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

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

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

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

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

Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team.

Accepted for publication October 4, 2013.
Published as an Early Online Release November 15, 2013.

From the Department of Pathology, St Jude Medical Center, Fullerton, California (Dr Fitzgibbons); the Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts (Dr Dillon); the Department of Pathology, Kaiser Permanente-Los Angeles Medical Center, Los Angeles, California (Dr Alsabeh); the Department of Pathology, Jefferson Hospital, Jefferson Hills, Pennsylvania (Dr Berman); the Breast Oncology Program, University of Michigan Comprehensive Cancer Center, Ann Arbor (Dr Hayes); the Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, New York (Dr Hicks); the Division of Surgical Oncology, Massachusetts General Hospital, Boston (Dr Hughes); and the Department of Anatomic Pathology, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada (Dr Nofech-Mozes).

The authors have no relevant financial interest in the products or companies described in this article.
doi: 10.5858/arpa.2013-0566-CP

Reprints: Patrick L. Fitzgibbons, MD, Department of Pathology, St Jude Medical Center, 101 E Valencia Mesa Dr, Fullerton, CA 92835, (e-mail: Patrick.Fitzgibbons@stjoe.org).

BIOMARKER REPORTING TEMPLATE

Breast

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

+ Data elements preceded by this symbol are not required.

RESULTS

Estrogen Receptor (ER)

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

Progesterone Receptor (PgR)

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

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

***HER2* (by Immunohistochemistry)**
___ Negative (score 0)
___ Negative (score 1+)
___ Equivocal (score 2+)
___ Positive (score 3+)
___ Indeterminate (explain): _____
___ Not performed

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

***HER2* (by In Situ Hybridization)**
___ Negative (not amplified)
___ Equivocal
___ Positive (amplified)
___ Indeterminate (explain): _____
___ Not performed
___ Pending
Number of observers: _____
Number of invasive tumor cells counted: _____
___ Using dual probe assay
Average number of *HER2* signals per cell: _____
Average number of *CEP17* signals per cell: _____
HER2:CEP17 ratio: _____
___ Using single probe assay
Average number of *HER2* signals per cell: _____
+ Aneusomy (as defined by vendor kit used):
+ ___ Not present
+ ___ Present
+ Heterogeneous signals:
+ ___ Absent
+ ___ Present
+ Percentage of cells with amplified *HER2* signals: ____ %

+ **Ki-67**
+ Percentage of positive nuclei: ____ %

+ **Multiparameter Gene Expression/Protein Expression Assay**
+ Name of assay: _____
+ ___ Low risk
+ ___ Moderate risk
+ ___ High risk
+ Recurrence score: _____

Cold Ischemia and Fixation Times Meet the Requirements Specified in the Latest Version of the ASCO/CAP Guidelines
___ Yes
___ No

METHODS

Fixative
___ Formalin
___ Other (specify): _____

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

Primary Antibody
___ SP1
___ 6F11
___ 1D5
___ Other (specify): _____

Progesterone Receptor
___ FDA cleared (specify test/vendor): _____
___ Laboratory-developed test

Primary Antibody
___ 1E2
___ 636
___ 16
___ SP2
___ 1A6
___ Other (specify): _____

+ **ER and PgR Scoring System**
+ ___ Allred
+ ___ Other (specify): _____

***HER2* (by Immunohistochemistry)**
___ FDA approved (specify test/vendor): _____
___ Laboratory-developed test

Primary Antibody
___ 4B5
___ HercepTest™
___ A0485
___ SP3
___ CB11
___ Other (specify): _____

***HER2* (by In Situ Hybridization)**
___ FDA approved (specify test/vendor): _____
___ Laboratory-developed test

+ **Ki-67**
+ Primary Antibody
+ ___ MIB1
+ ___ SP6
+ ___ Other (specify): _____

+ **Image Analysis**
+ ___ Not performed
+ ___ Performed (specify method): _____

+ **Biomarkers Scored by Image Analysis (select all that apply)**
+ ___ ER
+ ___ PgR
+ ___ HER2
+ ___ Ki-67
+ ___ Other (specify): _____

+ **COMMENT(S)**
_____
_____
_____

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

EXPLANATORY NOTES

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

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

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

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

Estrogen Receptor and Progesterone Receptor Testing

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

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

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

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

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

- Exposure of tumor cells to heat (eg, carcinomas transected by using cautery during surgery)
- Prolonged cold ischemic time, which may result in antigenic degradation; 1 hour or less is preferable[13,14]
- Underfixation or overfixation; fixation for at least 6 hours in buffered formalin is recommended,[5] and prolonged fixation can also diminish immunoreactivity[11,15]
- Type of fixative: ER is degraded in acidic fixatives, such as Bouin picroformol-acetic fixative and B-5 sodium acetate-sublimate; formalin should be buffered to ensure pH range between 7.0 and 7.4
- Decalcification, which may result in loss of immunoreactivity[8]
- Nonoptimized antigen retrieval
- Type of antibody
- Dark hematoxylin counterstain obscuring faintly positive diaminobenzidine (DAB) staining

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

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

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

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

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

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

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

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

Two methods of quantifying ER by using both the intensity and the percentage of positive cells are the Allred score[17] (Table 2) and the H score[18] (Table 3). The 2 systems classify carcinomas into similar, but not identical, groups.[19] If high-affinity antibodies are used with sensitive detection systems, most carcinomas will fall into clearly positive (score 7 or 8) or clearly negative (score 0) categories by the Allred score.[20,21] A small group of carcinomas (<1% of total) show intermediate levels of immunoreactivity.

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

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

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

HER2 Testing

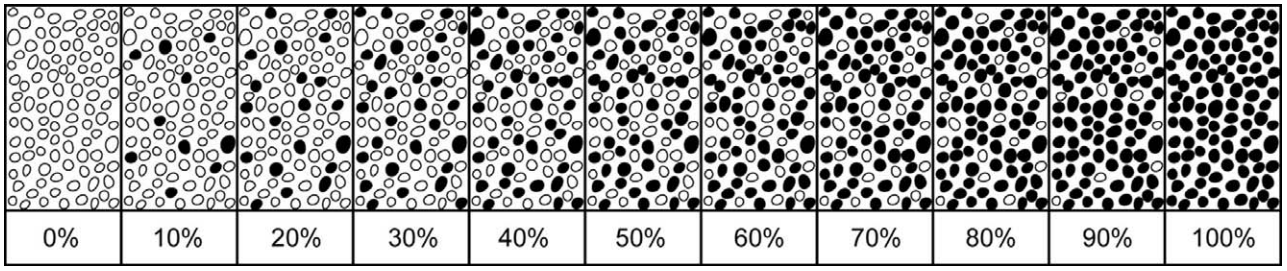
Scientific Rationale—A subset of breast carcinomas (approximately 15%–20%) overexpress human epidermal growth factor receptor 2 (HER2; HUGO Nomenclature, ERBB2). Protein overexpression is usually due to gene amplification. Assays for gene copy number, messenger RNA (mRNA) quantity, and protein generally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

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

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

HER2 Testing by Immunohistochemistry

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



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

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

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

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

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

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

Table 3. H Score[a] for Estrogen and Progesterone Receptor Evaluation

Cell Signal	Calculation of H Score: Percentage of Cells	Calculation of H Score: Value Multiplied
Cells with no signal		% × 0 = 0
Cells with weak signal		% × 1 =
Cells with moderate signal		% × 2 =
Cells with strong signal		% × 3 =
Total score =		

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

- *Improper antibody titration (concentration too low)*.

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

- *Tissue controls*.—External controls must stain as expected. There are no normal internal controls for HER2 protein assessment by IHC.
- *Correlation With Histologic and Other Biomarker Results*.—If the HER2 test is negative by IHC but the tumor has characteristics associated with HER2 positivity (see above), repeating the test by ISH should be considered.

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

HER2 Testing by In Situ Hybridization

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

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

- Prolonged fixation in formalin (>1 week)[23]
- Fixation in nonformalin fixatives[24]
- Procedures or fixation involving acid (eg, decalcification), which may degrade DNA[25]
- Insufficient protease treatment of tissue

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

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

Important issues in interpreting ISH are the following:

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

Table 4. Reporting Results of HER2 Testing by Immunohistochemistry

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

[a] Readily appreciated using low-power objective and observed within a homogeneous and contiguous population of invasive tumor cells.
[b] Must order reflex in situ hybridization test (same specimen) or new test (new specimen, if available, using immunohistochemistry or in situ hybridization).

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

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

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

Result	Average No. *HER2* Signals/Cell
Negative (not amplified)	<4.0
Equivocal[a]	≥4.0 and <6.0[b]
Positive (amplified)	≥ 6.0[b]

[a] Must order a reflex test (same specimen using dual-probe in situ hybridization or immunohistochemistry) or a new specimen, if available, using in situ hybridization or immunohistochemistry.
[b] Observed in a homogeneous and contiguous population of ≥10% of invasive tumor cells.

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

Result	Criteria
Negative (not amplified)	*HER2/CEP17* ratio <2.0 AND average HER2 copy number <4.0 signals/cell
Equivocal[b]	*HER2/CEP17* ratio <2.0 AND average HER2 copy number ≥4.0 but <6.0 signals/cell[a]
Positive (amplified)	*HER2/CEP17* ratio ≥2.0[a] (regardless of average HER2 copy number) or Average HER2 copy number ≥6.0 signals/cell[a] (regardless of ratio)

[a] Observed in a homogeneous and contiguous population of ≥10% of invasive tumor cells.
[b] Must order a reflex test (same specimen using immunohistochemistry), test with alternative in situ hybridization chromosome 17 probe, or order a new test (new specimen, if available, ISH or IHC).
Abbreviations: IHC, immunohistochemistry; ISH, in situ hybridization.

Ki-67 Testing

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

Multigene Expression Assays

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

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

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

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

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

We thank Susan C. Lester, MD, PhD, (previous lead contributor to the CAP breast cancer protocols), Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts; and Margaret B. Adamo, BS, RHIT, AAS, CTR Surveillance Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

References

1. National Comprehensive Cancer Network. Clinical practice guideline in oncology. Version 3.2013. http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf. Accessed July 23, 2013.

2. Harris L, Fritsche H, Mennel R, et al; American Society of Clinical Oncology. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol*. 2007;25(33):5287–5312.

3. Pusztai L, Viale G, Kelly CM, Hudis CA. Estrogen and HER-2 receptor discordance between primary breast cancer and metastasis. *Oncologist*. 2010;15(11):1164–1168.

4. Arslan C, Sari E, Aksoy S, Altundag K. Variation in hormone receptor and HER-2 status between primary and metastatic breast cancer: review of the literature. *Expert Opin Ther Targets*. 2011;15(1):21–30.

5. 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 [published correction appears in *Arch Pathol Lab Med*. 2010;134(8):1101].*Arch Pathol Lab Med*. 2010;134(6):907–922.

6. Wolff AC, Hammond ME, Schwartz JN, et al; American Society of Clinical Oncology/College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med*. 2007;131(1):18–43.

7. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) Clinical Practice Guideline Update [published online ahead of print October 7, 2013]. *Arch Pathol Lab Med*. doi:10.5858/arpa.2013-0953-SA.

8. Arber JM, Arber DA, Jenkins KA, Battifora H. Effect of decalcification and fixation in paraffin-section immunohistochemistry. *Appl Immunohistochem*. 1996;4(4):241–248.

9. Yaziji H, Taylor CR, Goldstein NS, et al; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. *Appl Immunohistochem Mol Morphol*. 2008;16(6):513–520.

10. Allred DC. Problems and solutions in the evaluation of hormone receptors in breast cancer [comment]. *J Clin Oncol*. 2008;26(15):2433–2435.

11. Arber DA. Effect of prolonged formalin fixation on the immunohistochemical reactivity of breast markers. *Appl Immunohistochem Mol Morphol*. 2002;10(2):183–186.

12. Fitzgibbons PL, Murphy DA, Hammond EH, Allred DC, Valenstein PN. Recommendations for validating estrogen and progesterone receptor Immunohistochemistry assays. *Arch Pathol Lab Med*. 2010;134(6):930–935.

13. Yildiz-Aktas IZ, Dabbs DJ, Bhargava R. The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. *Mod Pathol*. 2012;25(8):1098–1105.

14. Neumeister VM, Anagnostou V, Siddiqui S, et al. Quantitative assessment of effect of preanalytic cold ischemic time on protein expression in breast cancer tissues. *J Natl Cancer Inst*. 2012;104(23):1815–1824.

15. Oyama T, Ishikawa Y, Hayashi M, Arihiro K, Horiguchi J. The effects of fixation, processing and evaluation criteria on immunohistochemical detection of hormone receptors in breast cancer. *Breast Cancer*. 2007;14(2):182–188.

16. Viale G, Regan MM, Maiorano E, et al. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1–98. *J Clin Oncol*. 2007:25(25):3846–3852.

17. Harvey JM, Clark GM, Osborne CK, Allred DC. 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;17(5):1474–1481.

18. McCarty KS Jr, Miller LS, Cox EB, Konrath J, McCarty KS Sr. Estrogen receptor analyses: correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med*. 1985;109(8):716–721.

19. Shousha S. Oestrogen receptor status of breast carcinoma: Allred/H score conversion table. *Histopathology*. 2008;53(3):346–347.

20. Collins LC, Botero ML, Schnitt SJ. Bimodal frequency distribution of estrogen receptor immunohistochemical staining results in breast cancer: an analysis of 825 cases. *Am J Clin Pathol*. 2005:123(1):16–20.

21. Nadji M, Gomez-Fernandez C, Ganjei-Azar P, Morales AR. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *Am J Clin Pathol*. 2005:123(1);21–27.

22. Turbin DA, Leung S, Cheang MC, et al. Automated quantitative analysis of estrogen receptor expression in breast carcinoma does not differ from expert pathologist scoring: a tissue microarray study of 3,484 cases. *Breast Cancer Res Treat*. 2008;110(3):417–426.

23. Selvarajan S, Bay B-H, Choo A, et al. Effect of fixation period on HER2/neu gene amplification detected by fluorescence in situ hybridization in invasive breast carcinoma [published correction appears in *J Histochem Cytochem*. 2003;51(2):267].*J Histochem Cytochem*. 2002;50(12):1693–1696.

24. Willmore-Payne C, Metzger K, Layfield LJ. Effects of fixative and fixation protocols on assessment of Her-2/neu oncogene amplification status by fluorescence in situ hybridization. *Appl Immunohistochem Mol Morphol*. 2007;15(1):84–87.

25. Brown RS, Edwards J, Bartlett JW, Jones C, Dogan A. Routine acid decalcification of bone marrow samples can preserve DNA for FISH and CGH studies in metastatic prostate cancer. *J Histochem Cytochem*. 2002;50(1):113–115.

26. Gunn S, Yeh IT, Lytvak I, et al. Clinical array-based karyotyping of breast cancer with equivocal HER2 status resolves gene copy number and reveals chromosome 17 complexity. *BMC Cancer*. 2010;10:396. doi:10.1186/1471-2407-10-396.

27. Dowsett M, Nielsen TO, A'Hern R, et al; International Ki-67 in Breast Cancer Working Group. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in breast cancer working group. *J Natl Cancer Inst*. 2011;103(22):1656–1664.

28. Ross JS, Hatzis C, Symmans WF, Pusztai L, Hortobagyi GN. Commercialized multigene predictors of clinical outcome for breast cancer. *Oncologist*. 2008;13(5):477–493.