ASCO/CAP Guideline Recommendations for IHC Testing of ER and PgR in Breast Cancer

Arch Pathol Lab Med, 134:907-22, 2010
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SCHOOL OF MEDICINE
ASCO/CAP Guidelines for IHC Testing of ER and PgR in Breast Cancer

IHC Assays for ER and PgR
Must be Comprehensively Validated

Quantitative Scoring of Results
Percent or Proportion of Positive Cells
Intensity of Positive Cells

Interpretation of Results
Calibrated to Response to Endocrine Therapy

≥ 1% Expressing Cells
"Positive"
Expect 70-80%
Responsive to Endocrine Therapy

Report Results
Scores
Interpretation

<1% Expressing Cells
"Negative"
Expect 20-30%
Not Responsive
to Endocrine Therapy

Retest and Confirm If:
External Control Negative
Internal Control Negative
Low Histological Grade**
Lobular Subtype**
Tubular Subtype**
Mucinous Subtype**
Other...

*Probably single most helpful recommendation for improving accuracy
**Not required if internal control positive
**What is Comprehensive Validation?**

**Technical:** The assay should be specific, sensitive, reproducible, calibrated to clinical outcome, interpreted, and reported in a relatively uniform manner. There should be comprehensive ongoing quality assurance.

**Clinical:** The factor should identify groups of patients with significantly different risks of relapse, survival, or treatment response – demonstrated in multiple large studies (ideally randomized clinical trials).

**Useful:** Actually used by physicians to make important treatment decisions.

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Comprehensively Validated IHC Assays for Measuring ERα and PgR in Breast Cancer
(identified in ASCO/CAP Guidelines)

<table>
<thead>
<tr>
<th>Estrogen Receptor</th>
<th>Reference</th>
<th>Antibody</th>
<th>Cutpoint for &quot;Positive&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvey. J Clin Oncol 17:1474, 1999</td>
<td>6F11</td>
<td>Allred Score ≥3 (1-10% weakly positive cells)</td>
<td></td>
</tr>
<tr>
<td>Cheang. J Clin Oncol 24:5637, 2006</td>
<td>SP1</td>
<td>≥1%</td>
<td></td>
</tr>
<tr>
<td>Phillips. Appl IHC Molec Morphol15:325, 2007</td>
<td>ER.2.123 + 1D5</td>
<td>Allred Score ≥3 (1-10% weakly positive cells)</td>
<td></td>
</tr>
<tr>
<td>Dowsett. J Clin Oncol 26:1059, 2008</td>
<td>6F11</td>
<td>H-score &gt;1 (≥1%)</td>
<td></td>
</tr>
</tbody>
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<tbody>
<tr>
<td>Mohsin. Modern Pathol 17:1545, 2004</td>
<td>1294</td>
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<tr>
<td>Dowsett. J Clin Oncol 26:1059, 2008</td>
<td>312</td>
<td>≥10%</td>
<td></td>
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</table>
A Few Words about Validation in Your Laboratory

It is NOT mandatory to use the same validated antibodies and assays identified in the Guidelines.

It IS mandatory to get the same results.

It IS mandatory to provide ongoing proof of equivalent results.

Using the same validated antibodies and assays is a very reasonable thing to do.

FDA approval does not necessarily mean that there has been comprehensive validation of the assay.
A Few Words About Scoring Results

Examples of Satisfactory Methods:

*H-Score* (McCarty, Arch Pathol Lab Med 109:716, 1985)
*Allred-Score* (Allred, Mod Pathol 11:155, 1998)
*Quick-Score* (Rhodes, J Clin Pathol 53:125, 2000)
Percent and intensity positive – by computer (many methods)
Percent and intensity positive – by human (absolute, point-counting)

Clinical Relevance:

Tam = Tam+Chemo in postmenopausal patients with HIGH ER
e.g. Albain. Lancet Oncol. 11:55, 2010
Neoadjuvant endocrine therapy = restricted to patients with HIGH ER
e.g. Ellis. JNCI 100:1380, 2008

⇒ *Must avoid using IHC assays that are too sensitive (saturated)*
A Few Words about Controls

Multiple purposes
- confirm satisfactory performance of assay
- confirm satisfactory condition of sample

Perfect control does not currently exist
Certain normal tissues are satisfactory practical external controls
(e.g. endometrium; normal breast = external and internal control)
  - readily available
  - relatively stable reactivity
  - broad range of expression
  - ~90% normal breast tissue with positive epithelial cells...but not 100%
  - do NOT use breast cancers (abnormal variable expression)

Routinely score and record results to illuminate problems
  - utilizing same control daily can be very helpful

Every batch of cases must have positive and negative controls
Every pathologist must review the controls before scoring case
Essential Elements of Quality Assurance and Proficiency Testing

**Confirm accuracy of results**
- reasonable distribution (70-85% ER-positive; 60-75% PgR+)
- stable with time (e.g. day of week; quarter to quarter)
- >90% concordance vs. independent expert

**Demonstrate reproducibility of results**
- >90% vs. in-house or outside expert

**Demonstrate concordance of results**
- >90% between pathologists
- >90% by same pathologist

**Comprehensive training of pathologists**
- educational conferences (e.g. review important new publications)
- training conferences (e.g. calibrate scoring between pathologists)

**Comprehensive training of other laboratory personnel**
- educational and training conferences
- monitor reagents (e.g. lot numbers; expiration dates)
- monitor and regulate fixation time

**Designate an in-house expert Medical Director**
- with true expertise (requires substantial training and commitment)
- monitor quality of slides daily before disseminating to other pathologists
- go-to person for general oversight; problem solving; feedback, etc.

**Advertise quality of performance**
- reassurance to others (e.g. annual report at tumor board)

**Comprehensive record keeping**
- all above and more
Example of Comprehensive Report

LEFT BREAST MASS, CORE BIOPSY:
Invasive ductal carcinoma

<table>
<thead>
<tr>
<th>Factor</th>
<th>Proportion Score (PS)</th>
<th>Intensity Score (IS)</th>
<th>Total Score (TS)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen Receptor</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>Positive</td>
</tr>
<tr>
<td>Progesterone Receptor</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Comments:
Estrogen receptor (antibody 6F11) and progesterone receptor (antibody 1294) were evaluated by immunohistochemistry (IHC) on routine formalin-fixed paraffin-embedded tissue utilizing comprehensively validated assays (J Clin Oncol 17;1474, 1999; Mod Pathol 17:1545, 2004) in compliance with ASCO/CAP guidelines (Arch Pathol Lab Med 134:907, 2010; J Clin Oncol 28:2784, 2010).

The results were scored and interpreted using the Allred Score (J Clin Oncol 17;1474, 1999). PS (proportion of positive tumor cells): 0=none; 1<1/100th; 2=1/100th-1/10th; 3>1/10th-1/3rd; 4>1/3rd-2/3rds; 5>2/3rds. IS (average intensity of positive tumor cells): 0=none; 1=weak; 2=intermediate; 3=strong. TS = PS+IS (range 0-8); **positive >2**.

Formalin fixation time: 20 hours.
**Essential Reading**


Current issues in ER and HER2 testing by IHC in breast cancer. Mod Pathol. 2008 May;21 Suppl 2:S8-S15


Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol. 1999 May;17(5):1474-81

Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays. JNCI. 2006 Nov 1;98(21):1571-81.


