HER2 Testing in Breast Cancer:

Putting Guidance Into Practice

Based on the 2013 ASCO/CAP Guideline for HER2 Testing in Breast Cancer

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HER2 testing in breast cancer

• About 15% to 20% of breast tumors are HER2-positive.\textsuperscript{1}

• HER2-positive breast cancer is aggressive and likely to metastasize.\textsuperscript{16,17}

• HER2 testing is the only means for determining eligibility for HER2-directed therapy.\textsuperscript{1}

• A coordinated, multidisciplinary approach to HER2 testing is essential for
  – Obtaining dependable HER2 test results
  – Establishing an accurate disease diagnosis
  – Personalizing patient care
Roadmap for HER2 testing in breast cancer

Abbreviation: ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; HER2, human epidermal growth factor receptor 2.
Quality patient care should involve the entire multidisciplinary team

- Oncologist
- Surgeon
- Interventional radiologist
- Pathologist
- OR staff
- Pathology and diagnostics lab technician

Abbreviation: OR, operating room.
Communication is important for patient care

**The Multidisciplinary Testing Team**

**PREANALYTIC**
- Tumor Sampling
- Sample Incision
- Fixation
- Sample Processing
- Transport to Diagnostics Lab

**ANALYTIC**
- Testing
- Image Analysis

**POSTANALYTIC**
- Scoring and Interpretation
- Reporting

**APPLY ASCO/CAP**
- Histopathologic Discordance
- Reflex Testing
- References

- Patient
- Oncologist
- Surgeon, Interventional Radiologist
- Pathologist
- Diagnostics Lab Director, Laboratory Technicians

**Development and implementation of the treatment plan**

- Tissue acquisition
- Requests HER2 test
- Sample processing
- Test interpretation
- Results reporting
• HER2 testing begins when the tissue sample is removed from the patient.

• Ensure that members of the multidisciplinary testing team understand the overall roadmap for HER2 testing, as well as their specific roles in it.

• Promote interdisciplinary communication: it is important for quality HER2 testing and accurate patient diagnosis.
  – The pathologist plays a pivotal role in the collaborative approach to personalized patient care.

• Encourage complete reporting according to guideline recommendations, as it supports collaboration between pathologists and oncologists when testing uncertainties or discrepancies occur.
The preanalytic phase

- Begins when the tissue sample is removed from the patient
- Ends when the sample undergoes IHC or ISH analysis
- Involves input from different practitioners across multiple disciplines
- Defines tumor sample quality

Abbreviations: IHC, immunohistochemistry; ISH, in situ hybridization.
Tumor Sampling

The 2013 revision to the ASCO/CAP guideline reiterated the importance of sample quality in HER2 testing.

- Every case of primary, recurrent, or metastatic breast cancer should be evaluated for HER2, ER, and PR.\textsuperscript{1,2}

- Needle or excisional biopsy specimens are adequate for HER2 testing.\textsuperscript{1}

- Proper tissue sample handling is important for obtaining reliable test results.\textsuperscript{1,3}

- Some tissue-handling practices can introduce artifacts that may cause challenges for HER2 test result interpretation.\textsuperscript{1,3}

According to ASCO/CAP, a HER2 test result must be reported as indeterminate if technical issues, such as inadequate specimen handling, prevent one or both tests (IHC or ISH) from being reported as positive, negative, or equivocal.\textsuperscript{1}

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.
HER2 status may change between first diagnosis and recurrence

• According to ASCO/CAP,¹
  – HER2 testing should be conducted in cases of metastatic breast cancer (on a metastatic site if stage IV and if a specimen is available).
  – HER2 testing is especially recommended for a patient who previously tested HER2-negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.

  ![HER2 status diagram]

• According to the NCCN, patients should be rebiopsied upon first recurrence of disease to help ensure accurate assessment of tumor histology and appropriate treatment; HER2 testing should be considered when original results were HER2-negative or unknown.⁵

Abbreviation: NCCN, National Comprehensive Cancer Network.
HER2 heterogeneity can cause challenges in HER2 testing

- Recent literature suggests that HER2 heterogeneity may identify a subset of patients who could benefit from HER2-targeted therapy.\(^6\)
- According to ASCO/CAP,\(^6\)
  - All samples should be surveyed for HER2 heterogeneity.
  - When HER2 heterogeneity is a factor, an excisional biopsy specimen may constitute a more representative tumor sample.
ASCO/CAP recommends that after gross inspection and margin designation, breast tumor specimens should be sliced before being placed in fixative.\textsuperscript{1,3}

**Sample incision/sectioning is critical to downstream processes**

Abbreviation: NBF, neutral buffered formalin.
According to ASCO/CAP, uncut samples should not be fixed. It may take longer for fixative to penetrate larger samples, such as excisional samples, than smaller samples, such as core-needle biopsy samples. Inadequate fixation may compromise the HER2 test result.
ASCO/CAP guidance regarding fixative type remains consistent with previous recommendations.\textsuperscript{1-3}

- Breast tumor samples intended for ER, PR, and HER2 testing be fixed in a sufficient amount of 10\% NBF.
- 10\% NBF is compatible with both HER2 IHC and HER2 ISH testing.
- If an alternative fixative is used, this must be documented in the report.
In the HER2 testing guideline, ASCO/CAP recognizes that insufficient fixation can compromise the HER2 test result.¹

- To ensure complete fixation, breast tumor samples should remain in 10% NBF for a minimum of 6 hours regardless of sample size.¹,²,⁸
- Fixation time should not exceed 72 hours.¹,²

If the initial HER2 test result for a core-needle biopsy sample is negative and the fixation time was inadequate, a new test must be ordered on an excisional biopsy specimen.¹
Good preanalytic practices foster specimen quality

Samples should be fixed for 6 to 72 hours.¹

Limit cold ischemia time to 1 hour.¹

Samples should be fixed in 10% NBF.¹
• Evaluate all cases of invasive and metastatic breast cancer for HER2 status.

• Collect either needle biopsy or excisional specimens for HER2 testing.
  – Repeat testing of an excisional sample may be required in cases of questionable specimen quality or unreliable results from a needle-biopsy specimen.

• Follow recommended tumor sample–handling practices to preserve specimen quality.
  – Limit cold ischemic time to 1 hour.
  – Fix breast tissue samples in a sufficient volume of 10% NBF for at least 6 but no more than 72 hours.

• Send samples intended for HER2 analysis only to labs that meet validation, proficiency, and accreditation requirements.
The analytic phase

- Begins with initiation of IHC or ISH assay
- Ends when assay is complete and result has been analyzed
- Analytic expertise provided by highly trained technologists and pathologists
- Defines integrity of assay result
HER2 IHC and ISH provide complementary information about the biology of the breast tumor sample.\textsuperscript{11,12}

- ASCO/CAP preferentially recommends the use of FDA-approved IHC and ISH assays to evaluate HER2 status.\textsuperscript{1}
  - Standardized control materials must be used with every test run.\textsuperscript{1}
  - Standardized control materials must include cell lines or tumor blocks with well-defined results.\textsuperscript{1,3}
HER2 IHC evaluates the amount of HER2 protein present

• Labeled antibodies allow visualization and evaluation of the amount of HER2 protein present in a sample.\textsuperscript{13}

• Anti-HER2 antibodies are available independently of FDA-approved kits.
  – Kits have been optimized for use with the specific antibody included with each kit.

Antibodies vary in sensitivity and specificity for HER2 and in their sensitivity to antigen retrieval.\textsuperscript{3}

\*Images used with permission.\textsuperscript{4}
HER2 ISH evaluates the number of HER2 genes present

- Labeled probes bind to the HER2 gene, allowing detection and quantification of the number of HER2 genes present in a cell.¹⁴
- In dual-probe assays, the CEP17 probe is used to control for chromosome number and alterations to pericentromeric DNA.¹⁵

Abbreviation: CEP17, probe for enumerating the number of chromosomes 17.

* Images used with permission.
Considerations for IHC analysis

• Score infiltrating ductal carcinoma only.\textsuperscript{6}

• For a positive result, more than 10\% of the tumor must show circumferential membrane staining.\textsuperscript{6}
  – Membrane staining must be intense and resemble chicken wire.\textsuperscript{6}

• Incomplete or pale membrane staining should be ignored.\textsuperscript{6}

When a HER2 test is rejected, testing should be repeated using the same or an alternate FDA-approved assay.\textsuperscript{1}
Practice points: the analytic phase

- Perform HER2 analysis using FDA-approved assays.*
- Ensure that standardized control materials are used with every test run.
- Repeat HER2 testing if a test is rejected for inadequate specimen handling, the presence of obscuring artifact, or analytic failure.
- Assay for HER2 protein overexpression by IHC.
  - Positively stained IHC slides should show complete and intense membrane staining in more than 10% of the invasive component.
- Assay for HER gene amplification by ISH.
  - Count at least 20 cells in 2 sections of the invasive component and have a pathologist confirm the results.
- Automation promotes consistency in assay performance and in result interpretation.

* If an accredited laboratory opts to use a laboratory-developed test (LDT), analytic performance of the LDT must be prospectively validated in the same clinical laboratory that will perform it, and the test must have documented analytic validity.¹
The postanalytic phase

- Begins with assay result interpretation
- Ends when test result has been reported
- Requires pathologists to apply ASCO/CAP 2013 scoring and interpretation criteria
- Defines disease diagnosis
ASCO/CAP IHC interpretation criteria have been updated

• The 2013 update to the ASCO/CAP guideline contains revisions to the recommended IHC scoring and interpretation criteria.\textsuperscript{1,3}
  – IHC 3+ is defined as HER2-positive staining in greater than 10\% of the invasive component.\textsuperscript{*}
• The HER2-equivocal (IHC 2+) category has been expanded to include samples that stain HER2-positive in 10\% or less of the invasive component.\textsuperscript{1,3}
• Definitions for HER2-negative categories (IHC 1+ and IHC 0) have been updated.\textsuperscript{1,3}

According to ASCO/CAP, when > 10\% of the invasive component of a sample exhibits circumferential membrane staining that is intense and complete, the sample should be scored as HER2-positive.\textsuperscript{1}

\*,\textsuperscript{1,3} Observed in a homogeneous and contiguous population.
2013 ASCO/CAP HER2 IHC interpretation criteria

HER2 testing (invasive component) by validated IHC assay

Batch controls and on-slide controls show appropriate staining

Circumferential membrane staining that is complete, intense, and within > 10% of tumor cells:
- IHC 3+ positive

Circumferential membrane staining that is incomplete and/or weak/moderate and within > 10% of tumor cells:
- IHC 2+ equivocal

Incomplete membrane staining that is faint/barely perceptible and within > 10% of tumor cells:
- IHC 1+ negative

No staining observed or Membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of tumor cells:
- IHC 0 negative

Must order a reflex test (same specimen using ISH), or order a new test (new specimen if available, using IHC or ISH)

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*Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.
Equivocal HER2 IHC results require a reflex or new test

### Equivocal result for HER2 IHC is 2+

- **2007 Guideline**
  - Complete membrane staining that is nonuniform or weak in intensity with obvious circumferential distribution in at least 10% of cells

- **2013 Guideline**
  - Circumferential membrane staining that is incomplete and/or weak/moderate and within > 10% of invasive tumor cells
  - Complete and circumferential intense membrane staining within ≤ 10% of invasive tumor cells

IHC 2+ test results must be reported as *equivocal, and*

- Reflex testing *must* be ordered (same specimen, ISH assay)
  - or
- A new test *must* be ordered (new specimen, if available, either assay).
**2013 ASCO/CAP HER2 ISH interpretation criteria**

**HER2 testing (invasive component) by validated ISH assay**

Batch controls and on-slide controls show appropriate hybridization

**HER2/CEP17 ratio ≥ 2.0**

Average HER2 copy number ≥ 4.0 signals/cell

**ISH positive**

Average HER2 copy number < 4.0 signals/cell

**ISH positive**

Average HER2 copy number ≥ 6.0 signals/cell

**ISH positive**

Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell

**ISH equivocal**

Average HER2 copy number < 4.0 signals/cell

**ISH negative**

**HER2/CEP17 ratio < 2.0 or Single-probe assay**

Must order a reflex test (same specimen using IHC), test with alternative ISH chromosome 17 probe, or order a new test (new specimen if available, ISH or IHC)

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*a This hybrid ISH algorithm combines the information from the single-probe (Figure 2) and dual-probe (Figure 3) ISH assay algorithms in Wolff AC, Hammond MEH, 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 clinical practice guideline update [published online October 7, 2013]. Arch Pathol Lab Med. doi:10.5858/arpa.2013-0953-SA with permission from Archives of Pathology & Laboratory Medicine. Copyright 2013 College of American Pathologists.*

*b Observed in a homogeneous and contiguous population.

*c Rare scenario.
Equivocal HER2 ISH results require a reflex or new test

**2007 Guideline**

Equivocal result for HER2 ISH

- FISH HER2/CEP17 = 1.8 to 2.2
  
  or

- Average HER2 copy number = 4 to 6 HER2 signals/nucleus for test systems without an internal control

**2013 Guideline**

Equivocal result for HER2 ISH

- Single probe: Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell*

  or

- Dual probe: HER2/CEP17 < 2.0 and average HER2 copy number ≥ 4.0 and < 6.0 signals/cell*

ISH-equivocal test results must be reported as equivocal, and:

- Reflex testing *must* be ordered (same specimen, IHC assay)

  or

- A new test *must* be ordered (new specimen, if available, either assay).

* Observed in a homogeneous and contiguous population.
Repeat testing for questionable HER2 test results

Indications for Repeat HER2 Testing

1. Indeterminate result: technical issues such as inadequate specimen handling, the presence of crush or edge artifact, or assay failure prevent test results from being reported as positive, negative, or equivocal (repeat test on an alternate sample).

2. Apparent discordance between the HER2 test results and certain histopathologic features.

3. A negative HER2 test result and limited invasive component in the core-biopsy sample (repeat test on excisional sample).

4. HER2 test result for core-biopsy sample remains equivocal after both IHC and ISH testing (repeat test on excisional sample).

If the pathologist or oncologist observes apparent histopathologic discordance after HER2 testing, the need for additional HER2 testing should be discussed.\(^1\)
## When to repeat test for histopathologic discordance

### Initial Result

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Repeat Test?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Should not</td>
</tr>
<tr>
<td>Positive</td>
<td>Should</td>
</tr>
</tbody>
</table>

#### Negative

Histologic grade 1 carcinoma of the following types:
- Infiltrating ductal or lobular carcinoma, ER- and PR-positive
- Tubular (at least 90% pure)
- Mucinous (at least 90% pure)
- Cribriform (at least 90% pure)
- Adenoid cystic carcinoma (90% pure) and often triple-negative

#### Positive

Histologic grade 1 carcinoma of the following types:
- Infiltrating ductal or lobular carcinoma, ER- and PR-positive
- Tubular, mucinous, or cribriform (at least 90% pure in each case)
- Adenoid cystic carcinoma (≥ 90% pure) and often triple-negative

#### Negative on a core-needle biopsy sample

If any of the following are observed, repeat testing on an excisional specimen must be ordered:
- Grade 3 tumor
- Limited invasive tumor in core biopsy sample
- Excisional sample contains high-grade tumor that is morphologically distinct from that in the core biopsy sample
- HER2 test result of core biopsy sample remains equivocal after reflex testing
- Questions regarding core biopsy specimen handling or testing process

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Reporting elements for IHC

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Reporting elements for ISH⁶

Information in this sample report is fictional and intended for illustrative purposes only. Image used with permission.⁴
• Interpret HER2 test results using the 2013 ASCO/CAP guideline criteria:
  – IHC 3+: Circumferential membrane staining that is complete, intense, and in > 10% of invasive tumor cells
  – ISH+: \( \frac{HER2}{CEP17} \) ratio \( \geq 2.0 \) or \( HER2 \) copy number \( \geq 6.0 \)
• Perform reflex testing for all equivocal HER2 test results.
• Repeat HER2 testing in cases of discordance between the HER2 test result and certain histopathologic features.
• Repeat HER2 testing for indeterminate or persistently equivocal results (core-needle sample).
Discordance between the HER2 result and histopathologic features*

This case exhibits discordance.¹

- The core-needle biopsy sample is high-grade, but the HER2 test result is negative.
  - IHC 1+: incomplete membrane staining that is faint/barely perceptible and within > 10% of tumor cells†

According to ASCO/CAP, repeat HER2 testing of an excisional sample must be ordered.¹

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† Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.
Repeat testing is indicated due to discordance

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• Cribriform (at least 90% pure)  
• Adenoid cystic carcinoma (90% pure) and often triple-negative | Should not |
| **Positive**    | Histologic grade 1 carcinoma of the following types:  
• Infiltrating ductal or lobular carcinoma, ER- and PR-positive  
• Tubular, mucinous, or cribriform (at least 90% pure in each case)  
• Adenoid cystic carcinoma (≥ 90% pure) and often triple-negative | Should |
| **Negative on a core-needle biopsy sample** | If any of the following are observed, repeat testing on an excisional specimen must be ordered:  
• Grade 3 tumor  
• Limited invasive tumor in core-biopsy sample  
• Excisional sample contains high-grade tumor that is morphologically distinct from that in the core-biopsy sample  
• HER2 test result of core-biopsy sample remains equivocal after reflex testing  
• Questions regarding core-biopsy specimen handling or testing process | Must |

According to ASCO/CAP, if the initial result for a core-biopsy specimen is negative and there is apparent histopathologic discordance, a section of the tumor from the excisional specimen should be tested.¹

References


