Eastern HEALTH

FAX TRANSMISSION

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RE:	**Article**

Bev,

This is the article I was referencing.

Bob

BCU 7 | 2004

Editor's Note

Team in need of a coach

Every medical oncology fellow quickly learns about interdisciplinary cancer care, but thank God for the American College of Surgeons' mandate for tumor boards, because without them, we might be strangers. Personally, I don't like to think about any surgeon, radiation oncologist or medical oncologist not regularly attending one of these valuable meetings. However, the truth is that we really don't report to anyone, and our collaboration is pretty much voluntary.

This issue of our audio series attempts to demonstrate how critical it is that interdisciplinary team members talk to each other. We begin with the local control guys, and Pat Borgen and Frank Vicini comment on a plethora of surgical and radiation therapy research issues that profoundly affect systemic management decisions.

For example, Dr Vicini is the principal investigator of a critical NSABP-RTOG randomized clinical trial evaluating partial breast irradiation (PBI). This historic collaboration between two premier collaborative clinical trial groups will provide much-needed answers about PBI, albeit many years from now. In the interim, the pace at which this accelerated and patient-friendly treatment strategy permeates into the nonprotocol management algorithm utilized in the community treatment setting is anyone's guess.

While we wait for definitive research results, patients should seek input from every team member regarding the advisability of PBI and which technique is preferable. Pat Borgen cautions us that local control may have much more of an impact on long-term survival than previously recognized, and one might imagine that PBI could either have a deleterious effect (if it results in suboptimal local tumor control) or could be a more effective modality (because treatment can be implemented prior to chemotherapy).

With an increasing number of patients receiving taxane-based adjuvant regimens that can take up to six months to complete, earlier radiation therapy could have a potential antitumor advantage.

From a quality of life perspective, avoiding six weeks of daily treks for radiation therapy is appealing, particularly after the physical and emotional trauma of adjuvant chemotherapy. However, patients will surely want to know what their medical oncologist has to say on this issue before they opt for an unproven treatment modality.

Input from Craig Allred, the pathologist for the interdisciplinary team collaborating on this issue of *Breast Cancer Update*, is unfortunately very disheartening. I have nothing personal against pathologists or Craig, who is a really nice man, but if Adam Brufsky's interview provides ample documentation that contemporary systemic therapy of breast cancer is essentially target-driven, then Craig's comments leave us wondering if we have the ability to measure the most critical targets every oncologist must consider — ER, PR and HER2 status. (My apologies to Phillip Roth for that very long sentence.)

I keep expecting some rebel breast cancer patient advocacy group to stage a massive protest at the NCI to demand that pathologists provide impeccable ER, PR and HER2 assays. At the present time, however, women are going to continue to relapse unnecessarily or receive suboptimal palliative care because we can't get their pathology right. Even if recent history tells us that our usually capable nation is not totally effective in military intelligence gathering, we should be able to at least gather accurate

information for the war on cancer.

Maybe we need more than ACOS-mandated tumor boards. Maybe we need someone to rally and guide the entire team — including nurses, pharmacists, radiologists, psychologists, social workers and others — and take a deep breath, and really figure out how to work together better so patients can receive the very best care we have.

— Neil Love, MD
NLove@ResearchToPractice.net

Select publications

Gene expression profiling studies have reconfirmed the previously realized biologic importance of ER in breast cancer. Perou et al[59] published the results of their breast cancer gene expression analysis in 2000 and found that expression profile patterns largely separated tumors into ER positive and ER negative categories. These findings have been confirmed by others using different sampling methods and expression profiling techniques.[60–62] Results from gene microarray studies have further categorized breast cancers into several major subtypes based on their patterns of gene expression, including the ER positive luminal subtype and the ER negative basal subtype.[59,60,63] The existence of these breast cancer phenotypes have been verified by immunohistochemical studies of protein expression.[64,65]

ER has complex relationships with other biomolecules relevant in breast cancer. The majority of cancers express ER and HER2 in an inverse manner, and a subset of tumors (approximately 10%) express both.[66–69] Although individual luminal cells of the normal breast rarely co-express ER and the proliferation marker Ki-67, a substantial proportion of breast cancer cells show this coexpression.[70] The interactions of ER with growth factors and signal transduction molecules appear to be important in the development of resistance to endocrine therapy.[71]

Although ER often retains its functionality during endocrine therapy, evidence suggests that adaptive signal transduction pathways stimulate tumor progression independent of ER-ligand interactions.[72] Currently, clinical ER testing assesses for the presence or absence of detectable ER protein regardless of its functional state.

ER TESTING

Interlaboratory Variability

Multiple reports addressing interlaboratory variability for ER testing have been published in the past several years, mostly from European institutions.[23,25,73–76] The most notable of these studies were conducted by Rhodes and colleagues under the auspices of the United Kingdom's national external quality assessment scheme for immunocytochemistry (NEQAS-ICC).[25,75,76] The NEQAS-ICC is presently comprised of 200 participating laboratories from 26 countries in Europe and Asia. For its first published comparative study, the NEQAS-ICC investigators circulated to participating laboratories unstained composite tumor sections known to possess low, medium, or high ER levels.[25] Only 37% of the participating laboratories were able to obtain a positive result for the presence of ER in tumors with low ER levels using the traditional 10% staining cutoff, but 66% reported a positive result if a 1% cutoff was used.[25]

The high rates of interlaboratory variability found through the NEQAS-ICC quality assessment scheme prompted further investigation into the causative factors of such variability. In a second study, tumors fixed and processed by the NEQAS-ICC centralized laboratory were assayed by the participants, and the results were compared with those obtained using tumors fixed and processed by the participating laboratories themselves.[76] Overall testing results were found to be equivalent for the two sets of tumors, validating the scheme's quality assurance mechanism (ie, distribution of unstained composite tumor sections). Moreover, their findings strongly suggested that preanalytical variables (tissue handling, fixation, and processing) do not greatly affect ER testing results using IHC.

In a later NEQAS-ICC report, the length of time for heat antigen retrieval was identified as the most important variable for improving ER testing standardization.[75] Additionally, using an elegant statistical analysis of their ER testing results over 2 years, NEQAS-ICC ranked their participants as "high assay sensitivity" or "low assay sensitivity" laboratories. NEQAS-ICC high assay sensitivity laboratories had a mean rate of positive ER testing for all patients of 77% (compared with 72% for low sensitivity laboratories).[21] Obviously, ER testing results for an individual laboratory will depend to some extent on the characteristics of the patient population studied, especially patient age and the clinical setting in which the testing is performed (eg, primary cancers versus recurrences or metastases). Nevertheless, interlaboratory comparisons of testing results such as those provided in the NEQAS-ICC studies could assist in identifying specific laboratories that could benefit from technical improvements in their ER testing methodologies.

Additional interlaboratory comparisons of ER testing performed in Austria and Sweden addressed staining technique and scoring reproducibility, respectively.[73,74] Although variation was demonstrated in both of these studies, the authors concluded that improvements in testing could be made through (automation) and training. A German study demonstrated poor reproducibility of ER testing using tissue microarrays with ER detection failure rates similar to those reported by the NEQAS-ICC.[77]

Layfield et al[23] published results demonstrating a disagreement rate of 26% among three laboratories in the United States independently testing 35 breast cancers for ER using IHC. That study was a follow-up to an earlier laboratory survey (in the form of questionnaires) that also demonstrated poor standardization for ER testing.[27] The more recent of the two studies is the only published interlaboratory comparison of ER testing in the United States in which unstained slides were circulated.[23]

ER testing findings for intraductal carcinoma from NSABP Protocol B-24 have recently been presented by Allred et al.[22] The predictive value of a positive ER status for response to tamoxifen therapy was demonstrated by these data. Additionally, it was observed that cases analyzed by participating institutions using non-standardized methods were more frequently ER negative compared with those tested by a centralized IHC laboratory (where a clinically validated and standardized testing method was used). The findings of Layfield[23] and by NSABP B-24[22] indicate that significant interlaboratory variability for ER testing does occur in the United States.

Currently, there are legitimate concerns worldwide that ER immunohistochemical testing methodologies are insufficiently standardized and that clinically significant false negative rates exist.[24,78] The interlaboratory comparisons of Rhodes et al[25] and Layfield et al[27] have convincingly revealed interlaboratory variability in ER testing methodologies and results. A concerted effort by laboratories to adopt reproducible and clinically validated testing standards for ER

IHC will be necessary to properly address this problem. If successfully implemented, standardization of ER testing could serve as a paradigm for the multitude of predictive markers that will likely be assayed by IHC in the future.

Technical Considerations

Standardization of ER detection methods (ie, specimen selection, processing, scoring, and quality measures) is of paramount importance for the accurate analysis of ER status and appropriate patient management. IHC is a commonly used and widely commercialized technique that already has achieved a marked level of standardization. As a complex multistep laboratory procedure, IHC requires highly trained personnel for its proper performance. Indeed, seemingly minor differences in testing procedures may lead to marked variability of results. An additional level of complexity is encountered when evaluating markers requiring quantitation, such as ER or HER2 for breast cancer. Multiple parameters, such as those listed in Table 1, should be considered when performing IHC to detect ER.[79,80] In the subsequent paragraphs we review these variables and discuss their importance.

When to Test

ER testing is indicated for all primary invasive breast carcinomas because of its proven prognostic and predictive value.[9,13,81,82] Many centers are now also performing ER testing in cases of ductal carcinoma in-situ (Fig. 3), a trend based primarily on the recently presented findings from NSABP Protocol B-24.[22] The true utility of ER testing for ductal carcinoma in-situ, however, remains controversial, and further studies are pending.

TABLE 1. Variables for ER Detection by Immunohistochemistry

Preanalytical variables
Timing of testing
Specimen type
Fixative type
Fixation time
Processing method
Analytical variables
Automated versus manual procedure
Antibody and titer
Antigen retrieval time
Blocking procedure
Detection kit used
Staining method
Interpretive variables
Manual scoring versus image analysis
Scoring systems
Scoring cutoffs
Quality assurance and control
Types of controls
Internal
External
Quantitative
Quality assurance procedures
External quality assessment programs

ER testing may also be indicated in the settings of recurrent and/or metastatic breast cancer (when a change of ER status would affect treatment decisions) because of potential alterations of the ER status of tumors over time.[83–86] It has been demonstrated that the ER status in approximately one third of breast cancers reverses during disease progression, both from positive to negative and from negative to positive.[85,86] These ER status conversions typically require several years to occur, but conversion from ER positivity to ER negativity has been documented in less than one year.[86] An ER status change to ER positive from ER negative may be beneficial to patients undergoing hormonal treatment.[83] Conversely, conversion to ER negative from ER positive can be associated with aggressive, therapy-resistant disease.[84] The ER status of the recurrent and metastatic disease should be considered as the current ER status of a given patient.

Types of Specimens

ER analysis by IHC is traditionally performed on formalin-fixed, paraffin-embedded histologic tumor sections chosen during diagnostic review of the hematoxylin and eosin-stained slides. Typically, tumors are sectioned from excisional or mastectomy specimens as part of the routine pathologic evaluation, and the amount of tumor available for analysis can vary widely based on the stage of disease. Analysis of ER in smaller-sized, paraffin-embedded specimens (such as needle biopsies) and air-dried or alcohol fixed direct smears can also be performed.[87]

Measurement of ER in large gauge needle core biopsies has been validated against results from excisional specimens in several studies.[88–90] Many centers, including ours, routinely assess breast tumor markers on needle core biopsy specimens (Fig. 4).[91] Intratumoral heterogeneity for ER expression can be biologic or artifactual in nature, and reduced staining is most often observed in the center of the tumor compared with periphery.[5,92] This heterogeneity does not substantially affect ER results obtained using needle core biopsy specimens. If the ER results measured on needle core biopsy are questioned (usually due to small tumor volume), repeat testing of the excision specimen is warranted.

The analysis of cytologic specimens for ER using immunocytochemistry (Fig. 5) has recently been reviewed by one of the authors (NS).[93] Prognostic and predictive markers of breast cancer, including ER, can be reliably assessed on cytologic material by IHC. Comparative studies have demonstrated concordance rates ranging from 80 to 90% for ER analysis of cytologic versus histologic specimens.[94–96] Clinically, ER analysis of cytologic specimens is important for patients receiving neoadjuvant chemotherapy and only when core needle biopsy is not available. In that setting, when response to therapy is dramatic, pretreatment cytologic smears of primary or meta-static disease may represent the only material available for ER analysis.

Tissue Handling, Fixation and Processing

Methods used for tissue handling, fixation, and processing can affect ER analysis by IHC. Gross examination of specimens and tissue submission techniques vary between institutions, but overall they are relatively standardized. It is

Biotech Histochem. 1992 Mar;67(2):110-7. Related Articles, Links

Quality assurance and standardization in immunohistochemistry. A proposal for the annual meeting of the Biological Stain Commission, June, 1991.

Taylor CR.

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Quality assurance, quality control, proficiency testing, reagent documentation and validation are standard parts of everyday practice in clinical laboratories throughout the United States. Immunohistochemical stains employ reagents and principles in common with immunoenzyme methods utilized in the clinical laboratory. However, immunohistochemistry has not routinely been subjected to similar standardization and quality assurance procedures that manufacturers and pathologists alike have applied to essentially the same techniques in the clinical laboratory environment. The current proposal was invited by the Biological Stain Commission with the charge of incorporating the findings of previous workshops on quality control in immunohistochemistry into a practical design for implementation. The status of quality assurance, quality control and standardization in immunohistochemistry is reviewed and a phased strategy for implementation is proposed.

Biotech Histochem. 1991;66(4):194-9.

The taming of immunohistochemistry: the new era of quality control.

Herman GE, Elfont EA.

Sinai Hospital, Department of Laboratory Medicine, Detroit, Michigan 48235.

The most critical factor for interpreting the results of immunohistochemistry is verification of antibody sensitivity and specificity. While some manufacturers supply material data sheets with this information, many do not. This paper describes a well-defined quality assurance program for testing immune reagents. This program can be used to provide commercial suppliers of antisera with analyses of their products destined for government licensure applications. This paper illustrates the protocol and explains the testing philosophy developed over the last eight years.

Publication Types:
- Review
- Review, Tutorial

Assessment of Tissue Estrogen and Progesterone Receptor Levels: A Survey of Current Practice, Techniques, and Quantitation Methods

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Abstract: The assessment of steroid hormone receptors in resected breast carcinoma tissue is currently the standard of practice. The traditional method for assessment of receptor status is the ligand binding assay. More recently, immunohistochemistry (IHC) has become a popular method for such testing. Despite the widespread use of IHC and the availability of many antibodies, standardization of quantitative IHC for assessment of estrogen and progesterone receptors has not been achieved. While the College of American Pathologists (CAP) offers a Quality Assurance (QA) program for IHC quantitation of estrogen receptor (ER) and progesterone receptor (PgR), no universal standard is currently recognized in assessment of ER and PgR by IHC. We surveyed 300 laboratories within the United States for their current practices regarding the assessment of ER and PgR status in breast cancer tissue specimens. Eighty usable responses were received. Forty-nine (61%) laboratories performed the assay in-house, while the remainder sent the material out for assay. All responding laboratories performing their steroid receptor analysis in-house used the IHC technique. Forty-three (80%) laboratories answering the question on material accepted for analysis performed the assay only on paraffin-embedded material, three (6%) used either paraffin block or frozen material, and two (4%) used only frozen material. Eighty-eight percent of laboratories performing steroid receptor analysis in-house used a manual quantitation technique. Four (8%) used computer-assisted image analysis, and a single laboratory used laser scanning cytometry. Eight different antibodies were used among the 44 laboratories documenting the antibody supplier, and for any given commercially prepared antibody a wide variety of dilutions were used, with the exception of the standard solution used with the Ventana antibody. Of the laboratories using manual estimation techniques, 61% simply estimated the percentage of positive cells, 29% evaluated both the intensity of staining and percentage of nuclei staining, 6% used formal H-score analysis, 2% evaluated only intensity of nuclear staining, and 2% mainly counted the percentage of nuclei staining for ER but used a formal H score in the assessment of PgR. Cutoff points for the separation of positive and negative results varied widely, with some laboratories assessing any demonstrable positivity as a positive result, while others required as many as 19% of the nuclei to stain before a specimen was declared positive. Standardization techniques differed considerably among laboratories. Eighty-six percent used the CAP program for QA. While all laboratories utilized some form of intralaboratory control for assessment of ER and PgR, the nature of that control varied from laboratory to laboratory. Our survey indicates that a majority of laboratories perform their steroid hormone receptor

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analysis in-house using IHC. There is considerable variability in the antibodies utilized, the dilutions applied, and the quantitation method and level of expression used to dichotomize specimens into positive and negative groups. Finally, no universal control for interlaboratory standardization appears to exist. ■

Key Words: breast carcinoma, estrogen receptor, progesterone receptor, steroid hormone assay

Estrogen receptor (ER) and progesterone receptor (PgR), by their interaction with their respective steroid hormones play important roles in regulating the proliferation and differentiation of normal breast epithelium (1). The level of steroid hormone receptor expression in breast carcinoma cells is believed to be associated with the responsiveness of the neoplastic cells to circulating estrogen and progesterone. During the past quarter century, many studies have measured tissue levels of ER and PgR by biochemical methods and correlated them with both prognosis and response to hormone therapy (2–5). Within the past decade, a variety of antibodies against both the ERs and PgRs have become available on a commercial basis. Many studies have compared the results of immunohistochemically determined steroid receptor values with those obtained by ligand binding analysis. In general, the correlation has been good (6–11). Despite agreement of results derived from individual antibodies used in immunohistochemistry (IHC) determinations and the ligand binding technique, significant variability has been documented in the results obtained by IHC using different commercially available antibodies (12,13). While variability between the results achieved by different antibodies may exist, the overall value of IHC-determined ER and PgR levels for the prediction of response to hormonal therapy and overall prognosis appears high (9,14–16). Some studies have documented IHC determination of ER to be superior to the ligand binding assay for the prediction of response to adjuvant endocrine therapy in breast cancer (17).

Despite the documented value and accuracy of ER and PgR assayed by IHC, a wide variety of antibodies and quantitation techniques are currently in use. The extent to which these variabilities in technique and material affect the predictive value and standardization of IHC determination of ER and PgR is unknown. Of significance, there is no universally accepted control for standardization of the assays of ER and PgR by IHC. Hence interlaboratory comparisons of steroid receptors as determined by IHC may not be entirely valid. The authors are aware of only a single quality assurance/quality control (QA/QC) program within the United States for IHC determination of steroid receptors, again bringing into question the overall consistency of results obtainable between laboratories. Despite such problems, the overall robustness of the method appears to compensate for the lack of precision associated with the technique. Several laboratories are working to refine protocols and standardization methods and Riera et al. (18) have recently proposed tissue cell culture lines as a universal control.

In order to clarify the current status of ER and PgR level determinations, we surveyed 300 laboratories to determine their methods and interpretative approach for the estimation of steroid hormone receptors in breast tissue and whether they take part in a regional or national QA/QC program in this field. Herein we report the results of that survey.

MATERIALS AND METHODS

Questionnaires were mailed with return addressed and stamped envelopes to 300 hospital pathology departments. The first 200 questionnaire recipients were drawn from the College of American Pathologists (CAP) directory by randomly selecting four pathologists from each state. An additional 100 pathologists with interest and expertise in the area of breast pathology were selected on the basis of a literature search for publications concerning estrogen and progesterone receptors.

The questionnaire contains questions relating to size and type of hospital practice. The questionnaire asked if they routinely ordered ER and PgR assays on newly diagnosed breast carcinomas and whether this analysis was done in-house or if it was sent out. If they performed in-house analysis, questions about the method used [IHC, dextran-coated charcoal (DCC) assay, polymerase chain reaction (PCR), or flow cytometry], type of material accepted [paraffin embedded, frozen tissue, or fine needle aspiration (FNA)], type of antibody, and dilution employed were included. The pathologists were also asked how quantitation was performed (image analysis, manually calculating the nuclei staining percentage, estimating the intensity of staining, H score), what the cutoff point was for differentiating positive and negative results, what protocol was used for standardization, and what controls were used. Lastly, the laboratories were asked whether they participated in any of the interinstitutional QA programs and, if so, which one.

In addition, 150 questionnaires were mailed with return addressed and stamped envelopes to hematology/

oncology departments in all 50 states. One hundred and ten oncologists were program directors at teaching hospitals. The remaining were randomly selected oncologists based at community hospitals. The questionnaire contains questions relating to the method used by the laboratory for the analysis of ER and PgR, the quantitation method, and the cutoff point used to separate positive and negative results. The questionnaire asked whether their treatment approach changed following a switch from DCC to IHC, whether they equated immunohistochemical expression of ER and PgR to specific femtomol values, and whether they required quantitation or merely positive and negative results. The oncologists were also asked whether they were influenced in choice of therapy by PgR status, or whether they gave tamoxifen regardless of steroid hormone receptor status to all postmenopausal patients. They were also asked if ER and PgR status had ever changed during treatment. Finally, their opinion regarding current recommendations (19,20) on chemotherapy and endocrine therapy and the duration of such treatment (21) (2 years versus 5 years) was sought.

Following mailing of the survey questionnaire, 3 months were allowed to pass before closure of the data collection period, allowing for adequate response time. The responses were entered on a spreadsheet program (Excel 7.0, Microsoft, Redmond, WA) and analyzed.

RESULTS

Responses were received from 80 of the 300 questionnaire recipients, geographically representing 35 states. Thirty (38%) were obtained from institutions describing themselves as community/general hospitals, seven from nonacademic tertiary care centers, 41 from academic tertiary care centers, 1 from a reference laboratory, and 1 did not indicate the type of institution. Respondents were almost equally distributed between hospitals with fewer than 250 beds (22), hospitals between 250 and 500 beds (26), and hospitals with more than 500 beds (29). Three respondents did not state the size of their institution.

Seventy-seven of the 80 (96%) responding practices routinely performed ER and PgR analysis on both in situ and invasive carcinoma of the breast. Two performed it only on invasive carcinoma, and a single respondent did not indicate his practice pattern. Both institutions performing ER and PgR analysis only on invasive carcinomas were community hospitals. Forty-nine of the 80 (61%) respondents performed steroid receptor hormone analysis in their own laboratories, 30 routinely sent tissue out for such analysis, and 1 laboratory performed in-house IHC but sent out tissue to have DCC ligand binding assay performed on some specimens. Table 1 shows the distribution of institutions performing steroid receptor analysis by hospital type. Academic tertiary care hospitals were most likely to perform the assays within their institution. Similarly, hospitals with more than 500 beds were more likely to perform steroid receptor analysis in their own laboratories. Community/general hospitals were most likely to send out ER and PgR analyses (23; 77%). A wide variety of academic tertiary care medical centers and specialty commercial laboratories were used for referral of tissue for steroid hormone analysis.

All institutions performing ER and PgR analysis in-house used immunohistochemistry. A single institution performed in-house IHC but sent out material for ligand binding assay in selected cases. Forty-three (80%) of the institutions answering the question on tissue acceptable for analysis performed ER/PgR analysis only on paraffin tissue, 6 (11%) performed the analysis on paraffin-embedded and FNA material, 2 (4%) performed the assay on paraffin-embedded, frozen, and FNA material, while 2 (4%) performed the assay only on frozen material. One (2%) respondent performed the analysis on paraffin-embedded and frozen material.

Methods of quantitation varied among the laboratories responding to our survey. Forty-two (88%) used a manual counting method for quantitation, 4 (8%) used computer-aided image analysis, and 1 (2%) used laser scanning cytometry. One additional laboratory (2%) used computer-assisted image analysis between 1988 and 1998 but recently changed to a manual technique. Of the laboratories utilizing a manual counting method for quantitation of ER and PgR levels, 30 (61%) manually counted the number of tumor cell nuclei staining positively and calculated a staining percentage. Fourteen respondents (29%) used both the percentage of positive nuclei and the intensity of staining. Three laboratories (6%) used formal H-score analysis. One laboratory (2%) measured only the intensity of staining. A single

Table 1. Hospital Type

Type	Number	Percentage
Community/general	30	37.5
Nonacademic tertiary	7	8.75
Academic tertiary	41	51.25
Reference laboratory	1	1.25
No response	1	1.25
Total	80	100

Table 2. Suppliers of Antibodies Used for ER Analysis by Responding Laboratories

Type	Number	Percentage
Dako	17	39
Ventana	15	34
Novacastra	3	7
AMAC	2	5
Immunotech	4	9
Abbott	1	2
Biogenex	1	2
Zymed	1	2
Total	44	100

Table 3. Range of Dilutions Used

ER	
Dako	5–2,000
Ventana	Predilution
AMAC	50–500
Novacastra	40–200
Immunotech	50–400
PR	
Dako	20–160
Biogenex	25–350
Novacastra	50–100

laboratory manually counted the nuclei staining percentage for ER but employed the H-score technique for PgR analysis.

Table 2 shows the different commercial suppliers of antibodies used for IHC analysis of ER and PgR. Antibodies were supplied by eight different companies, and antibody dilutions varied considerably, as indicated in Table 3. Cutoff points for separation of positive and negative results varied widely among laboratories. Even when the H-score system was used, the cutoff point was not uniform. Table 4 shows the cutoff points reported by the respondents. Some laboratories accepted any visually detectable staining as indicative of positive ER while others required the nuclei staining percentage to be as high as 20% before a tissue specimen was considered positive. Two laboratories (4%) did not interpret the results as either positive or negative, but simply estimated the nuclei staining percentage and intensity of staining present, leaving interpretation to the clinicians.

Protocols for standardization are listed in Table 5. There was considerable variation, with reliance on manufacturer protocols, DCC validation, or CAP survey. The controls used for standardization were also inconsistent among laboratories, with the majority of institutions (42; 74%) using known positive and negative cases as their controls. Table 6 shows the types of controls used by the responding institutions. Sixty-nine of the 80 institutions responding (86%) took part in the CAP program. The remaining 11 institutions did not take part in any QA/QC program.

Only 26 (17%) useful responses were received from the 150 questionnaires sent out to directors of hematology/oncology programs (representing 17 states). All 26 respondents routinely ordered steroid hormone receptor analysis on all newly diagnosed breast carcinomas. One of these did not routinely request such an analysis on intraductal carcinoma. Twenty-five respondents believed the technique used for the analysis was IHC, while one received information from the ligand binding (DCC)

Table 4. Criteria Used to Separate Positive and Negative Results

	Number	Percentage
10% positive cells	15	34
5% positive cells	12	28
Any positivity	4	9
Combination formula (number of cells and intensity)	3	7
<9% = negative, 9–19% = borderline, >19% = positive	2	4
Different criteria for ER and PgR	2	4
20% ER, 5% PgR		
20% ER, 10% PgR		
Number and intensity supplied for clinician interpretation	2	4
Combination of number and intensity		
10% positive with >2 (scale of 1–4)	1	2
H score of 50	1	2
H score of 10	1	2
San Antonio score		
1–2 = negative, 3 = borderline, 4–8 = positive	1	2
Intensity (SI 0–3), PP = percentage 0–4, IS = SI × PP		
0–1 = negative, 2 or more = positive	1	2
Total	45	100

Table 5. Protocols Used for Standardization

Protocol	Number
Positive and negative controls	3
DCC validation	3
Ventana automated stainer	2
Dako protocol	2
Do not know (DNK)	1
In-house standardized protocol (Techmate instrumentation) and standardized commercial reagents	1
Parallel testing for new kits and new antibody lot	1
CAP survey	1
Modified Techmate	1
All slides reviewed by the director	1
Ventana/microwave antigen retrieval	1
No protocol	1
DCC and reference laboratories	1
HIER target antigen retrieval (Dako)	1
CAS 2000	1
Manufacturers' guidelines/journals/textbooks	1

analysis. Of the 25 individuals experiencing a shift in analytic technique from DCC to IHC, only one changed their treatment approach because of the modification in technique. Thirteen of 25 (52%) hematologists/oncologists equated negative or low IHC values of ER and PgR with specific femtomol values. The other 12 did not directly correlate IHC results with femtomol levels.

In agreement with the results of the survey of pathologists, there was considerable variation in the cutoff points used by hematologists and oncologists to separate positive from negative ER results. These values ranged from 1 to 30%. Four hematologists/oncologists (18%) did not know the value used for stratifying ER results into positive and negative. Table 7 shows the distribution of cutoff points used by the responding hematologists and oncologists. Eleven respondents required quantitation in their practice, while 16 required only a statement of positive or negative. Thirteen of 25 respondents agreed with the recommendations for treatment of early breast cancer as stated in the *British Journal of Cancer* (19) and in the *Review of Seminars in Oncology* (20).

Table 7. Distribution of Thresholds for Establishing ER Positivity Used by Hematologists/Oncologists

Threshold	Number	Percentage
No response	7	26
10%	5	19
Do not know	4	15
10 fmol	4	15
5%	2	7
30%	2	7
1%	1	4
20% = negative, <30% = borderline, >30% = positive	1	4
Laboratory does it	1	3
Total	27	100

Sixteen of 25 (64%) responding hematologists/oncologists would not treat an ER-negative carcinoma in a postmenopausal patient with tamoxifen. Twenty-three of 27 responding hematologists/oncologists stated that they were influenced in their treatment decisions by the presence or absence of PgR positivity in the neoplasm. Twenty-five of 26 hematologists/oncologists stated that they had modified their treatment plans based on the ER or PgR status of the patient. Finally, 20 of 27 responding hematologists/oncologists stated that they had not changed the duration of treatment after publication of the Swedish Breast Cancer Cooperative Group results in *Journal of the National Cancer Institute* (21).

DISCUSSION

Since the recognition of the relationship between ER and PgR levels in breast carcinoma and patient prognosis and response to hormonal therapy, the assessment of steroid hormone receptors has become a widely accepted component in the examination of breast carcinomas (23). Initially assessment of ER and PgR levels was performed using a ligand binding technology (DCC) (2–5). More recently, ER and PgR assessment by IHC has become popular, if not the predominant technique. Many

Table 6. Type of Controls Used for ER and PgR Analysis

Control	Number	Percentage
Known positive and negative cases	42	71
Internal and external breast tissue control	9	17
EIA assayed breast cancers	1	2
Endometrium	1	2
ER, breast; PgR, endometrium	1	2
Abbott ER-ICA, PgR-ICA control slides for frozen and cytology	1	2
Control cases with H < 10, 10–100, and >100	1	2
Multitumor blocks	1	2
Total	57	100

studies have correlated the results of IHC with those obtained by the ligand binding method (6–12), and have confirmed the relationship of ER and PgR with patient prognosis and response to hormonal therapy (1,14–17). Despite the near uniformity in finding a high correlation between IHC and ligand binding assay results and good predictive value for IHC, these studies have used a variety of antibodies, antibody dilutions, staining and quantitation techniques, and cutoff points (23). The variation within the literature is reflected within community practice. Many of the issues relating to IHC determination of steroid hormone receptor levels reflect basic issues in quantitative IHC as discussed by a panel of experts during a recent meeting of the European Section of the International Academy of Pathology (Nice, France, October 1998).

In order to assess the current status of ER and PgR assay methods in the United States, we undertook a mail survey investigating the methodologies, controls, quantitation techniques, and cutoff points utilized by a variety of academic and nonacademic laboratories. Our study found a wide variation in the antibodies, dilutions, quantitation techniques, and cutoff points used by the respondents. While these variations do not in and of themselves negate the clinical significance of steroid hormone receptor analysis, the existence of such variations raises the potential for clinically significant discordance in reported steroid hormone receptor values between laboratories.

Commercial and large academic medical center laboratories perform approximately 38% of all ER and PgR assays, but the majority of such assays are performed in-house by local laboratories. Nearly all of these laboratories report using IHC for the assessment of ER and PgR. A number of observations can be made on the basis of this study. First, a confounding variable for interlaboratory comparisons of steroid hormone receptor results is the variety of materials accepted by various laboratories. Eighty-one percent of laboratories accept only paraffin-embedded material for analysis, but 11% used only frozen tissue or both frozen and paraffin-embedded tissue for analysis. In addition, 4% also accepted FNA specimens. Second, various antibodies were used. The laboratories in our study employed antibodies supplied by eight different manufacturers. Seventy-three percent of institutions used an antibody supplied by either Dako (Carpinteria, CA) or Ventana (Tucson, AZ). As shown in Table 3, even when using the same antibody, various institutions employed widely different antibody dilutions for their assays. These differences in antibodies and dilutions may have significant impact on the quantitative assessment of ER and PgR by IHC.

Third, methods for the quantitation of IHC results varied considerably between respondents. The majority (88%) of laboratories completing the survey questionnaire used various manual techniques for quantitation in which estimates of nuclei staining percentage were made. Multiple manual quantitation methods exist in addition to simply estimating the nuclei staining percentage. Twenty-nine percent of laboratories using a manual quantitation technique employed a technique where both the number of positive cells and the intensity with which the cell nuclei stained were estimated. Six percent used formal H-score analysis (6). We did not obtain information on the threshold of staining intensity used to accept a nucleus as positive for quantitation purposes. Neither did we obtain information on cell selection techniques. Clearly differences in counting technique can affect whether a neoplasm is designated positive or negative for ER and PgR. Recommendations for cell counting have been published (11).

Fourth, the cutoff points used for the assignment of breast cancer cell populations as positive for ER or PgR differ between laboratories. Our survey documented at least a fourfold variation in the nuclei staining percentage used by laboratories for the assignment of positivity. Twelve laboratories (27%) used a 5% cutoff point to designate a specimen as positive and four laboratories (9%) used a value of 20% as their cutoff point. Fifteen laboratories (33%) used a 10% nuclear positivity rate as an indicator of a positive ER level. Variability in cutoff point results in discordance of results even when methodologic aspects of the assay are identical. Such variability in threshold for positivity highlights the need for laboratories to include cutoff points in their reports. Thresholds used with other quantitation methods (H score) also varied. A few laboratories simply assessed the number of positively staining cells and the intensity of staining present, allowing the clinicians to interpret the data. Thus wide variability in practice exists and such variability may have a significant impact on the documentation of the presence or absence of clinically significant levels of ER and PgR.

Standardization protocols varied widely among the respondents to our survey. Only 22 respondents (28%) answered our query concerning their standardization protocol. Of these, three used DCC validation as their standardization technique, while a majority relied on manufacturer's guidelines, previously tested positive and negative intralaboratory controls, or simply review

of all slides by the laboratory director. Controls for the standardization of ER and PgR assays varied among the laboratories. The majority (52%) used previously assayed tissue blocks known to be positive and negative. This offered a degree of intralaboratory consistency but did not allow external verification of laboratory assay levels. Nine laboratories utilized internal breast tissue controls for the assessment of steroid hormone receptors in associated neoplastic tissue. Occasionally laboratories utilized endometrium as a positive control. As stated by Riera et al. (18), no universal control exists for external validation of steroid hormone receptor assays by IHC. The lack of such a control complicates comparisons between laboratories using different antibodies, dilutions of antibodies, and modifications of the IHC technique. Recently, cultured cells have been suggested as a control for quantitative immunocytochemical analysis of ER levels (18). Widespread utilization of such a standard control should increase the comparability of ER results performed at different laboratories.

Only 26 responses (17%) from our survey of 150 directors of hematology/oncology programs were received, meaning conclusions based on this small dataset should be made with caution. However, certain trends were noted. First, the respondents routinely ordered ER and PgR analysis on all newly diagnosed breast carcinomas, implying general acceptance by oncologists for measurement of ER and PgR. The majority of responding oncologists did not alter their treatment approach when their laboratory switched from the DCC to the IHC methodology. Variability existed among clinicians in how they equated IHC expression to femtomol values. Approximately half of the respondents did not equate negative or low IHC values with specific femtomol values.

In agreement with our laboratory survey findings, the threshold for calling a result positive varied widely among oncologists. Values associated with a positive result by IHC varied from 1 to 30%. Of equal importance, 59% of the responding hematologists/oncologists treating breast cancer patients did not require quantitative data but merely desired a statement by the laboratory as to whether the assay was positive or negative. This finding is of particular interest in light of a recent study showing that very high levels of ER are associated with an unfavorable prognosis (22). Simply dividing ER values into positive and negative may yield incomplete and misleading information. The reporting of femtomol equivalents, nuclei staining percentage, or the stratification of results into negative, borderline, intermediate, and high levels may be more clinically useful.

There does not appear to be uniformity in approach to the interpretation and utilization of ER and PgR data by oncologists. Only 13 respondents (48%) agreed with recently published recommendations concerning the use of endocrinology and chemotherapy in patients with breast cancer (19,20). The majority of oncologists responding to our questionnaire acknowledged that ER or PgR status had modified their treatment of patient's with breast cancer (92%). Despite this reliance on steroid hormone receptor assay results, fully one-third of responding oncologists would treat a postmenopausal patient whose carcinoma was ER negative with tamoxifen. The results of the Swedish BCCG study (21) appeared to change the treatment approach of only a minority of oncologists responding to our survey (26%).

The oncologists varied significantly in the threshold they used to classify specimens as positive or negative. Reported cutoff points for positivity varied from any staining to a cutoff point of at least 30% of nuclei staining. When specific percentages were given, there was a sixfold variation in the cutoff point (5–30%). Such variability in interpretative thresholds renders interlaboratory comparisons of ER and PgR results difficult if only positive and negative assessments are reported. Interlaboratory comparisons are more easily achieved if laboratories record the nuclei staining percentage, cutoff point used, and interpretation of the results rather than simply reporting the specimen as positive or negative.

The CAP QA program is commonly used by laboratories assessing ER and PgR in breast tissue, but its level of success in ensuring interlaboratory uniformity was not assessed by this survey. Further studies into the effectiveness of this program would be of value both to pathology laboratories performing steroid hormone receptor analysis as well as to oncologists interpreting the results.

Steroid hormone receptor assay by IHC appears robust enough to maintain the correlation with prognosis established by DCC, despite the many variations discussed. This interpretation is supported by the observation that the majority of studies in the literature using variable techniques, antibodies, titers, and cutoff points still report good correlation of the IHC results with DCC assays and demonstrate good predictive and prognostic value for the test (1,6–17). As in many areas of surgical pathology, reliability may exceed reproducibility (24). This may lead some clinicians and pathologists to adopt a nihilistic approach to standardization. However, it may be that important prognostic information associated with steroid hormone receptor levels is being

concealed by the imprecision of current IHC methods. The development of a universal control and improved standardization methods should improve the validity of interlaboratory comparison of the results of ER and PgR measurement by the IHC technique.

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The use of the program should be as a shared utility between a health professional and a patient. **It is not for use by patients in the absence of health professional input**.

The reasons for this are:

- First, the interpretation of some of the prognostic and staging information for any given person's case can be difficult even for an experienced professional. One reason is that surgical and pathologic reports are difficult to interpret. If information from these sources is misinterpreted very erroneous conclusions may result.
- Second, there is a concern that someone reviewing their prognosis alone (viewing them on this website) may find the information emotionally overwhelming. Although this may be unlikely since usually uninformed cancer survivors over estimate their risk of negative outcome (and Adjuvant! makes many people feel relieved and empowered), it seems reasonable to guard against the rare instances where great emotional stress happens in an unsupportive situation. By including at least both a health professional and the patient, social support and sense of moving toward a positive course of action is more assured.

If you are a patient and want to see these estimates for yourself, it is suggested that you have your health care professional register to use this website so that they may generate and go over the results with you.

The best way to review of the results is by generating the printouts, rather than capturing results directly from the computer screen.