and PgR. This practice is based on the results of a retrospective subset analysis of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-24 clinical trial comparing tamoxifen versus placebo after lumpectomy and radiation, which has thus far been reported only in abstract form. There was a significant 40% to 50% reduction in subsequent breast cancer (ipsilateral and contralateral) restricted to patients with ER-positive DCIS at 10 years of follow-up, and a full manuscript has recently been submitted for peer review (personal communication from NSABP, September 2009). Because the results are scientifically reasonable and consistent with previous studies of invasive/metastatic breast cancer, the Panel sees value in assessing ER in patients with DCIS. However, because there are unlikely to be any validation studies, the Panel leaves it up to patients and their physicians to decide on testing, rather than making a formal recommendation. Breast recurrences should also always be tested to ensure that prior negative results of ER and/or PgR were not falsely negative and to evaluate the specimen for biologic changes since the previous testing.

What Strategies Can Ensure Optimal Performance, Interpretation, and Reporting of Established Assays?

Summary and recommendations.—The Panel considered those strategies that would ensure optimal performance of ER/PgR testing, interpretation, and reporting and was heavily influenced by the previous experience with the implementation of the elements included in the ASCO/CAP HER2 testing guideline. This guideline included measures to improve standardization of preanalytical variables, type of fixative and duration of tissue fixation, antibodies and controls, and assay interpretation.

What Are the Preanalytic, Analytic, and Postanalytic Variables That Must Be Controlled to Ensure That the Assays Reflect the Tumor ER and PgR Status?

Preanalytic standardization: tissue handling.—The warm and cold ischemic times are widely accepted as important variables in the analysis of labile macromolecules such as proteins, RNA, and DNA from clinical tissue samples. Warm ischemia time is the time from the interruption of the blood supply to the tumor by the surgeon to the excision of the tissue specimen; cold ischemia time is the

time from excision to the initiation of tissue fixation. Numerous studies have documented the progressive loss of activity of these labile molecules after the surgical interruption of blood flow, leading to tissue ischemia, acidosis, and enzymatic degradation.^{28–30} The contribution to this macromolecular degradation by the warm ischemic interval is currently under study. The standardization of the time between tissue removal and the initiation of fixation is an important step to help ensure that differences in levels of protein expression for clinically relevant targets such as ER are biologically meaningful and are not an artifact related to the manner in which the tissue was handled.

The breast resection specimen should be fixed as quickly as possible in an adequate volume of fixative (optimally 10-fold greater than volume of the specimen). The time of tissue collection (defined as the time that the tissue is handed from the surgical field) and the time the tissue is placed in fixative both must be recorded on the tissue specimen requisition to document the time to fixation of the specimen. The pathologist should effectively communicate this priority to all members of the breast care management team so processes are put in place to make sure these times are routinely recorded. It is the responsibility of the surgeon and operating room staff or the radiologist and his/her staff obtaining the specimen to document the collection time, and it is the responsibility of the pathologist and laboratory staff to document the fixation start time. Every effort should be made to transport breast excision specimens with a documented or suspected cancer from the operating room to the pathology laboratory as soon as they are available for an immediate gross assessment. The time from tumor removal to fixation should be kept to ≤ 1 hour to comply with these recommendations.

On receipt in the pathology laboratory, these specimens should be oriented and carefully inked for surgical margin assessment and then carefully sectioned at 5-mm intervals and placed in 10% NBF. Gauze pads or paper towels should be placed in between tissue slices to assist with the penetration of formalin into all areas of the tissue sample if the specimen will be further sectioned and placed into tissue cassettes at a later time. If gross tumor is easily identifiable, a small portion of tumor and fibrous normal breast tissue can be included together in a cassette and placed immediately into fixative at the time of the initial gross evaluation. This will initiate good tissue fixation and also ensure that normal breast elements are available as an internal positive control that have been handled and fixed in a manner that is identical to the tumor tissue. In situations where excision specimens are obtained remotely from the grossing laboratory, the pathologists should work with personnel in the remote operating suites to ensure that the sample is bisected through the tumor and promptly placed in NBF before transport. The time to insertion of tumor sample into fixative and the time of removal of the tumor from the patient should be noted on the specimen requisition by the remote personnel. Although less optimal than immediate gross examination of the fresh sample by the pathologist, this process is preferable to storage of the sample in the refrigerator unfixated or in fixative without sectioning.

Preanalytic standardization: type of fixative.—Only 10% NBF should be used as the fixative for breast tissue specimens. Higher or lower concentrations of NBF are not

acceptable. This recommendation is based on published literature regarding the expected or characteristic immunoreactivity for ER in breast cancer, which has been accrued over many years and has been clinically validated with patient outcomes in numerous clinical trials.³¹ In addition, FDA approval for assay kits analyzing ER and HER2 explicitly states that formalin fixation should be used and that the FDA approval for the kits is not applicable if an alternative fixative is used. If the laboratory uses a formalin alternative for fixation, the assay must be validated against NBF fixation, and the laboratory director assumes responsibility for the validity of these assay results.

Preanalytic standardization: duration of tissue fixation.—Breast tissue specimens must be fixed in 10% NBF for no less than 6 hours and for not more than 72 hours before processing.^{32,33} Further information about the need for standardization of tissue fixation appears in the unabridged version of this guideline.

Analytic standardization: antibody selection for ER testing.—The selection of antibodies for ER and PgR IHC testing should be restricted to those reagents that have well-established specificity and sensitivity and have been clinically validated, demonstrating good correlation with patient outcomes in published reports. Alternatively, the results of laboratory-selected antibodies should be at least 90% concordant with those of the clinically validated assay for the ER- and PgR-positive category and 95% concordant with those for the ER- or PgR-negative category that have been correlated with clinical outcomes of endocrine treatment. The Panel determined that the antibodies for ER that have met these criteria are clones 1D5, 6F11, SP1, and 1D5+ER.2.123, whereas the antibodies for PgR include clones 1294 and 312 (Table 3). There is a single FDA 510(k)-cleared ER/PgR kit. Published reports have demonstrated that each of these antibodies is equivalent or superior to LBAs in terms of correlation with outcome and/or benefit from endocrine therapy (Tables 2 and 3). Antibodies sold as research use only or investigational use only or developed by the testing facility may not be used in ER and PgR testing. Use of research use only, investigational use only, and laboratory-developed antibodies in an assay is not compliant with these guidelines.

Analytic standardization: control samples for ER and PgR IHC assays.—Positive and negative controls should be included with every ER and PgR IHC assay batch run. Batch controls are used to monitor assay performance over time and to detect a loss of sensitivity or assay analytic drift. Acceptable batch controls include cell lines with defined receptor content varying from high positive to negative and including at least one intermediate level of receptor content. Other acceptable external controls include endometrial tissue with known receptor content. On-slide external controls and internal normal epithelial elements should be used to help ensure that all reagents were dispensed onto the slide containing a test sample and that the assay is performing properly. The internal positive control must display a heterogeneous staining pattern of the luminal cells, with a mixture of a variable number of cells exhibiting weak, moderate, and intense immunoreactivity. If the assay only highlights a few cells among the normal breast epithelium with a homogeneous staining pattern, then the risk of a false-negative assess-

Table 4. IHC ER/PgR Testing Interpretation Criteria

Review controls (external standard and internal normal breast epithelium if present). If not as expected, the test should be repeated and not interpreted.

Provide an interpretation of the assay as receptor positive, receptor negative, or receptor uninterpretable.

Positive interpretation requires at least 1% of tumor cells showing positive nuclear staining of any intensity.

Receptor negative is reported if < 1% of tumor cells show staining of any intensity.

Receptor uninterpretable is reported if the assay controls are not as expected or the preanalytic or analytic conditions do not conform to the guideline and there is no tumor cell staining in the absence of normally stained intrinsic epithelial elements.

Report the percentage of cells with nuclear staining using either estimation or quantitation. Quantitation may be done either by image analysis or manually.

Entire slide should be reviewed to assess the tumor-containing areas. Cytology samples with limited tumor cells and little tumor staining must have at least 100 cells counted.

Report an average intensity of tumor cell nuclei recorded as strong, moderate, or weak.

A score may be provided if the scoring system is specified.

Quantitative image analysis is encouraged for samples with low percentages of nuclear staining or in cases with multiple observers in the same institution. It is also a valuable way to quantify intensity and assure day-to-day consistency of control tissue reactivity.

If cytoplasmic staining occurs, repeat assay or perform on another sample.

Reject sample if normal ducts and lobules do not show obvious staining of some cells with variable intensity in the presence of totally negative tumor cells.

Reject sample if there are obscuring artifacts such as decalcification of sample or staining only of necrotic debris.

In samples with DCIS only, the type of DCIS should be mentioned and the DCIS may be scored for ER/PgR; in patients with invasive disease and DCIS, ER/PgR should be reported only for the invasive component. DCIS staining pattern may also be provided in a comment.

The ER and PgR results should fit the clinical profile of the patient being evaluated: Consider the type of invasive cancer and the grade of the cancer in interpretation; some cancer types like lobular, mucinous, and tubular carcinoma are almost always strongly ER positive and only rarely ER negative.

Abbreviations: IHC, immunohistochemistry; ER, estrogen receptor; PgR, progesterone receptor; DCIS, ductal carcinoma in situ.

ment of the tumor ER and/or PgR is higher as a result of an insufficient sensitivity of the reaction to detect the tumor cells with a weak to moderate immunoreactivity. The normal breast tissue also represents a useful built-in negative control of the staining because the myoepithelial cells and the stromal cells must invariably show a negative result. In some specimens, there are no internal control elements (normal breast epithelium); in this case, the pathologist must exercise judgment as to whether the assay can be interpreted based on the level of ER and/or PgR positivity of the tumor cells, the histologic type of the tumor, the fixation status of the tumor, and the status of external controls.

To ensure that there has not been analytic drift because of subtle differences in technique or dilution, controls with intermediate reactivity or controls covering a spectrum of expression should be scored and recorded daily (percent positive tumor cells and intensity of staining) using laboratory standard scoring system or image analysis. It is not appropriate to use a single strong positive control tissue to evaluate assay performance.

If an external or internal control does not produce the expected reaction, the result of patient testing must not be reported. Instead, the assay should be repeated with the standard reagents under the standard conditions until acceptable ER and/or PgR reactivity of control material is

achieved. No patient material should be reported until controls react appropriately.

If the particular histologic type of breast cancer is unlikely to be ER negative (tubular, mucinous, or lobular morphology or Nottingham score of 1), the tumor should also be subjected to confirmatory testing, such as sending the same specimen to a reference laboratory for retesting or by repeating the assay on another block or on a separate breast cancer specimen.

Postanalytic standardization: interpretation of IHC assays for ER and PgR.—The interpretation of ER and PgR assays should include an evaluation of both the percentage of positive tumor cell nuclei and the intensity of the staining reaction. The level of expression of ERs in different breast tumors demonstrates a broad dynamic range that can vary by several hundred-fold. There is still no consensus about what level of expression constitutes the equivocal range for ER/PgR, and this terminology should not be used in the report. Table 4 lists interpretation guidelines.

Postanalytic standardization: reporting of ER and PgR by IHC.—The elements to be reported are listed in Tables 5 and 6. The staining of normal breast elements, if present within the specimen, should also be reported as an additional check on the IHC assay performance.

Postanalytic standardization: ER and PgR IHC assay internal quality control and validation.—A comprehensive quality control program for ER/PgR IHC analyses should include all aspects of the total test including periodic trend analysis to help ensure an appropriate and expected number of ER-positive breast cancers in the patient population served by the laboratory. Table 7 lists specific suggestions; additional suggestions are provided in a separate publication.³

What Is the Regulatory Framework That Allows for Increased Scrutiny?

The Clinical Laboratory Improvement Act of 1998 (CLIA 88) provides stringent quality standards for highly

Breast Cancer Hormone Receptor Guideline, IHC—Hammond et al

Table 5. Elements to Be Included in Accession Slip for ER and PgR Assays

Patient identification information
Physician identification
Date of procedure
Clinical indication for biopsy
Specimen site and type of specimen
Collection time
Time sample placed in fixative
Type of fixative
Fixation duration

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

Table 6. Reporting Elements for ER and PgR IHC Assays

Patient identification information*
Physician identification*
Date of service*
Specimen site and type*
Specimen identification (case and block number)*
Fixative
Cold ischemia time (time between removal and fixation)
Duration of fixation
Staining method used
Primary antibody and vendor
Assay details and other reagents/vendors
References supporting validation of assay (note: most commonly, these will be published studies performed by others that the testing laboratory is emulating)
Status of FDA approval
Controls (high protein expression, low-level protein expression, negative protein expression, internal elements or from normal breast tissue included with sample)
Adequacy of sample for evaluation
Results*
Percentage of invasive tumor cells exhibiting nuclear staining†
Intensity of staining: strong, medium, or weak
Interpretation:
Positive (for ER or PgR receptor protein expression), negative (for ER or PgR protein expression), or uninterpretable
Internal and external controls (positive, negative, or not present)
Standard assay conditions met/not met (including cold ischemic time and fixation parameters)
Optional score and scoring system
Comment: Should explain reason for uninterpretable result and or any other unusual conditions, if applicable; may report on status of any DCIS staining in the sample; should also provide correlation with histologic type of the tumor; may provide information about laboratory accreditation status

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; IHC, immunohistochemistry; FDA, US Food and Drug Administration.

* Report should contain these elements as a minimum. Other information must be available in the laboratory for review and/or appear on the patient accession slip.

† There is no recommendation in this guideline concerning whether specimens containing only ductal carcinoma in situ should be tested for ER/PgR.

complex tests, which include all predictive cancer factor assays. This legislation also requires application of external controls to assure compliance with CLIA standards. These external controls include required successful performance on external proficiency surveys (or alternative external assessment of assay accuracy) and on-site biennial inspection of laboratories performing highly complex tests with defined criteria and actions required when performance is deemed deficient. On-site inspections may be performed by the Centers for Medicare and Medicaid Services or its agents or by various deemed private accreditors, including CAP, The Joint Commission, and COLA (formerly known as Commission on Office Laboratory Accreditation).

The FDA regulates medical devices as a result of the 1976 Medical Devices Amendments Act. ER and PgR

testing reagents and kits, which have potentially high impact on patient mortality and morbidity, have been the subject of several guidance documents and reports referencing FDA opinion on the subject.³⁴

After review of the legislation and applicable regulations, the Panel agreed that the current regulatory framework provided sufficient justification for the guideline recommendations without modification, just as it had for the previously published ASCO/CAP HER2 guideline. Other countries such as Australia and New Zealand have similar requirements.

What Are the Optimal External Quality Assurance Methods to Ensure Ongoing Accuracy in ER/PgR Testing?

Summary and recommendations.—The guideline is based on regulatory requirements of CLIA 88, published studies,

Table 7. CAP Laboratory Accreditation Elements Requiring Documentation

Validation of test method before reporting patient results
Use and following of standard operating procedures with appropriate elements and sign-offs
Qualifications, responsibilities, and training of personnel involved in testing
Proper labeling of samples and reagents
Proper storage and handling of samples and reagents
Equipment calibration, maintenance, QC, and remedial action; proficiency testing performance and corrective actions when 100% not achieved
Internal QA plan for entire testing process, evidence that it is followed, and identified problems monitored and resolved effectively
Quality of tests for interpretation
Ongoing competency assessment of technologists and pathologists*
Report adequacy and quality, including required dates and times
Recordkeeping for entire test process and record retention
Accurate, timely submission of results

Abbreviations: CAP, College of American Pathologists; QC, quality control; QA, quality assurance.

* Competency assessment is monitored by periodic or continuous review of performance of those doing tests against peers. When failure is documented, remediation is undertaken.

previous CAP experience,^{1,2} experience of other groups,³¹ and the Panel's consensus.

Currently there are no regulatory requirements for proficiency testing of ER or PgR assays in the United States. CLIA regulations require alternative assessment schemes for ER and PgR as substitutes for mandated successful performance on external proficiency testing. However, proficiency testing can be used to meet the alternative assessment requirement if it is available. The current guideline will make successful performance in proficiency testing mandatory. There are mandatory requirements for successful performance in proficiency testing in Australia and New Zealand, which had been in place since 2001.

The guidelines also require enhanced levels of scrutiny at the time of laboratory inspection beyond those required by CLIA. The Panel recommends that ER and PgR testing be performed in a CAP-accredited laboratory or in a laboratory that meets the additional accreditation requirements set out within this guideline.

External quality assurance (laboratory accreditation).—Beginning in 2010, the CAP Laboratory Accreditation Program will require that every CAP-accredited laboratory performing ER and/or PgR testing participate in a proficiency testing program directed to these analytes. Other Centers for Medicare and Medicaid Services-approved certifying or accrediting organizations that wish to evaluate laboratory compliance with this guideline must bring their accreditation programs in conformance with this and other requirements.

The CAP Laboratory Accreditation Program will monitor performance in the required proficiency testing. Performance less than 90% (described in detail in the following section) will be considered unsatisfactory and will require internal or external response consistent with accreditation program requirements. Responses must include identification of the cause of the poor performance, actions taken to correct the problem, and evidence that the problem has been corrected. Competency of the laboratory personnel performing the ER/PgR testing, including the pathologists, is an important aspect of the laboratory proficiency. Competency of testing personnel and pathologists must be assured by the laboratory director of each facility in a manner consistent with CLIA. Competency assessments must be documented, and documentation shall be evaluated at the time of laboratory inspection accreditation. The checklist of requirements for laboratories is presented in Table 7.

Proficiency testing requirements.—All laboratories reporting ER and/or PgR results must participate in a guideline-concordant proficiency testing program specific for each assay and method used. To be concordant with this guideline, proficiency testing programs must distribute specimens at least twice per year including a sufficient number of challenges (cases) to ensure adequate assessment of laboratory performance. For programs with ≥ 10 challenges per event, satisfactory performance requires correct identification of at least 90% of the graded challenges in each testing event. Laboratories with less than 90% correct responses on graded challenges in a given proficiency testing event are at risk for the next event. Laboratories that have unsatisfactory performance will be required to respond according to accreditation program requirements up to and including suspension of

ER and/or PgR testing for the applicable method until performance issues are corrected. In some Canadian provinces and within the United Kingdom, the method of proficiency testing is different. In Canada, laboratories may participate in proficiency testing that uses sections of tissue microarrays offered by the Canadian Immunohistochemistry Quality Control (an academic program associated with the Canadian Association of Pathologists) or tumor samples or sections of cell blocks with characterized cell lines. Many Canadian laboratories also participate in CAP proficiency testing programs or European programs. The results may or may not be used for laboratory accreditation depending on the province. Laboratories receive unstained materials and must return those materials to a central laboratory for review and comment. The Australasian program developed by the Royal College of Pathologists of Australasia Quality Assurance Program consists of two components. Laboratories are sent unstained sections from tissue microarray blocks and are required to stain these and return them for central review and scoring. In addition, laboratories are required to submit de-identified data on the ER/PgR and HER2 status of reported breast cancers for evaluation of acceptable performance. Enrollment and participation in these programs are mandatory.

How Can These Efforts Be Implemented and the Effects Measured?

Plans to ensure compliance with guideline.—ASCO and CAP will provide educational opportunities (print, online, and society meetings) to educate health care professionals, patients, third-party payers, and regulatory agencies. In addition, CAP is producing a certificate program for pathologists that will assess their competency in following both the hormone receptor and the HER2 guideline recommendations. CAP will urge its members and participants in accreditation and proficiency testing programs to optionally append a statement to individual results or laboratory informational or promotional materials indicating that the laboratory's ER/PgR assays have been validated and performed in accordance with ASCO/CAP ER testing guidelines, provided that all of the guideline conditions are met.

ASCO and CAP will work to coordinate these recommendations with those of other organizations, such as the National Comprehensive Cancer Network, the Commission of Cancer of the American College of Surgeons, the American Joint Committee on Cancer, and patient advocacy organizations.

We are confident that these guidelines and measures developed for testing of ER, PgR, and HER2 will improve performance of laboratories using these and future predictive testing methods. CAP will actively review results of proficiency testing and laboratory accreditation activities and periodically publish performance results.

CAP will also work to include quality monitoring activities of ER and PgR testing in its programs designed for ongoing quality assessment, similar to its Q-tracks and Q-probes. In Australasia, participation in the programs is mandatory and linked to laboratory accreditation. In Australia and New Zealand, the laboratory accreditation is linked to funding of testing for laboratories ensuring compliance.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Employment or Leadership Position: Jared N. Schwartz, Aperio (C). **Consultant or Advisory Role:** Mitch Dowsett, Dako (C); D. Craig Allred, Genomic Health (C), Clariant (C), Dako (C); Sunil Badve, Dako (C); Neal S. Goldstein, Clariant (C); Giuseppe Viale, Dako (C). **Stock Ownership:** D. Craig Allred, Clariant. **Honoraria:** Glenn Francis, Roche Ventana Medical Systems; Giuseppe Viale, Dako. **Research Funding:** Hironobu Sasano, Ventana Japan. **Expert Testimony:** None. **Other Remuneration:** Glenn Francis, Roche Ventana Medical Systems.

After the guideline manuscript was completed, Jared N. Schwartz assumed an Employment or Leadership Position with Aperio and resigned as co-chair of the Expert Panel.

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APPENDIX

Definitions

Analyte-specific reagent.—Antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents, which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biologic specimens [21CFR864.4020(a)].

Research use only (RUO).—Products that are in the laboratory research phase of development (ie, either basic research or the initial search for potential clinical utility) and not represented as an effective in vitro diagnostic product (21CFR809.10).

Investigational use only (IUO).—A product being shipped or delivered for product testing before full commercial marketing (for example, for use on specimens derived from humans to compare the usefulness of the product with other products or procedures that are in current use or recognized as useful) (21CFR809.10).

Clinical laboratory.—A facility for the biologic, microbiologic, serologic, chemical, immunohematologic, hematologic, biophysical, cytologic, pathologic, or other examination of materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings. These examinations also include procedures to determine, measure, or otherwise describe the presence or absence of various substances or organisms in the body. Facilities only collecting or preparing specimens (or both) or only serving as a mailing service and not performing testing are not considered laboratories (42CFR493.2).

US Food and Drug Administration (FDA)–cleared test.—A test that has been cleared by the FDA after analysis of data showing substantial performance equivalence to other tests being marketed for the same purpose. Such tests typically follow the 510(k) approval route (21CFR807).

FDA-approved test.—A test that is classified as a class III medical device and that has been approved by the FDA through the premarket approval process (21CFR814.3).

Laboratory modified test.—An FDA-cleared or FDA-approved test that is modified by a clinical laboratory, but not to a degree that changes the stated purpose of the test, approved test population, specimen type, specimen handling, or claims related to interpretation of results.

Laboratory developed test (LDT).—A test developed within a clinical laboratory that has both of the following characteristics: is performed by the clinical laboratory in which the test was developed and is neither FDA cleared nor FDA approved.

Note: All laboratory modified tests are, by definition, LDTs. An LDT may or may not use analyte-specific reagent, RUO, or IUOs; the type of reagents and devices used does not affect whether a test is classified as an LDT. A laboratory is considered to have developed a test if the test procedure or implementation of the test was created by the laboratory performing the testing, irrespective of whether fundamental research underlying the test was developed elsewhere or reagents, equipment, or technology integral to the test was purchased, adopted, or licensed from another entity.

Validation of a test.—Confirmation through a defined process that a test performs as intended or claimed.

Note: There is no universally acceptable procedure for validating tests. The process for validating tests must take into account the purpose for which a test is intended to be used, claims made about the test, and the risks that may prevent the test from serving its intended purpose or meeting performance claims. Even FDA-approved and FDA-cleared tests require limited revalidation in clinical laboratories (a process often referred to as verification) to establish that local implementation of the test can reproduce a manufacturer's validated claims. Tests that use reagents or equipment that have not been validated (such as RUOs or IUOs) typically pose increased risks that require more extensive validation, as do tests used in more loosely controlled settings. The determination of whether a test has been adequately validated requires professional judgment.

Table A1. Panel Members	
Panel Member	Institution
M. Elizabeth H. Hammond, MD, FCAP, Co-Chair Antonio C. Wolff, MD, FACP, Co-Chair Daniel F. Hayes, MD, Co-Chair D. Craig Allred, MD, FCAP, Steering Committee Member Mitch Dowsett, PhD, Steering Committee Member Sunil Badve, MD Robert L. Becker, MD, Ex-Officio Patrick L. Fitzgibbons, MD, FCAP Glenn Francis, MBBS, FRCPA, MBA Neil S. Goldstein, MD, FCAP Malcolm Hayes, MD David G. Hicks, MD, FCAP Susan Lester, MD Richard Love, MD Lisa McShane, PhD Keith Miller, MD C. Kent Osborne, MD Soonmyung Paik, MD Jane Permutter, PhD, Patient Representative Anthony Rhodes, PhD Hironobu Sasano, MD Jared N. Schwartz, MD, PhD, FCAP Fred C.G.J. Sweep, PhD Sheila Taube, PhD Emina Emilia Torlakovic, MD, PhD Giuseppe Viale, MD, FRCPATH Paul Valenstein, MD, FCAP Daniel Visscher, MD Thomas Wheeler, MD, FCAP R. Bruce Williams, MD, FCAP James L. Wittliff, MD, PhD Judy Yost, MA, MT (ASCP), Ex Officio	Intermountain Healthcare, University of Utah School of Medicine, UT The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, MD University of Michigan Comprehensive Cancer Center, University of Michigan Health System, MI Washington University School of Medicine in St Louis, MO Royal Marsden Hospital, United Kingdom Eastern Cooperative Oncology Group, Indiana University, IN US Food and Drug Administration, Center for Devices and Radiological Health, Office of In Vitro Diagnostic Device Evaluation and Safety St. Jude Medical Center, CA Princess Alexandra Hospital, Australia Advanced Diagnostics Laboratory, MI University of British Columbia, Canada University of Rochester, NY Brigham and Women's Hospital, MA Ohio State University, OH National Cancer Institute, Biometric Research Branch, Division of Cancer Treatment and Diagnosis, MD UK NEQAS, United Kingdom Baylor College of Medicine, TX National Surgical Adjuvant Breast and Bowel Project, PA Gemini Group, MI University of the West of England, Bristol, UK NEQAS Tohoku University School of Medicine, Japan Presbyterian Hospital, NC Radboud University, Nijmegen, the Netherlands ST Consulting, Glen Echo, MD Royal University Hospital, Saskatoon, Canada European Institute of Oncology, and University of Milan, Italy St. Joseph Mercy Hospital, Ann Arbor, MI University of Michigan, Ann Arbor, MI Baylor College of Medicine, TX The Delta Pathology Group, Shreveport, LA University of Louisville, KY CMS, Division of Laboratory Services (CLIA), MD

Abbreviations: UK NEQAS, United Kingdom National External Quality Assessment Service; CMS, Centers for Medicare and Medicaid Services; CLIA, Clinical Laboratory Improvement Act.

Verification of a test.—An abbreviated process through which a clinical laboratory establishes that its implementation of an FDA-approved and FDA-cleared test performs in substantial conformance to a manufacturer's stated claims.

Analytic validity.—A test's ability to accurately and reliably measure the analyte (measurand) of interest. The elements of analytic validity include the following, as applicable.

- **Accuracy.** The closeness of agreement between the average value obtained from a large series of measurements and the true value of the analyte. Note: Technically, the term accuracy refers to the measure of the closeness of a single test result to the true value, not the average of multiple results. The definition of accuracy used here is what metrologists call trueness of measurement and describes the popular (but technically incorrect) meaning of the word accuracy.
- **Precision.** The closeness of agreement between independent results of measurements obtained under stipulated conditions (the International Organization of Standardization, 1993).
- **Reportable range.** For quantitative tests, the span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response and over which results will be reported. For semiquantitative, binary, and nominal/

categoric tests, the reportable range is all of the values that can be reported by the test system (eg, 2+, 3+, "positive," "negative," *Escherichia coli*, *Staphylococcus aureus*).

- **Analytic sensitivity.** For quantitative tests (including semiquantitative tests), analytic sensitivity is the lowest amount of analyte (measurand) in a sample that can be detected with (stated) probability, although perhaps not quantified as an exact value. For qualitative tests (binary and nominal/categoric tests), analytic sensitivity is the proportion of instances in which the analyte/measurand/identity is correctly detected, within a stated CI.

Table A2. Invited Guests to Open Session December 2008 Panel Meeting

Invited Guests	Affiliation
Steven Shak, MD	Genomic Health, Redwood City, CA
Kenneth J. Bloom, MD Patrick Roche, PhD	Clariant, Aliso Viejo, CA Ventana Medical Systems, Tucson, AZ
Allen M. Gown, MD	PhenoPath Laboratories, Seattle, WA
David L. Rimm, MD, PhD Hadi Yaziji, MD Richard Bender, MD Roseanne Welcher	Yale University, New Haven, CT Ancillary Pathways, Miami, FL Agendia, Huntington Beach, CA Dako, Glostrup, Denmark

- **Analytic specificity.** Ability of a measurement procedure to measure solely the measurand/analyte.

Note: Analytic validity is expressed in the context of a defined set of test conditions (including standard operating procedures and permissible specimen types) and an ongoing quality management regimen (including, as applicable, ongoing quality control, periodic assay recalibration, and external proficiency testing or alternative external testing). If the test conditions or quality management regimen changes, the analytic validity of a test may change.

Clinical validity.—A test's ability to detect or predict a disorder, prognostic risk, or other condition or to assist in the management of patients. The elements of clinical validity include the following, as applicable.

- **Clinical sensitivity (clinical detection rate).** The proportion of individuals with a disorder, prognostic risk, or condition who are detected by the test.
- **Clinical specificity.** The proportion of individuals without a disorder, prognostic risk, or condition who are excluded by the test.
- **Reference limits.** A value or range of values for an analyte that assists in clinical decision making. Reference values are generally of two types—reference intervals and clinical decision limits. A reference interval is the range of test values expected for a designated population of individuals. This may be the central 95% interval of the distribution of values from individuals who are presumed to be healthy (or

normal). For some analytes that reflect high-prevalence conditions (such as cholesterol), significantly less than 95% of the population may be healthy. In this case, the reference interval may be something other than the central 95% of values. A clinical decision limit represents the lower or upper limit of a test value at which a specific clinical diagnosis is indicated or specified course of action is recommended.

- **Clinical utility.** The clinical usefulness of the test. The clinical utility is the net balance of risks and benefits associated with using a test in a specific clinical setting. Clinical utility does not take into consideration the economic cost or economic benefit of testing and is to be distinguished from cost-benefit and cost-effectiveness analysis. Clinical utility focuses entirely on the probabilities and magnitude of clinical benefit and clinical harm that result from using a test in a particular clinical context.

Note 1: The qualities listed in this appendix represent the primary performance measurements that are used to describe the clinical capabilities of a test. Other measures of clinical validity may be applicable in particular circumstances.

Note 2: Clinical validity is expressed in the context of a defined test population and a defined testing procedure. If the test population changes (eg, a change in the prevalence of disease) or the testing procedure changes, the clinical validity of a test may change.

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Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Breast

Patrick L. Fitzgibbons, MD; Deborah A. Dillon, MD; Randa Alsabeh, MD; Michael A. Berman, MD; Daniel F. Hayes, MD; David G. Hicks, MD; Kevin S. Hughes, MD; Sharon Nofech-Mozes, MD; for the Members of the Cancer Biomarker Reporting Committee, College of American Pathologists

The College of American Pathologists offers these templates to assist pathologists in providing clinically useful and relevant information when reporting results of biomarker testing. The College regards the reporting elements in the templates as important elements of the biomarker test report, but the manner in which these elements are reported is at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice.

The College developed these templates as educational tools to assist pathologists in the useful reporting of relevant information. It did not issue them for use in litigation, reimbursement, or other contexts. Nevertheless, the College recognizes that the templates might be used by hospitals, attorneys, payers, and others. The College cautions that use of the templates other than for their intended educational purpose may involve additional considerations that are beyond the scope of this document.

TEMPLATE FOR REPORTING RESULTS OF BIOMARKER TESTING OF SPECIMENS FROM PATIENTS WITH CARCINOMA OF THE BREAST

Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are

performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team.

BIOMARKER REPORTING TEMPLATE

Breast

Note: Required elements in this template comply with the most recent versions of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines on HER2 and hormone receptor testing. Reporting elements are required only if applicable and only for tests performed. If some studies were performed on different specimen(s), the specimen number(s) should be provided.

+ Data elements preceded by this symbol are not required.

RESULTS

Estrogen Receptor (ER)

- ☐ Positive (percentage of cells with nuclear positivity: %)
- + Average intensity of staining:
 - + ☐ Weak
 - + ☐ Moderate
 - + ☐ Strong
- ☐ Negative
 - ☐ Internal controls present and ER positive (as expected)
 - ☐ Internal controls present but ER negative
 - ☐ Internal controls absent
 - ☐ Cannot be determined (explain):

Progesterone Receptor (PgR)

- ☐ Positive (percentage of cells with nuclear positivity: %)
- + Average intensity of staining:
 - + ☐ Weak
 - + ☐ Moderate
 - + ☐ Strong
- ☐ Negative
 - ☐ Internal controls present and PgR positive (as expected)
 - ☐ Internal controls present but PgR negative

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The authors have no relevant financial interest in the products or companies described in this article.

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☐ Internal controls absent
☐ Cannot be determined (explain): _____

HER2 (by Immunohistochemistry)

☐ Negative (score 0)
☐ Negative (score 1+)
☐ Equivocal (score 2+)
☐ Positive (score 3+)
☐ Indeterminate (explain): _____
☐ Not performed

Percentage of cells with uniform intense complete membrane staining: ____ %

HER2 (by In Situ Hybridization)

☐ Negative (not amplified)
☐ Equivocal
☐ Positive (amplified)
☐ Indeterminate (explain): _____
☐ Not performed
☐ Pending

Number of observers: _____

Number of invasive tumor cells counted: _____

☐ Using dual probe assay
Average number of *HER2* signals per cell: _____
Average number of *CEP17* signals per cell: _____
HER2:CEP17 ratio: _____

☐ Using single probe assay
Average number of *HER2* signals per cell: _____

+ Aneusomy (as defined by vendor kit used):

+ ☐ Not present
+ ☐ Present

+ Heterogeneous signals:

+ ☐ Absent
+ ☐ Present

+ Percentage of cells with amplified *HER2* signals: ____ %

+ Ki-67

+ Percentage of positive nuclei: ____ %

+ Multiparameter Gene Expression/Protein Expression Assay

+ Name of assay: _____

+ ☐ Low risk
+ ☐ Moderate risk
+ ☐ High risk

+ Recurrence score: _____

Cold Ischemia and Fixation Times Meet the Requirements Specified in the Latest Version of the ASCO/CAP Guidelines

☐ Yes
☐ No

METHODS

Fixative

☐ Formalin
☐ Other (specify): _____

Estrogen Receptor

☐ US Food and Drug Administration (FDA) cleared (specify test/vendor): _____
☐ Laboratory-developed test

Primary Antibody

☐ SP1
☐ 6F11
☐ 1D5
☐ Other (specify): _____

Progesterone Receptor

☐ FDA cleared (specify test/vendor): _____
☐ Laboratory-developed test

Primary Antibody

☐ 1E2
☐ 636
☐ 16
☐ SP2
☐ 1A6
☐ Other (specify): _____

+ ER and PgR Scoring System

+ ☐ Allred
+ ☐ Other (specify): _____

HER2 (by Immunohistochemistry)

☐ FDA approved (specify test/vendor): _____
☐ Laboratory-developed test

Primary Antibody

☐ 4B5
☐ HercepTest™
☐ A0485
☐ SP3
☐ CB11
☐ Other (specify): _____

HER2 (by In Situ Hybridization)

☐ FDA approved (specify test/vendor): _____
☐ Laboratory-developed test

+ Ki-67

+ Primary Antibody
+ ☐ MIB1
+ ☐ SP6
+ ☐ Other (specify): _____

+ Image Analysis

+ ☐ Not performed
+ ☐ Performed (specify method): _____

+ Biomarkers Scored by Image Analysis (select all that apply)

+ ☐ ER
+ ☐ PgR
+ ☐ HER2
+ ☐ Ki-67
+ ☐ Other (specify): _____

+ COMMENT(S)

Note: Time to fixation (cold ischemia time) and time of fixation are required elements but may be reported in this template or in the original pathology report.

EXPLANATORY NOTES

It is recommended that hormone receptor and HER2 testing be done on all primary invasive breast carcinomas and on recurrent or metastatic tumors.¹⁻⁴ If hormone receptors and HER2 are both negative on a core biopsy, repeat testing on a subsequent specimen should be considered, particularly when the results are discordant with the histopathologic findings. When multiple invasive foci are present, the largest invasive focus should be tested. Testing smaller invasive carcinomas is also recommended if they are of different histologic type or higher grade. Other biomarker tests (eg, Ki-67 or multigene expression assays) are optional and are not currently recommended for all carcinomas. Fresh tissue should not be used for special studies (eg, RNA expression profiling or investigational studies) unless the invasive carcinoma is of sufficient size that histologic evaluation and ER, PgR, and HER2 assessment will not be compromised.

Guidelines published by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) require recording specific preanalytic and analytic variables that can affect test results.⁵⁻⁷ Such variables include the following:

- Cold ischemia time (time between tissue removal and initiation of fixation) *and* time of fixation. Alternatively, laboratories may record the time the specimen was removed from the patient and the time the specimen was placed in formalin.
- Type of fixative, if other than buffered formalin
- Treatment of the tissue that could potentially alter immunoreactivity (eg, decalcification)⁸
- Status of controls:
 - *Internal*.—Normal epithelial cells positive or negative for ER and PgR
 - *External*.—Type and expected level of expression
- Adequacy of sample for evaluation
- Primary antibody clone
- Regulatory status (FDA cleared versus laboratory-developed test)

Information regarding assay validation or verification should be available in the laboratory. Any deviation(s) from the laboratory's validated methods should be recorded. Appropriate positive and negative controls should be used and evaluated.

Estrogen Receptor and Progesterone Receptor Testing

Scientific Rationale—Normal breast epithelial cells have receptors for estrogen and progesterone and proliferate under their influence. Most breast carcinomas also express those receptors and may be stimulated to grow by those hormones. Removal of endogenous hormones by oophorectomy or blocking hormonal action pharmacologically (eg, with tamoxifen or aromatase inhibitors) can slow or prevent tumor growth and prolong survival.

Clinical Rationale—Hormone receptor status is determined primarily to identify patients who may benefit from hormonal therapy.² About 75% to 80% of invasive breast cancers are positive for ER and PgR, including almost all well-differentiated cancers and most moderately differentiated cancers, and studies have shown a substantial survival benefit from endocrine therapy among patients with ER⁺ tumors.⁵ True ER[−], PgR⁺ carcinomas are extremely rare, but patients with such tumors are also considered eligible for

hormonal therapy. Receptor status is only a weak prognostic factor.

Method—Hormone receptor status is most often determined in formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry (IHC). Only nuclear staining is considered positive. Use of single-gene expression assays are not recommended for routine use.

Quality Assurance—There are many tissue and technical variables that can affect test results,^{5,9-11} and the assays must be validated to ensure their accuracy.¹² External proficiency testing surveys for ER and PgR are invaluable tools to help ensure that assays perform as expected, and they are available from the CAP and other organizations.

False-Negative Results.—Failure to detect ER or PgR is the greatest problem with this assay because patients may not receive effective therapy. To avoid false-negative results, appropriate internal and external controls should be positive. If internal controls are not present, consider repeating the test on another specimen (if available). Reasons for false-negative results include the following:

- Exposure of tumor cells to heat (eg, carcinomas transected by using cautery during surgery)
- Prolonged cold ischemic time, which may result in antigenic degradation; 1 hour or less is preferable^{13,14}
- Underfixation or overfixation; fixation for at least 6 hours in buffered formalin is recommended,⁵ and prolonged fixation can also diminish immunoreactivity^{11,15}
- Type of fixative: ER is degraded in acidic fixatives, such as Bouin picoformal-acetic fixative and B-5 sodium acetate-sublimate; formalin should be buffered to ensure pH range between 7.0 and 7.4
- Decalcification, which may result in loss of immunoreactivity⁸
- Nonoptimized antigen retrieval
- Type of antibody
- Dark hematoxylin counterstain obscuring faintly positive diaminobenzidine (DAB) staining

False-Positive Results.—False-positive results occur less frequently.¹⁶ Rare reasons would be the use of an impure antibody that cross-reacts with another antigen or misinterpretation of entrapped normal cells or of an in situ component as invasive carcinoma. False-positive tests can also be generated by image analysis devices that mistakenly count overstained nuclei. It has been suggested that highly sensitive assays may detect very low levels of ER in cancers that will not respond to hormonal therapy, but that has not been proven by a clinical trial.

False-negative and false-positive results can be reduced by paying attention to the following:

- *Staining of normal breast epithelial cells*: Normal epithelial cells serve as a positive internal control and should always be assessed. If the normal cells are negative, repeat studies on the same specimen or on a different specimen should be considered. If normal cells are not present (eg, core biopsy) and the test results are negative, testing should be repeated on another block or subsequent specimen.
- *External controls (must stain as expected)*: These controls help ensure that the reagents have been appropriately dispensed onto the slide with the clinical sample.
- *Correlation with histologic type and grade of the cancer*: The study should be repeated if the results are discordant (eg, ER[−], low-grade carcinoma).

Table 1. Reporting Results of Estrogen Receptor and Progesterone Receptor Testing		
Result	Criteria	Comments
Positive	Immunoreactive tumor cells present ($\geq 1\%$)	The percentage of immunoreactive cells may be determined by visual estimation or quantitation. Quantitation can be provided by reporting the percentage of positive cells or by a scoring system, such as the Allred score or the H score.
Negative	$<1\%$ immunoreactive tumor cells present	

Reporting Guidelines—ASCO and the CAP have issued recommendations for reporting the results of immunohistochemical assays for ER and PgR (Table 1).⁵ Studies using both IHC and the ligand-binding assay suggest that patients with higher hormone receptor levels have a higher probability of response to hormonal therapy, but expression as low as 1% positive staining has been associated with clinical response. As a result, the guidelines recommend classifying all cases with at least 1% positive cells as receptor positive.⁵ For patients with low ER expression (1%–10% weakly positive cells), the decision on endocrine therapy should be based on an analysis of its risks and potential benefits.

Definition of a Negative Result—The ASCO/CAP guidelines recommend that carcinomas with $<1\%$ positive cells be considered negative for ER and PgR.⁵ In the Allred system (see Table 2), the survival of patients whose carcinomas had a score of 2 (corresponding to $<1\%$ weakly positive cells) was similar to that of patients whose carcinomas were completely negative for ER.¹⁷ Therefore, a score of 2 was considered a negative result. Carcinomas with $<1\%$ positive cells and intensity scores of 2 or 3 would have a total score of 3 or 4 and be considered positive. These are rare carcinomas, and their response to hormonal therapy has not been studied specifically.

Quantification of ER and PgR—There is a wide range of receptor levels in cancers, as shown by the biochemical ligand binding assay and as observed with IHC. Patients whose carcinomas have higher levels have improved survival when treated with hormonal therapy.^{16,17} Quantification systems may use only the proportion of positive cells or may include the intensity of immunoreactivity:

- **Number of positive cells:** The number of positive cells can be reported as a percentage or within discrete categories (Figure).
- **Intensity:** The intensity refers to degree of nuclear positivity (ie, pale to dark). The intensity can be affected by the amount of protein present, as well as the antibody used and the antigen retrieval system. In most cancers,

there is heterogeneous immunoreactivity with pale to darkly positive cells present.

Two methods of quantifying ER by using both the intensity and the percentage of positive cells are the Allred score¹⁷ (Table 2) and the H score¹⁸ (Table 3). The 2 systems classify carcinomas into similar, but not identical, groups.¹⁹ If high-affinity antibodies are used with sensitive detection systems, most carcinomas will fall into clearly positive (score 7 or 8) or clearly negative (score 0) categories by the Allred score.^{20,21} A small group of carcinomas ($<1\%$ of total) show intermediate levels of immunoreactivity.

Quantitation can also be performed using the proportion of positive cells. In one study, carcinomas were scored as 0 ($<1\%$ positive), 1 (1%–25% positive), 2 ($>25\%$ –75% positive), and 3 ($>75\%$ positive).²² The same results were obtained when scored by visual analysis or by image analysis. The proportion of positive cells correlated with the results of the biochemical assay and with prognosis. In another study, carcinomas with small numbers of positive cells (between 1% and 10%) had a prognosis between cancers with no or rare positive cells ($<1\%$) and cancers with more than 10% positive cells.¹⁶

HER2 Testing

Scientific Rationale—A subset of breast carcinomas (approximately 15%–20%) overexpress human epidermal growth factor receptor 2 (HER2; HUGO Nomenclature, ERBB2). Protein overexpression is usually due to gene amplification. Assays for gene copy number, messenger RNA (mRNA) quantity, and protein generally give similar results; gene amplification correlates with protein overexpression in about 95% of cases. In a small subset of carcinomas (probably $<5\%$), protein overexpression may occur by different mechanisms. Overexpression is both a prognostic and predictive factor.

Clinical Rationale—HER2 status is primarily evaluated to determine patient eligibility for anti-HER2 therapy. It may identify patients who have a greater benefit from anthracycline-based adjuvant therapy.

Methods—HER2 status can be determined in formalin-fixed, paraffin-embedded tissue by assessing protein expression on the membrane of tumor cells using IHC or by assessing the number of *HER2* gene copies using in situ hybridization (ISH). When both IHC and ISH are performed on the same tumor, the results should be correlated. The most likely reason for a discrepancy is that one of the assays is incorrect, but in a few cases, there may be protein overexpression without amplification, amplification without protein overexpression, or marked intratumoral heterogeneity.

HER2 Testing by Immunohistochemistry

Factors altering the detection of HER2 by IHC have not been studied as well as they have for ER and PgR. It is recommended that tissue be fixed in buffered 10% formalin

Table 2. Allred Score ^a for Estrogen and Progesterone Receptor Evaluation			
Positive Cells, %	Proportion Score	Intensity	Intensity Score
0	0	None	0
<1	1	Weak	1
1–10	2	Intermediate	2
11–33	3	Strong	3
34–66	4		
≥ 67	5		

^a The Allred score combines the percentage of positive cells and the intensity of the reaction product in most of the carcinoma.¹⁷ The 2 scores are added together for a final score with 8 possible values. Scores of 0 and 2 are considered negative. Scores of 3–8 are considered positive.

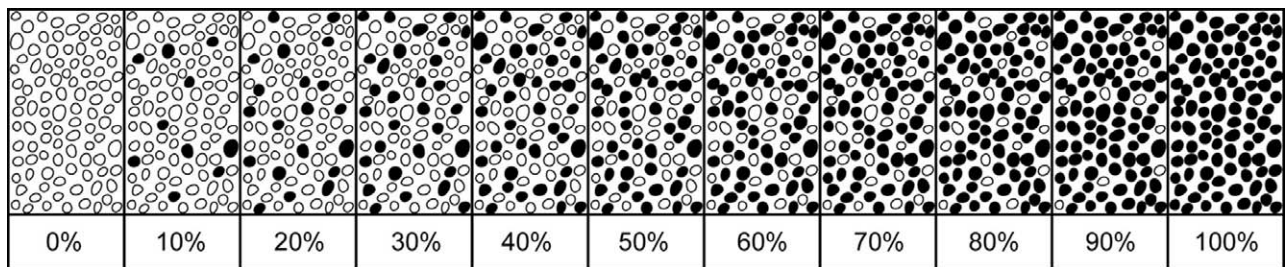


Figure. Quantification of immunohistochemical findings. The percentage of positive cells can be visually estimated.

for at least 6 hours unless another fixative has been validated. External proficiency testing surveys for HER2 are available from the CAP and other organizations. These surveys are invaluable tools to ensure that the laboratory assays are working as expected.

False-positive IHC results for HER2 may be due to the following:

- **Edge artifact.**—This is usually seen in core biopsies, where cells near the edges of the tissue stain stronger than those in the center, possibly because antibody pools at the sides. Specimens with stronger staining at the edge of the tissue should be interpreted with caution.
- **Cytoplasmic positivity.**—Cytoplasmic positivity can obscure membrane staining and make interpretation difficult.
- **Overstaining (strong membrane staining of normal cells).**—Overstaining may be due to improper antibody titration (concentration too high).
- **Misinterpretation of ductal carcinoma in situ (DCIS).**—High-grade DCIS is often HER2⁺. In cases with extensive DCIS relative to invasive carcinoma (particularly micro-invasive carcinoma), HER2 scoring may mistakenly be done on the DCIS component. Care must be taken to score only the invasive component.

False-negative IHC results for HER2 may be due to the following:

- **Prolonged cold ischemia time.**
- **Tumor heterogeneity.**—When a negative result is found, but only a small biopsy sample was tested, repeat testing on a subsequent specimen with a larger area of carcinoma should be considered, particularly if the tumor has characteristics associated with HER2 positivity (ie, tumor grade 2 or 3, weak or negative PgR expression, increased proliferation index).

Table 3. H Score ^a for Estrogen and Progesterone Receptor Evaluation		
Cell Signal	Calculation of H Score	
	Percentage of Cells	Value Multiplied
Cells with no signal		% × 0 = 0
Cells with weak signal		% × 1 =
Cells with moderate signal		% × 2 =
Cells with strong signal		% × 3 =
Total score =		

^a The H score is determined by multiplying the percentage of cells demonstrating each intensity (scored from 0 to 3) and adding the results.¹⁸ There are 300 possible values. In this system, <1% positive cells is considered to be a negative result.

- **Improper antibody titration (concentration too low).**
False-negative and false-positive results can be reduced by paying attention to the following:
- **Tissue controls.**—External controls must stain as expected. There are no normal internal controls for HER2 protein assessment by IHC.
- **Correlation With Histologic and Other Biomarker Results.**—If the HER2 test is negative by IHC but the tumor has characteristics associated with HER2 positivity (see above), repeating the test by ISH should be considered.

Reporting Guidelines—ASCO and the CAP have issued recently updated recommendations for reporting the results of HER2 testing by IHC (Table 4).^{6,7}

HER2 Testing by In Situ Hybridization

Fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and silver-enhanced in situ hybridization (SISH) studies for *HER2* determine the presence or absence of gene amplification. Some assays use a single probe to determine the number of *HER2* gene copies present, but most assays include a chromosome enumeration probe (CEP17) to determine the ratio of *HER2* signals to copies of chromosome 17. Although 10% to 50% of breast carcinomas have more than 2 CEP17 copies, only 1% to 2% of carcinomas show true polysomy (ie, duplication of the entire chromosome).

Failure to obtain results with ISH may be due to the following:

- Prolonged fixation in formalin (>1 week)²³
- Fixation in nonformalin fixatives²⁴
- Procedures or fixation involving acid (eg, decalcification), which may degrade DNA²⁵
- Insufficient protease treatment of tissue

External proficiency-testing surveys for HER2 by ISH are available from the CAP and other organizations. These surveys are invaluable tools to ensure that the laboratory assays are working as expected.

Reporting Guidelines—ASCO and the CAP have issued recently updated recommendations for reporting the results of HER2 testing by ISH (Tables 5 and 6).^{6,7}

Important issues in interpreting ISH are the following:

- **Identification of invasive carcinoma:** A pathologist should identify on the hematoxylin-eosin (H&E) or HER2 IHC slide the area of invasive carcinoma to be evaluated by ISH.
- **Identification of associated DCIS:** In some cases, DCIS will show gene amplification, whereas the associated invasive carcinoma will not. The ISH analysis must be performed on the invasive carcinoma.

Table 4. Reporting Results of HER2 Testing by Immunohistochemistry

Result	Criteria
Negative (score 0)	- No staining observed or - Incomplete, faint/barely perceptible membrane staining in $\leq 10\%$ of invasive tumor cells
Negative (score 1+)	Incomplete, faint/barely perceptible membrane staining in $> 10\%$ of invasive tumor cells^a
Equivocal (score 2+) ^b	- Incomplete and/or weak to moderate circumferential membrane staining in $> 10\%$ of invasive tumor cells or - Complete, intense, circumferential membrane staining in $\leq 10\%$ of invasive tumor cells^a
Positive (score 3+)	Complete, intense, circumferential membrane staining in $> 10\%$ of invasive tumor cells^a

^a Readily appreciated using low-power objective and observed within a homogeneous and contiguous population of invasive tumor cells.

^b Must order reflex in situ hybridization test (same specimen) or new test (new specimen, if available, using immunohistochemistry or in situ hybridization).

Some cancers have a low level of HER2 expression, as determined by equivocal results by both IHC and ISH analysis. Repeat testing may be helpful to exclude possible technical problems with the assays but often does not result in definitive positive or negative results.

Either the number of *HER2* genes or the ratio of *HER2* to *CEP17* can be used to determine the presence of amplification. In most carcinomas, both methods give the same result. In unusual cases, the 2 methods give different results, usually because of variation in the number of *CEP17* signals. Some studies have shown that chromosome 17 abnormalities can lead to alterations of the *HER2:CEP17* ratio, potentially leading to equivocal or incorrect ISH results.²⁶ In such cases, gene copy number may be a more accurate reflection of HER2 status. If there is a second contiguous population of cells with increased HER2 signals/cell, and that cell population consists of more than 10% of the tumor cells on the slide (defined by image analysis or by visual estimation of the ISH or IHC slide), a separate counting of at least 20 nonoverlapping cells must also be done within that cell population and also be reported. An overall random count is not appropriate in this situation.

Table 5. Reporting Results of HER2 Testing by In Situ Hybridization (Single-Probe Assay)

Result	Average No. <i>HER2</i> Signals/Cell
Negative (not amplified)	< 4.0
Equivocal ^a	≥ 4.0 and $< 6.0^b$
Positive (amplified)	$\geq 6.0^b$

^a Must order a reflex test (same specimen using dual-probe in situ hybridization or immunohistochemistry) or a new specimen, if available, using in situ hybridization or immunohistochemistry.

^b Observed in a homogeneous and contiguous population of $\geq 10\%$ of invasive tumor cells.

Table 6. Reporting Results of HER2 Testing by In Situ Hybridization (Dual-Probe Assay)

Result	Criteria
Negative (not amplified)	<i>HER2/CEP17</i> ratio < 2.0 AND average <i>HER2</i> copy number < 4.0 signals/cell
Equivocal ^b	<i>HER2/CEP17</i> ratio < 2.0 AND average <i>HER2</i> copy number ≥ 4.0 but < 6.0 signals/cell ^a
Positive (amplified)	<i>HER2/CEP17</i> ratio $\geq 2.0^a$ (regardless of average <i>HER2</i> copy number) or Average <i>HER2</i> copy number ≥ 6.0 signals/cell ^a (regardless of ratio)

^a Observed in a homogeneous and contiguous population of $\geq 10\%$ of invasive tumor cells.

^b Must order a reflex test (same specimen using immunohistochemistry), test with alternative in situ hybridization chromosome 17 probe, or order a new test (new specimen, if available, ISH or IHC).

Abbreviations: IHC, immunohistochemistry; ISH, in situ hybridization.

Ki-67 Testing

Ki-67 is a nuclear protein found in all phases of the cell cycle and is a marker of cell proliferation. The monoclonal antibody MIB-1 is the most commonly used antibody for assessing Ki-67 in formalin-fixed, paraffin-embedded tissue sections. The percentage of Ki-67⁺ tumor cells determined by IHC is often used to stratify patients into good and poor prognostic groups, but there is a lack of consensus on scoring, on the definition of low versus high expression, on the appropriate cutoff point for positivity, and on which part of the tumor should be scored (eg, leading edge, hot spots, overall average).²⁷ There is also a paucity of data on the effects of preanalytic variables (eg, ischemic time, length of fixation, antigen retrieval) on Ki-67 staining. For these reasons, routine testing of breast cancers for Ki-67 expression is not currently recommended by either ASCO or the National Comprehensive Cancer Network (NCCN).

Multigene Expression Assays

Scientific Rationale—Breast cancers vary greatly in histologic appearance, expression of biomarkers, response to treatment, and prognosis. Assays that detect variations in gene expression by mRNA levels have confirmed the diversity of gene expression patterns underlying these observations.

Clinical Rationale—It may be possible to use multigene expression assays to identify specific tumor subtypes and improve our ability to assess prognosis and likelihood of response to specific treatments.²⁸

Methods—A variety of multigene and multiprotein expression assays are available, most of which are proprietary assays developed and performed by a single laboratory. Multigene assays detect gene expression patterns by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) or by hybridization of labeled nucleic acids derived from the tumor to a number of small, immobilized, synthetic DNA strands (microarrays). Using these methods, numerous gene products can be examined simultaneously in the same sample. Some of the assays have been optimized for use on formalin-fixed tissue, whereas others require frozen tissue.

Reporting Guidelines—Pathologists may choose to incorporate results of proprietary assays into their own

reports if that makes the information more accessible for patient care.

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