# Hormone receptors in breast cancer: Clinical utility and guideline recommendations to improve test accuracy

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## INTRODUCTION

The importance of steroid hormone receptors to the biology of breast cancer was recognized over 40 years ago, when it was observed that radiolabeled estrogens concentrated preferentially in the estrogen-influenced target organs of both animal and human breast cancers. These findings gave rise to the concept of an estrogen receptor (ER). It has since become clear that human breast cancers are dependent upon estrogen and/or progesterone for growth and that this effect is mediated through ERs and progesterone receptors (PRs). Not surprisingly, ERs and PRs are both overexpressed in malignant breast tissue.

New insights into hormone receptor biology and the increasing array of proteins that can modify their function have already translated into better therapies for breast cancer. As an example, a number of drugs that interact with the receptor, including selective ER modulators (SERMs) and selective ER downregulators, are approved for the treatment and prevention of breast cancer. At the same time, improved methods for assaying receptor proteins have led to less expensive and simpler measurements of ER and PR. Unfortunately, however, these methods have been fraught with variation that has resulted in inaccurate test results. Since these tests guide the use of endocrine therapy in patients with breast cancer, the American Society of Clinical Oncology and the College of American Pathologists convened a panel to address causes of variation related to measurement of ER and PR by immunohistochemistry (IHC) in 2010 [1,2]. The guideline was updated and reaffirmed in 2020 [3,4].

Here we review the clinical utility of hormone receptor testing in breast cancer and the guideline recommendations published in 2010 and reaffirmed in 2020 for measurement of ER and PR in tissue samples by IHC. An overview of ER biology, the mechanism of action of SERMs at the level of the hormone receptor, the use of endocrine therapy for adjuvant treatment of early-stage breast cancer and for treatment of metastatic disease, and an overview of prognostic factors for patients with breast cancer are discussed elsewhere.

- (See "Molecular biology and physiology of estrogen action".)
- (See "Mechanisms of action of selective estrogen receptor modulators and down-regulators".)
- (See "Prognostic and predictive factors in early, non-metastatic breast cancer".)
- (See "Adjuvant endocrine and targeted therapy for postmenopausal women with hormone receptor-positive breast cancer".)
- (See "Treatment for hormone receptor-positive, HER2-negative advanced breast cancer".)

## ESTROGEN AND PROGESTERONE RECEPTORS

Guidelines from the American Society of Clinical Oncology and College of American Pathologists recommend that both estrogen receptor (ER) and progesterone receptor (PR) analysis should be performed routinely in all invasive breast cancers, and the information should be used to select patients for endocrine therapy since there is evidence in clinical trials that PR positivity predicts endocrine responsiveness regardless of ER status. ER-negative, PR-positive tumors may be falsely ER negative [1,2,5-7].

The responsiveness of a tumor to endocrine therapy is an important parameter in breast cancer management. However, not all patients with breast cancer benefit from endocrine therapy. Tumor expression of ER and/or PR can best identify those women who are most likely to benefit from endocrine therapy. Tumors that are negative for ER and PR are unlikely to respond to endocrine therapy and are better served by cytotoxic chemotherapy. Largely due to their predictive value, measurement of these receptors has become a routine part of the evaluation of breast cancers. (See "Treatment for hormone receptor-positive, HER2-negative advanced breast cancer" and "Adjuvant endocrine and targeted therapy for postmenopausal women with hormone receptor-positive breast cancer".)

**Molecular biology** — ER and PR are both members of the nuclear hormone receptor superfamily that includes the androgen and retinoid receptors. These receptors are located in

the cytosol of target cells and operate as ligand-dependent transcription factors. Attachment of a lipid-soluble hormone to the ligand-binding domain results in unmasking of the DNA-binding sites on the receptor, followed by migration into the nucleus, and binding to specific hormone-responsive elements near the genes that are responsible for the physiologic actions of the hormone.

Subsequent steps include transcription of messenger RNA and ribosomal RNA and the eventual synthesis of new proteins. Two isoforms of the ER have been identified: ER-alpha and ER-beta. Although similar in many respects, the transcriptional properties of both receptors are different. In this review, we will focus on the measurement of ER-alpha only, since virtually all publications that provided evidence for guideline recommendations relate to measurement of this isoform. The molecular biology of the ER is discussed separately. (See "Molecular biology and physiology of estrogen action" and "Mechanisms of action of selective estrogen receptor modulators and down-regulators", section on 'Estrogen receptor-beta: A second ER isoform'.)

As with ER, the PR protein exists as two receptor isoforms (in this case, called A and B), but these forms are the products of the same gene. These isoforms of PR (A is a slightly truncated form of B) bind with one another to create homo- and heterodimers. There has been little work on the relative significance of these isoforms in clinical tissue specimens and their relevance to clinical decision making, although this is an active area of research.

**Predictive value** — ER expression predicts patients who will benefit from endocrine therapy. Although patients with PR-positive tumors also have better outcomes when treated with endocrine therapy, PR status is heavily dependent on ER; therefore, it does not appear that PR has independently predictive value, especially when the ER status is known. The predictive value of hormone receptor expression for responses to endocrine therapy is discussed separately. (See "Prognostic and predictive factors in early, non-metastatic breast cancer", section on 'ER predicting response to endocrine therapy'.)

**Prognostic value** — Multiple studies describe the relationship between hormone receptor levels as determined by immunohistochemistry and patient outcomes. Further discussion is covered separately. (See "Prognostic and predictive factors in early, non-metastatic breast cancer", section on 'Luminal subtypes'.)

## ASSAYS FOR ER AND PR

Originally, estrogen receptor (ER) and progesterone receptor (PR) quantitation was done by an assay involving the competitive binding of a radiolabeled steroid ligand to the receptor. Since

the early 1990s, such methods have largely been replaced by immunohistochemical (IHC) methods that rely upon the recognition of the receptor protein through the use of specific antibodies. In both cases, the receptor protein is either directly or indirectly measured. A third, newer technique is measurement of ER messenger RNA by either northern blot analysis or reverse-transcriptase polymerase chain reaction (RT-PCR), often as part of a multigene assay method (eg, Oncotype DX, MammaPrint) [8-10]. At present, these techniques are not recommended to replace traditional assays because they have not been used as predictive ER assays in clinical trials, although they have significant advantages for reproducibility. It is likely that they will become routine assays in the future [11].

**Ligand-binding assay** — The prototype method for the ligand-binding assay (LBA) is the dextran-coated charcoal (DCC) assay. Radiolabeled steroid ligand ([125I]-estradiol for ER, [3H]-progestin for PR) is first added to the supernatant of homogenized tissue (representing the cytosol) and incubated to allow labeled steroid to bind all available receptor protein [12]. DCC, which absorbs unbound steroid, is added, after which the charcoal with adherent unbound steroid is separated by centrifugation.

Since the receptor-bound portion remains in the supernatant and the free fraction is found in the charcoal precipitate, the bound and unbound fractions can then be quantified for a range of concentrations of radiolabeled steroid and the results used to create a multipoint Scatchard plot. From this Scatchard plot, the total concentration of receptor protein in the cytosol is obtained and is usually expressed as femtomoles of receptor protein per mg of total cytosol protein (fmol/mg).

**Antibody-based assays** — The availability of monoclonal antibodies to the ER in the 1980s had a profound and long-lasting impact on the methodologies for ER assay. Initially, quantification was by enzyme immunoassay (EIA) that still required preparation of cytosols from homogenates of fresh tissue but was more precise and far less laborious than the DCC assay. The results from the new structurally specific assay were highly correlated with the old functionally specific assay, and cutoffs approved by the US Food and Drug Administration (FDA) for EIA (ER positivity ≥15 fmol/mg cytosol protein) were mostly based upon correlative values with LBA (ER positivity ≥10 fmol/mg cytosol protein). In addition, use of an optimal cutting temperature embedding compound with frozen breast tumors did not alter either ER or PR results.

IHC application of hormone receptor-specific antibodies was initially successful only on sections of frozen tissue, but antigen retrieval methods developed in the early 1990s and new antibodies allowed their application to formalin-fixed and paraffin-embedded material. The ease of this approach, coupled with the availability of inexpensive reagents that were applicable to routine pathology specimens and the ability to evaluate small cancers and ensure that only invasive

tumor cells are assessed, has led to IHC becoming the near universal choice for ER and PR assay determinations since the early 1990s.

However, much of the data that provided the basis for our knowledge of the relationship between the presence of significant amounts of ER and response to endocrine therapy were derived using the LBA, and positive-negative cutoffs were developed by reference to LBA results. IHC methods have been subject to retrospective comparisons with established methods such as LBA, and there are some published reports indicating that IHC may be more predictive than LBAs in identifying patients who will derive benefit from endocrine therapy ( table 1) [6,13-23].

IHC assays that identify ER as a nuclear protein in cell fractionation experiments largely support this observation. Signaling through this mechanism is designated the **"genomic" signaling pathway**. However, emerging cell line data show that ER may signal through a separate, **"nongenomic" pathway** [24]. In this case, ER is found on the cell membrane and in the cell cytoplasm. This pathway is important, particularly when the epidermal growth factor receptors (including the human epidermal growth factor receptor 2 [HER2]) are also upregulated. ER binds to upregulated epidermal growth factor receptor (EGFR) and HER2 receptors, which initiates a cascade of growth factor signaling that leads to PR downregulation and alteration of the apoptotic pathway, a mechanism of tamoxifen resistance [25-27]. (See "Molecular biology and physiology of estrogen action", section on 'Non-genomic actions of estrogens'.)

IHC can be performed on thin sections of formalin-fixed, paraffin-embedded tissue, or on sectioned frozen specimens. Initially, 4 to 5 micron sections are cut and mounted on protein-coated glass slides. Sections are heated to uncover hidden protein epitopes and exposed to a primary anti-ER and/or anti-PR antibody [28]. A secondary antibody that recognizes the first, which is attached to an enzyme such as horseradish peroxidase, is then added. This linked enzyme converts substrates like diaminobenzidine into colored molecules upon exposure to a developer. Tissue sections are then counterstained, and the amount of ER or PR protein present is semi-quantitated according to the presence of nuclear staining, which may be heterogeneous in malignant epithelium ( picture 1) [29,30]. Staining of membranes or cytoplasm may be seen as valid staining, but should **not** be used to define ER or PR positivity. If such staining is seen in both the tumor and the internal control (normal ducts in the specimen), the assay should be repeated because it is likely that the staining is spurious.

IHC offers several advantages over LBA:

- It can be performed on a variety of samples including fine needle aspirates, core biopsies, small tumors, and cell blocks from body fluids such as pleural effusions, frozen tissue, or

fixed paraffin-embedded archival tissue.

- In contrast to the LBA, total receptor protein is measured, not just the unbound fraction. As a result, IHC is not affected by endogenous or exogenous steroids or tamoxifen.
- It is simple to perform and does not require specialized equipment.
- As noted above, in some reports, the ability to discriminate estrogen-responsive from estrogen-nonresponsive tumors is somewhat better with IHC than with LBAs [6,13,14,17].

For all of these reasons, IHC has rapidly become the predominant method for measuring ER and PR in clinical practice.

**Assay variability** — IHC provides two pieces of information: the percentage of positive cells and the intensity of staining within individual cells. Scoring systems (ie, percentage of positive cells versus staining intensity) as well as cutoff points (thresholds) separating positive and negative samples differ among individual laboratories [31-34].

The reported percentages of positive cells vary according to the method by which the observations are made. Some observers quantitate by overall assessment, some manually count areas of positivity, and others use image analysis [35,36]. In contrast to the significant body of data correlating the degree of tamoxifen benefit in ER-positive tumors to quantitative ER levels as assessed by LBA (including levels as low as 10 fmol/mg of cytosolic protein [37]), the importance of quantifying hormone receptor expression by IHC remains an open question. In the Stockholm Breast Cancer Study Group report, for example, the degree of tamoxifen benefit in ER-positive tumors correlated with ER levels by LBA but not with the percentage of cells stained ER-positive by IHC [23]. Reproducible quantitation of IHC results by computer-aided image analysis is valuable in this regard, providing a more accurate classification of ER-positive disease [38-45]. Some commercial methods are in use for HER2 testing, but few are utilized for ER and PR testing. One is commercially available [46], and another system is available as an open-source, web-based image analysis system [47]. Use of image analysis has suffered because of lack of published recommendations for equipment validation and testing requirements, although a validation guideline was published to address this [48]. Guideline recommendations for quantitative image analysis of biomarkers have been published by the College of American Pathologists in 2019 [49].

Other reports have highlighted the extent of variability in ER and PR assay results caused by a variety of factors. Results can vary substantially between laboratories because of differences in specimen handling, tissue fixation, antigen retrieval, and antibody type. Depending on the epitope recognized, different antibodies may only detect certain forms of the receptor, such as

ER-alpha or ER-beta, and may fail to detect a specific form or variant. In addition, interpretation of assay results is variable due to the variable threshold values for positive and negative [16,35,50-52]. These variations have resulted in serious issues with ER reliability prior to the development of a national guideline. (See 'Guideline recommendations to improve ER and PR testing accuracy' below.)

Assay testing issues have been highlighted in other parts of the world. For example, several Canadian studies demonstrated variations in policies and practices related to ER testing [53,54]. In addition, older studies from Asia reported that breast cancer was mostly ER negative. However, the percentage of ER-positive tumors in a series of 1000 patients from the Philippines was 68.9 percent after changes in fixation were instituted [55]. In other series from Vietnam and China [56], and Nigeria [57], over two-thirds of patients had exhibited ER- and PR-positive disease after implementation of standardized practices for procurement and formalin fixation of breast cancer specimens for hormone receptor assays.

Reports from large, multinational cooperative groups have addressed concordance between ER testing at the primary institutional site of patient enrollment and centrally by high-volume, academic laboratories [15,31,58]:

- In the Breast Cancer Intergroup trial Eastern Cooperative Oncology Group (ECOG) 2197, 11 percent of local ER-negative tests were scored positive upon central testing, for an overall concordance rate for ER of approximately 90 percent [58].

- ER and PR assay differences between local versus central reference laboratory testing were addressed in a study examining tumor blocks from almost 5000 patients enrolled from countries worldwide (except the United States and China) in the ongoing Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization (ALTTO) trial between June 2007 and November 2008 that were submitted for pre-randomization confirmation of HER2 status at the European Institute of Oncology [15]. Overall, 4.3 percent of the cases were found to be falsely positive for ER on central review, and 20 percent of cases were found to be falsely negative.

- Viale et al examined the prognostic and predictive value of both local and central hormone receptor expression on disease-free survival for 3650 patients who had been assigned to the single-agent arms in the Breast International Group 1-98 adjuvant trial evaluating letrozole versus tamoxifen versus combined therapy [31]. Using positivity criteria of ER and/or PR ≥10 percent of positive cells, central review confirmed 97 percent of tumors as hormone receptor positive. However, 69 percent of the small number of tumors (n = 105) said to be ER negative were actually ER positive.

All of these cited trials suffer from differences in methodology and thresholds between central and local institutional testing, which make it difficult to accurately quantify the magnitude of the difference between local versus central laboratory testing. In general, the ER false-negative rate (central versus local testing) was in the range of 10 percent, while the false-positive rate has been as high as 5 percent for ER. Having a guideline available that specifies the threshold for positive and negative assays will likely mitigate these accuracy concerns.

## GUIDELINE RECOMMENDATIONS TO IMPROVE ER AND PR TESTING ACCURACY

The guideline panel convened by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) reviewed the literature concerning estrogen receptor (ER) and progesterone receptor (PR) testing [1,2]. The data set, which included 216 studies assessing immunohistochemistry (IHC) methods related to a comparator (which in 17 studies was patient outcomes, such as disease-free survival), was the basis for the recommendations.

Guideline elements that were recommended to reduce assay variability included definition of appropriate specimen handling, fixation and analytical testing methods, thresholds for interpretation of positive and negative results, quality assurance methods, and monitoring strategies for individual laboratories. These elements are described in the following sections and summarized in the table and associated algorithm ( table 2 and algorithm 1).

### Specimen-handling methods

**Cold ischemic time** — Issues related to testing variation begin as soon as the breast tissue is removed from the patient. Both the warm and cold ischemic times are widely accepted as important variables in the analysis of labile macromolecules (eg, proteins, RNA, 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 specimen excision to the initiation of tissue fixation.

Numerous studies have documented the progressive loss of activity of labile macromolecules following the surgical interruption of blood flow, which leads to tissue ischemia, acidosis, and enzymatic degradation [59-61]. The contribution of the warm ischemic interval to this macromolecular degradation is under study.

Breast resection specimens 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 should be recorded in order to document the time to fixation.

Every effort should be made to transport specimens from the operating room to the pathology laboratory as soon as possible. Ideally, the time from tumor removal to fixation should be kept to one hour or less [1,2]. (See "HER2 and predicting response to therapy in breast cancer", section on 'Testing for HER2 expression'.)

After being received in the pathology laboratory, specimens should be oriented and carefully inked for surgical margin assessment, sectioned at 5 mm intervals, and placed in 10 percent neutral (phosphate) buffered formalin (NBF). If the excision specimen was obtained remotely from the grossing laboratory, the sample should be bisected through the tumor and promptly placed in NBF prior to transport. The time of removal of the tumor from the patient and the time to insertion of tumor sample into fixative should be noted by the remote operating suite personnel.

Studies have examined the effect of delays in tissue processing on ER negativity rates [62]. In this retrospective multicenter study of 5000 patients treated within the Intermountain Healthcare hospital system, all analytical and reporting considerations were done in a single location. The rates of ER negativity were significantly different and varied with both the site of tumor removal and the days on which surgery was performed. For patients having tumor resections on Sunday through Thursday at any site, rates of ER negativity were significantly lower than for those who had surgery on Friday or Saturday. This strongly suggested that specimen-handling issues (in particular, prolonged specimen handling ( figure 1)) were at fault.

Intermountain Healthcare conducted a follow-up study where times of tissue removal and time to fixation (cold ischemic time) were standardized and recorded by two of the six sites [63]. For the two sites, the average time to fixative was 18 minutes, and the average time in fixative was 18 hours. In this cohort, there was a lower prevalence of ER and PR negativity as compared with the four facilities that lacked standardized preanalytical specimen handling conditions, although only the difference in PR negativity rate (24 versus 30 percent) was statistically significant [63].

**Fixative type and duration** — Only 10 percent NBF should be used as the fixative for breast tissue [64-66]. If a laboratory uses a formalin alternative for fixation, the assay must first be validated against NBF fixation.

Breast tissue specimens (including core needle biopsies) must be fixed in 10 percent NBF for no less than six hours and not more than 72 hours before processing. The 2013 ASCO/CAP guideline update for human epidermal growth factor receptor 2 (HER2) testing has now reconciled the fixation time required for ER, PR, and HER2 testing so that any specimen for these testing procedures should be fixed 6 to 72 hours [67]. (See "HER2 and predicting response to therapy in breast cancer", section on 'Testing for HER2 expression'.)

Formalin is aqueous, completely dissolved formaldehyde. Formalin penetrates tissue at a rate of approximately 1 mm/hour, which is the reason why breast excision samples must be incised in a timely fashion to initiate formalin fixation throughout the tissue. Fixation does not begin until formaldehyde has penetrated into the tissue. However, permeation of tissue by formalin is not the same as the chemical reaction of fixation, which involves protein cross-linking by formaldehyde. Underfixation of breast tissue may lead to false-negative ER results. Overfixation is likely to be less problematic than underfixation, but could also potentially lead to false-negative results due to excessive protein cross-linking by formaldehyde [64,66].

**Analysis of ER and PR expression** — Testing for estrogen receptor (ER) and progesterone receptor (PR) by IHC must be performed using antibodies that have proven reliable in predicting patient outcome of endocrine treatment. A list of antibodies with acceptable clinical validation was provided in the guideline document [1,2], but any antibody that has been shown to predict endocrine therapy responses in published reports can be used for these assays.

Testing for ER and PR must be validated by each laboratory in a set of samples (40 to 80) that are compared with another laboratory using the same antibody method and threshold value for interpretation. Guidelines for IHC and biomarker validation have been published [68,69]. The testing method must also involve both positive and negative assay controls that are run concurrent with every clinical specimen.

**Interpretation of ER and PR tests** — The threshold for a positive result for hormone receptor expression was carefully considered by the guideline panel and is established as at least 1 percent of cancer cells staining for estrogen receptor (ER) or progesterone receptor (PR) [3]. In 2020, the panel recommended that cancers with 1 to 10 percent of cells staining for ER should be reported as a new category, "ER low positive." As previously, a sample is considered ER negative if <1 or 0 percent of tumor cell nuclei are immunoreactive. Previous cutoffs for ER and PR positivity by IHC ranged from 10 to 20 percent staining of cancer cells. IHC testing results of at least 1 percent positive-staining carcinoma cells (for either ER or PR) are associated with clinical response to endocrine therapy [13,15,18,70-72]. The panel readily acknowledged, however, that there are only limited data to support endocrine therapy benefits for cancers with 1 to 10 percent of cells staining ER positive [3,23,70,73-80]. Practically, this means that all

patients with ER or PR expression >1 percent should be offered endocrine therapy. However, if patients with ER expression of 1 to 10 percent do not tolerate treatment for whatever reason, it may be reasonable to discontinue therapy. Genomic assays can help determine whether such patients should receive chemotherapy, as discussed elsewhere. (See "Deciding when to use adjuvant chemotherapy for hormone receptor-positive, HER2-negative breast cancer", section on 'Overview of gene expression profiles'.)

By contrast, patients whose cancers demonstrate greater than 10 percent staining should be urged to continue endocrine therapy if at all possible due to strong evidence of beneficial effects. The administration of endocrine therapy for adjuvant and metastatic breast cancers is discussed separately. (See "Adjuvant endocrine and targeted therapy for postmenopausal women with hormone receptor-positive breast cancer" and "Treatment for hormone receptor-positive, HER2-negative advanced breast cancer".)

Importantly, ER and PR staining does not determine a diagnosis of malignancy, but rather dictates whether cancer cells (identified on the basis of morphology and evidence of invasion) are described as hormone receptor positive.

ER and PR tests should only be evaluated by a pathologist after assuring that the test has been done properly. This is done by reviewing the status of the external controls and also reviewing the status of internal controls for each breast cancer sample. Normal breast epithelium is usually ER- and PR-positive with at least 10 percent of cells expressing these receptors and therefore serve as a positive control for hormone receptor staining.

Review of internal controls is critical for proper interpretation because it assures that if present, the ER and PR receptors can be correctly identified in the tissue being examined. The entire sample of tumor cells is then carefully examined, and the percent of tumor cells expressing ER or PR receptor in the nuclei of the cells is recorded along with an assessment of the strength of the receptor staining (weak, moderate, strong, 1 to 3+ ( algorithm 1)).

When IHC assays replaced ligand-binding assays (LBAs) in the early-to-mid 1990s, relatively few clinical studies had been performed to establish optimum cut points for either assay. Instead, most studies simply compared the two and assumed that the IHC level that corresponded to the previously determined LBA cut point was also valid ( table 1). IHC appears to be equivalent or superior to LBA in predicting response to endocrine therapy.

**Reporting results** — Reporting elements that should be included are shown in the table ( table 3). The percentage of tumor cells, intensity of tumor cell staining, and the interpretation of the test as positive or negative should be provided. Tumors exhibiting less than 1 percent of tumor cells staining for ER or PR of any intensity should be considered

negative based upon data that such patients do not derive meaningful benefit from endocrine therapy.

The sample should only be considered negative in the presence of appropriately stained extrinsic and intrinsic controls. Any specimen that is negative by ER and/or PR IHC assay but lacks intrinsic elements (normal breast epithelium) should be reported as uninterpretable rather than negative. The assay should be repeated using another tumor block or specimen. The pathologist must always take specimen handling issues into consideration and correlate the results of the ER and PR assay with the histologic appearance of the tumor (eg, almost all grade 1 [low-grade] breast cancers are ER and PR positive ( table 4)). Results that conflict with clinical considerations should be discussed and perhaps repeated.

**Laboratory quality assurance monitoring** — The guideline is based on regulatory requirements of the Clinical Laboratory Improvement Amendments of 1988 (CLIA 88), as well as published studies of the experience of CAP and other groups, and expert panel consensus [50,81-83].

ER and PR testing should be performed in a CAP-accredited laboratory or in a laboratory that meets the additional accreditation requirements as set out within the guideline [1,2]. While mandatory proficiency testing has been a component of laboratory accreditation in Australia and New Zealand since 2001, there are no regulatory requirements for proficiency testing of ER or PR assays in the United States in order for laboratories to be accredited by CLIA. However, the current guideline makes successful performance in proficiency testing mandatory for accreditation. The CAP Laboratory Accreditation Program will monitor performance in the required proficiency testing. Competency of the laboratory personnel performing the ER/PR testing, including the pathologists, is an important aspect of the laboratory proficiency.

## SOCIETY GUIDELINE LINKS

Links to society and government-sponsored guidelines from selected countries and regions around the world are provided separately. (See "Society guideline links: Breast cancer".)

## SUMMARY

- Assay for tumor expression of estrogen receptors (ER) and progesterone receptors (PR) have established utility in the clinical management of patients with both early-stage and advanced breast cancer, and they should be routinely obtained on all tumor specimens. Receptor positivity is an important indicator of hormone responsiveness and identifies

tumors for which endocrine therapy is a valuable therapeutic option, both for adjuvant therapy and for advanced disease. (See 'Introduction' above.)

- Immunohistochemistry (IHC) has rapidly become the predominant method for measuring ER and PR in clinical practice. (See 'Antibody-based assays' above.)
- Other methods such as RNA expression assays are not recommended for routine use.
- Reports have highlighted the extent of variability in ER and PR IHC assay caused by a variety of factors including differences in specimen handling, tissue fixation, antigen retrieval, and antibody type. In addition, variability in interpretation of assay results is caused by variable threshold values for positive and negative. These variations have resulted in serious issues with ER reliability prior to the development of a national guideline. (See 'Assay variability' above.)
- We agree with the guidelines from a joint panel of the American Society of Clinical Oncology and the College of American Pathologists that provide recommendations to improve test accuracy ( table 5) and recommendations for reporting of results ( table 3) [1,2]. The use of guidelines, along with strategies to monitor compliance with them, should improve the accuracy of these assays and therefore improve the selection of patients for whom endocrine therapy is likely to provide clinical benefit. (See 'Guideline recommendations to improve ER and PR testing accuracy' above.)



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Topic 747 Version 28.0

## GRAPHICS

### Comparison of ER by ligand binding assay (LBA) and immunohistochemistry (IHC) in selected trials

| Reference | n | Original assay (cut off for positivity) | IHC assay concordance with original assay | Outcome according to biomarker |
|---|---|---|---|---|
| McCarty et al, 1985 | 134 patients | LBA (≥20 fmol/mg) | 89% | Objective clinical response to hormonal therapy: specificity 89%, sensitivity 93%. |
| Barnes et al, 1996 | 170 patients | LBA (≥20 fmol/mg) | 81% | Response to tamoxifen in 72% ER/PR positive and in 61% ER positive/PR negative; superior result with IHC. |
| Harvey et al, 1999 | 1982 patients | LBA (≥3 fmol/mg) | 71% | In multivariate analysis, ER status determined by IHC was significantly better than ER status by LBA at predicting better DFS; results were similar for prediction of overall survival. |
| Elledge et al, 2000 | 205 patients | LBA (≥3 fmol/mg) | 90% | Overall response rate 56% if LBA positive versus 60% if IHC positive. In multivariate analysis, there was significant correlation between IHC for ER and response to tamoxifen. |
| Thomson et al, 2002 | 332 patients | LBA (positive if ≥20 fmol/mg) | Spearman's rank correlation coefficient 0.55 | Significant interaction between IHC score of zero and lack of benefit from ovarian ablation. |
| Regan et al, 2006 | 571 patients | LBA (positive if ≥20 fmol/mg) | Concordance 88% (k = 0.66) in postmenopausal patients | Hazard ratios were similar for association between DFS and ER status or PR status by either LBA or IHC. In premenopausal women, IHC was better than LBA for predicting DFS. |

| | | | | |
|---|---|---|---|---|
| Khoshnoud et al, 2011 | 683 patients | LBA (positive if ≥50 fmol/mg) | Overall concordance rate 88% | Hazard ratios were similar for association of tamoxifen use with improved recurrence free survival regardless of assay used. |

DFS: disease-free survival; ER: estrogen receptor; fmol/mg: fentomoles per mg; IHC: immunohistochemical staining; LBA: ligand-binding assay; PR: progesterone receptor.

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*Modified from: Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010; 28:2784.*

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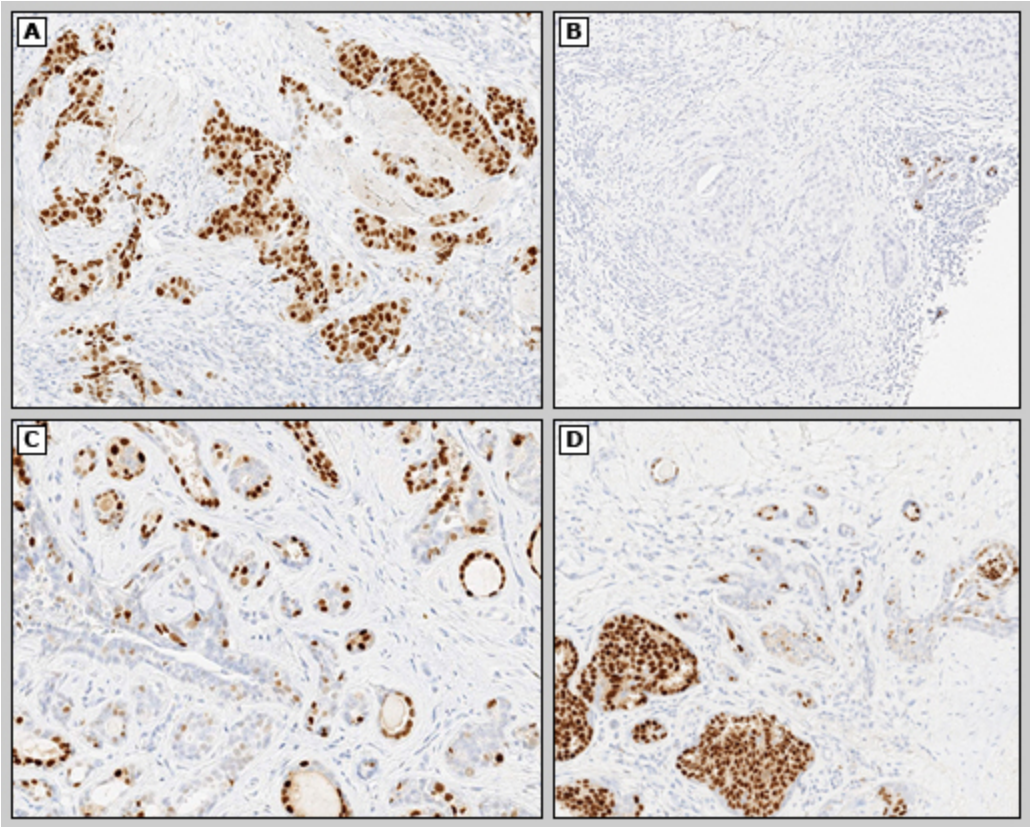
Graphic 56559 Version 12.0

## Estrogen receptor stain in breast cancer




(A) Strongly ER-positive invasive cancer. Tumor cells exhibit variable intensity of staining in >90% of the cells.

(B) Tumor cells stained for ER show no ER activity. Normal breast ducts, shown at right have variable positivity indicating that the assay can be interpreted.

(C) Breast cancer with variable ER activity in invasive tumor cells. Although many areas are negative, some are strongly positive and the proportion of positive cells is greater than 1%. No intrinsic normal elements (ducts) are seen, but they were also positive in this case.

(D) Illustrates intrinsic controls. Slide shows DCIS at the lower left and normal ducts at the upper right. The DCIS is strongly ER positive.

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ER: estrogen receptor; DCIS: ductal carcinoma in situ.

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Graphic 63384 Version 5.0

## Summary of all ASCO/CAP recommendations for ER and PR testing in breast cancer

| 2010 Recommendation | Updated recommendation |
|---|---|
| **Clinical question 1:** What are the optimum QA, specimen handling, positive threshold, scoring system, and reporting for determining potential benefit from endocrine therapy? | |
| **Optimal algorithm for ER/PR testing** | **Optimal algorithm for ER/PR testing** |
| Positive for ER or PR if finding that ≥1% of tumor cell nuclei are immunoreactive. | Samples with 1 to 100% of tumor nuclei positive for ER or PR are interpreted as positive. |
| Negative for ER or PR if finding that <1% of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PR (positive intrinsic controls are seen). | For reporting of ER (not PR), if 1 to 10% of tumor cell nuclei are immunoreactive, the sample should be reported as ER low positive with a recommended comment. |
| Uninterpretable for ER or PR 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. | A sample is considered negative for ER or PR if <1% or 0% of tumor cell nuclei are immunoreactive. |
| | A sample may be deemed uninterpretable for ER or PR if the sample is inadequate (insufficient cancer or severe artifacts present, as determined at the discretion of the pathologist), if external and internal controls (if present) do not stain appropriately, or if preanalytic variables have interfered with the assay's accuracy. |
| | Clinicians should be aware of and be able to discuss with patients the limited data on ER-low positive cases and issues with test results that are close to a positive threshold. |
| **Optimal testing conditions** | **Optimal testing conditions (no changes)** |
| Large (preferably multiple) core biopsies of tumor are preferred for testing if they are representative of the tumor (grade and type) at resection. | Large (preferably multiple) core biopsies of tumor are preferred for testing if they are representative of the tumor (grade and type) at resection. |
| Accession slip and report must include guideline-detailed elements. | Accession slip and report must include guideline-detailed elements. |
| **Optimal tissue handling requirements** | **Optimal tissue handling requirements (no changes)** |

| | |
|---|---|
| Time from tissue acquisition to fixation should be as short as possible. Samples for ER and PR 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. | Time from tissue acquisition to fixation should be as short as possible. Samples for ER and PR 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, use of slides cut more than 6 weeks before analysis is not recommended. | As in the ASCO/CAP HER2 guideline, use of unstained slides cut more than 6 weeks before analysis is not recommended. |
| The 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. | The 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. |
| **Optimal internal validation procedures** | **Optimal internal validation procedures** |
| Internal validation must be done before test is offered; refer to separate article on testing validation.[1] | This topic is deferred to the forthcoming CAP guideline update, principles of analytic validation of IHC assays, once available. There should be initial test validation/verification prior to reporting any clinical samples. Prior to that, previously recommended principles apply.[1, 2] |
| Validation must be done using a clinically validated ER or PR 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** | **Optimal internal QA procedures** |
| Ongoing quality control and equipment maintenance. | Ongoing quality control and equipment maintenance are required. |
| Initial and ongoing laboratory personnel training and competency assessment. | Initial and ongoing laboratory personnel training and competency assessment should be performed. |
| Use of SOPs, 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 | SOPs should be used that include 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 (or other appropriate |

| | |
|---|---|
| breast sections on each tested slide, wherever possible. | control) on each tested slide, wherever possible. External controls should include negative and positive samples as well as samples with lower percentages of ER expression (such as tonsil). On-slide controls are recommended. |
| Regular, ongoing assay reassessment should be done at least semiannually;[1, 2] revalidation is needed whenever there is a significant change to the test system. | Regular, ongoing assay reassessment should be done at least semiannually.[1] Revalidation is needed whenever there is a significant change to the test system.[2] |
| Ongoing competency assessment and education of pathologists. | Ongoing competency assessment and education of pathologists is required. |
| **Optimal external proficiency assessment** | **Optimal external proficiency assessment** |
| Mandatory participation in external proficiency testing program with at least two testing events (mailings) per year. | The laboratory performing ER and PR testing must participate in external proficiency testing or alternative performance assessment as required by its accrediting organization. |
| Satisfactory performance requires at least 90% correct responses on graded challenges for either test. | |
| **Optimal laboratory accreditation** | **Optimal laboratory accreditation** |
| On-site inspection every other year with annual requirement for self-inspection. | On-site inspection every other year should be undertaken with annual requirement for self-inspection. |
| **Clinical question 2:** What additional strategies can promote optimal performance, interpretation, and reporting of IHC assays, particularly in cases with low ER expression? | |
| No specific recommendations were specified in 2010 for low ER expression cases. | Laboratories should include ongoing quality control using SOPs for test evaluation prior to scoring (readout) and interpretation of any case (refer to UpToDate algorithm on scoring of IHC for ER in BC). |
| | Interpretation of any ER result should include evaluation of the concordance with the histologic findings of each case. Clinicians should also be aware of when results are highly unusual/discordant and work with pathologists to attempt to resolve or explain atypical reported findings (refer to UpToDate table on invasive breast cancer histopathologic concordance with ER staining). |
| | Laboratories should establish and follow an SOP stating the steps the laboratory takes to |

| | |
|---|---|
| | confirm or adjudicate ER results for cases with weak stain intensity or ≤10% of cells staining. |
| | The status of internal controls should be reported for cases with 0 to 10% staining. For cases with these results without internal controls present and with positive external controls, an additional report comment is recommended. |
| **Clinical question 3:** Are other ER expression assays acceptable for identifying patients likely to benefit from endocrine therapy? | |
| No assays other than IHC are recommended as testing platforms. | Validated IHC is the recommended standard test for predicting benefit from endocrine therapy. No other assay types are recommended as the primary screening test for this purpose. |
| **Clinical question 4:** Should DCIS be routinely tested for hormone receptors? | |
| ER and PR testing of DCIS is optional (no formal recommendation made to test or not test). | ER testing in cases of newly diagnosed DCIS (without associated invasion) is recommended to determine potential benefit of endocrine therapies to reduce risk of future breast cancer; PR testing is considered optional. |

ASCO: American Society of Clinical Oncology; CAP: College of American Pathologists; ER: estrogen receptor; PR: progesterone receptor; QA: quality assurance; NBF: neutral buffered formalin; HER2: human epidermal growth factor receptor 2; IHC: immunohistochemistry; SOP: standard operating procedure; BC: breast cancer; DCIS: ductal carcinoma in situ.

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*References:*

1. *Fitzgibbons PL, Murphy DA, Hammond ME, et al. Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. Arch Pathol Lab Med 2010; 134:930.*
2. *Torlakovic EE, Cheung CC, D'Arrigo C, et al. Evolution of quality assurance for clinical immunohistochemistry in the era of precision medicine. Part 3: Technical validation of immunohistochemistry (IHC) assays in clinical IHC laboratories. Appl Immunohistochem Mol Morphol 2017; 25:151.*



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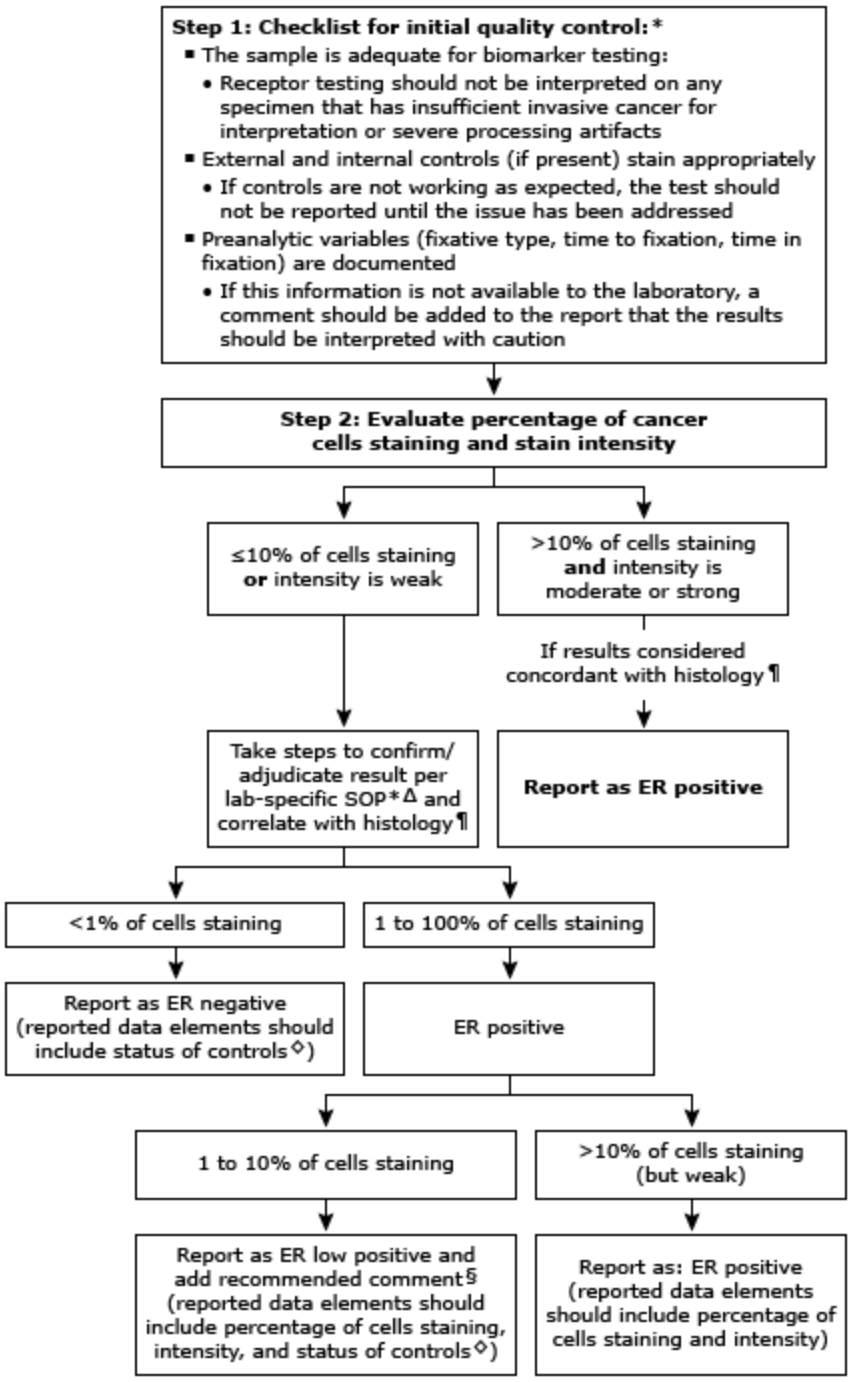
Graphic 130206 Version 1.0

## Recommendations for scoring (readout) and interpretation of IHC test to determine ER status in breast cancers




For PR testing, the same overall interpretation principles apply, but the reporting elements are only recommended for ER testing. PgR should be interpreted as either positive (if 1 to 100% of cells have nuclear staining) or negative (if 1 or 0% of cells have nuclear staining), with the overall percentage and intensity of staining reported.

IHC: immunohistochemistry; ER: estrogen receptor; SOP: standard operating procedure; PgR: progesterone receptor.

* Hormone receptor testing should only be done with a validated method and with appropriate laboratory procedures, including ongoing assay monitoring and pathologist competency assessment.

¶ Refer to UpToDate graphic on invasive breast cancer histopathologic concordance with ER staining.

Δ Steps to consider including in SOP:

- Re-review of controls
- A second reviewer to confirm interpretation
- Validated quantitative digital image analysis to confirm interpretation
- Comparison of result with any prior patient-specific results
- Retesting the same sample if analytic issues suspected (eg, controls did not work as expected)
- Repeating the test on a different block or subsequent specimen if there are no internal controls, preanalytic issues are suspected, or result is unusual or unexpected

◇ If no internal controls are present but external controls are positive, include comment: "No internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status."

§ For ER-low-positive results, include comment: "The cancer in this sample has a low level (1 to 10%) of ER expression by IHC. There are limited data on the overall benefit of endocrine therapies for patients with these results, but they currently suggest possible benefit, so patients are considered eligible for endocrine treatment. There are data that suggest invasive cancers with these results are heterogeneous in both behavior and biology and often have gene expression profiles more similar to ER-negative cancers."

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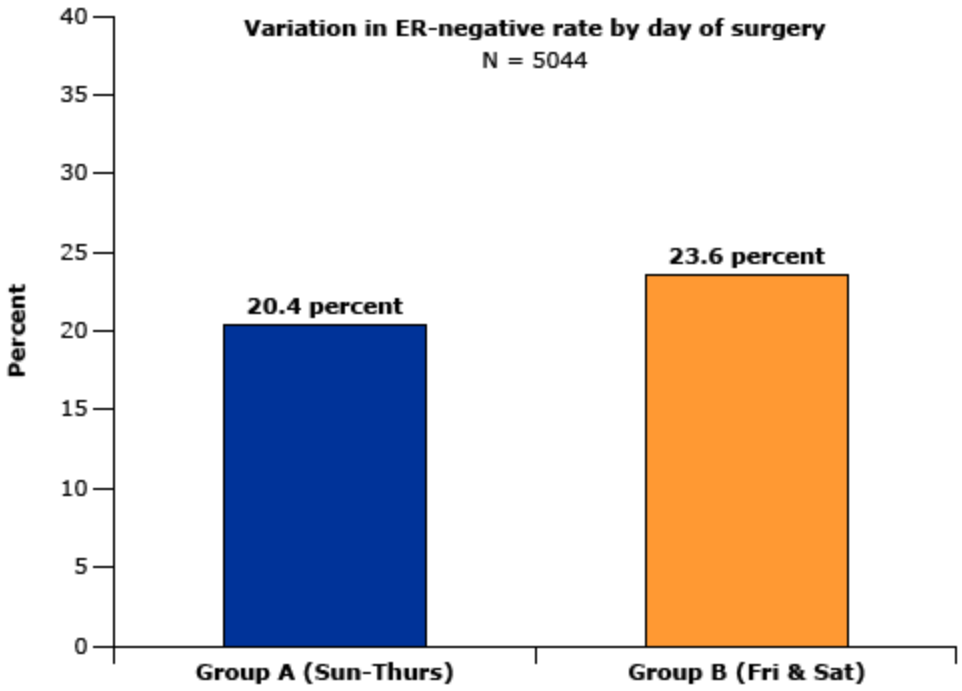
Graphic 130207 Version 1.0

## Immunohistochemistry data about ER-negative rate from the Intermountain Healthcare Study




ER: estrogen receptor.

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*Based on data from: Nkoy FL, Hammond ME, Rees W, et al. Arch Pathol Lab Med 2010; 134:606.*

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Graphic 76536 Version 2.0

## Reporting elements for ER and PR immunohistochemistry 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 utilized: |
| 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, weak |
| Interpretation: |
| - Positive (for ER or PR receptor protein expression), negative (for ER or PR protein expression), uninterpretable |
| - Internal and external controls (positive, negative, 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. |

Report should contain the elements in **green** as a minimum. Other information must be available in

the laboratory for review or appear on the patient accession slip.

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ER: estrogen receptor; PR: progesterone receptor.

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Graphic 78069 Version 4.0

## Invasive breast cancer histopathologic concordance with ER staining

| Highly unusual ER-negative results | Highly unusual ER-positive results |
|---|---|
| Low-grade invasive carcinomas of no special type (also known as invasive ductal carcinoma) | Metaplastic carcinomas of all subtypes |
| Lobular carcinomas (classic type) | Adenoid cystic carcinomas and other salivary gland-like carcinomas of the breast |
| Pure tubular, cribriform, or mucinous carcinomas | Secretory carcinoma |
| Encapsulated papillary and solid papillary carcinomas | Carcinomas with apocrine differentiation |

NOTE: If a result is considered highly unusual/discordant, additional steps should be taken to check the accuracy of the histologic type or grade as well as the preanalytic and analytic testing factors. This workup may include second reviews and repeat testing. If all results appear valid, the result can be reported with a comment noting that the findings are highly unusual and testing of additional samples may be of value to confirm the findings.

ER: estrogen receptor.

*Reprinted from: Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and progesterone receptor testing in breast cancer. American Society of Clinical Oncology/College of American Pathologists guideline update. Arch Pathol Lab Med 2020; 144(5):545-563. With permission from Archives of Pathology & Laboratory Medicine. Copyright © 2020 College of American Pathologists.*

Graphic 130208 Version 1.0

## Summary of guideline recommendations for ER and PR testing by immunohistochemistry in breast cancer patients

<table>
<tr><th colspan="2">Optimal algorithm for ER/PR testing</th></tr>
<tr><th>Recommendation</th><th>Comments</th></tr>
<tr><td>Positive for ER or PR if finding of ≥1% of tumor cell nuclei are immunoreactive.</td><td rowspan="2">These definitions depend on laboratory documentation of the following:<ol><li>Proof of initial testing validation using clinically validated antibody</li><li>Ongoing internal quality assurance procedures</li><li>Participation in external proficiency testing</li><li>Biennial accreditation by valid accrediting agency</li></ol></td></tr>
<tr><td>Negative for ER or PR if finding of <1% of tumors cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PR (positive intrinsic controls are seen).</td></tr>
<tr><th colspan="2">Optimal testing conditions</th></tr>
<tr><th>Recommendation</th><th>Comments</th></tr>
<tr><td rowspan="2">Large, preferably multiple core biopsies of tumor are preferred for testing if they are representative of the tumor (grade and type) at resection.</td><td>Specimen should be rejected and repeated on a separate sample if any of the following conditions exist:<ol><li>External controls are not as expected (scores recorded daily show variation)</li><li>Artifacts involve most of sample</li></ol></td></tr>
<tr><td>Specimen may also be rejected and repeated on another sample if:<ol><li>Slide has no staining of included normal epithelial elements and/or normal positive control on same slide</li><li>Specimen decalcified using strong acids</li><li>Specimen shows an ER-/PR+ phenotype (to rule out a false-negative ER assay or a false-positive PR assay)</li><li>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</li></ol></td></tr>
<tr><td>Interpretation follows guideline recommendation.</td><td><ul><li>Positive ER or PR requires ≥1% of tumor cells are immunoreactive; both average intensity and extent of staining are reported</li></ul></td></tr>
</table>

| | |
|---|---|
| | ▪ Image analysis is a desirable method of quantifying percentage of tumor cells which are immunoreactive<br>▪ H Score, Allred Score or Quick Score may be provided<br>▪ Negative ER or PR requires <1% of tumor cells with ER or PR staining |
| | Interpreters have method to maintain consistency and competency documented regularly. |
| Accession slip and report must include guideline-detailed elements. | |

| **Optimal tissue handling requirements** |
|---|
| **Recommendation** |
| Time from tissue acquisition to fixation should be as short as possible. Samples for ER and PR testing are fixed in 10% neutral buffered fomalin (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.<br><br>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. |
| Storage of slides for more than six weeks prior to 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. |

Guidelines from a combined panel from the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP).

ER: estrogen receptor; NBF: neutral buffered formalin; PR: progesterone receptor.

*Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010; 28:2784.*

Graphic 65263 Version 4.0

## Contributor Disclosures

**M Elizabeth H Hammond, MD, FCAP** No relevant financial relationship(s) with ineligible companies to disclose. **Daniel F Hayes, MD** Equity Ownership/Stock Options: Inbiomotion [Breast cancer]. Patent Holder: Immunicon Corporation [Inventor]; University of Michigan [Inventor]; University of Michigan [Inventor]. Grant/Research/Clinical Trial Support: AstraZeneca [Breast cancer]; Menarini Silicon Biosystems, LLC [Breast cancer]; Pfizer [Breast cancer]. Consultant/Advisory Boards: Artiman Ventures [Breast cancer]; BioVeca [Breast cancer]; Cepheid [Breast cancer]; EPIC Sciences, Inc [Breast cancer]; Freenome, Inc [Colorectal cancer]; Guardant [Oncology]; Lexent Bio [Breast cancer]; L-Nutra [Breast cancer]; Macrogenics [Breast cancer]; OncoCyte [Biomarkers]; Predictus BioSciences [Breast cancer]; Tempus [Oncology]; Turnstone Biologics [Breast cancer]; Xilis [GI cancer]. Other Financial Interest: Menarini Silicon Biosystems [Royalties from licensing of patent – Breast cancer]. All of the relevant financial relationships listed have been mitigated. **Sadhna R Vora, MD** No relevant financial relationship(s) with ineligible companies to disclose.

Contributor disclosures are reviewed for conflicts of interest by the editorial group. When found, these are addressed by vetting through a multi-level review process, and through requirements for references to be provided to support the content. Appropriately referenced content is required of all authors and must conform to UpToDate standards of evidence.

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