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.**

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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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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 ≥ 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.	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.
	Interpretation follows guideline recommendation.	Positive ER or PgR requires that ≥ 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.
	Accession slip and report must include guideline-detailed elements.	
Optimal tissue handling requirements	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 postanalytic 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 al[6] and Mohsin et al[10] 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.[11] 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,[12] 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
McCarty 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 (≥ 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 (≥ 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 ≥ 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 ≥ 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

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Reference	No. of Patients (eligibility)	Intervention (outcome)	Original Assay (cutoff)	Retrospective 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 ≥ 10% stained cells)	Concordance of 88% (κ = 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 recurrence[17] 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 recurrence[17] 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 value[10] 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 correlation 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 ≥ 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 ≥ 10% (high)
Cheang et al, 2006[15]	SP1	≥ 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 (≥ 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 ≥ 10% (high)
Phillips et al, 2007[16]	1294	Allred score ≥ 3 (1% to 10% weakly positive cells)
Dowsett et al, 2008[17]	312	≥ 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 unfixed 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 1A6, 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.

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.

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

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

CORRECTION

The article by Hammond et al, entitled American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer, that appears in the June 2010 issue (*Arch Pathol Lab Med*. 2010;134[6]:907-922) contained errors.

In the Recommendations section, under the heading ''What Are the Clinically Validated Methods That Can Be Used in This Assessment?'' and subheading ''Laboratory concordance with standards,'' references 71 and 60 were cited in the sixth and seventh sentences of the first paragraph, whereas references 11 and 12 should have been cited, respectively.

In the same section, under the heading ''What Are the Preanalytic, Analytic, and Postanalytic Variables That Must Be Controlled to Ensure That the Assays Reflect the Tumor ER and PgR Status?'' and subheading ''Analytic standardization: antibody selection for ER testing,'' the antibody 1A6 was inadvertently omitted from the third sentence, which should have read:

''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 **1A6,** 1294 and 312 (Table 3).''

The online version of the abridged version of this paper has been revised to reflect these corrections. In addition, the online version of the unabridged version of this paper that appears as a Web-only publication for the July 2010 issue of the *Archives* has been revised to reflect the second correction listed above.