HER2 Testing in Breast Cancer: Putting Guidance Into Practice
Based on the 2013 ASCO/CAP Guideline for HER2 Testing in Breast Cancer

Roadmap for HER2 testing in breast cancer

Quality patient care should involve the entire multidisciplinary team

Abbreviation: ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; HER2, human epidermal growth factor receptor 2.

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

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1. Ensure that members of the multidisciplinary testing team understand the overall roadmap for HER2 testing, as well as their specific roles in it.
2. Promote interdisciplinary communication: it is important for quality HER2 testing and accurate patient diagnosis.
3. The pathologist plays a pivotal role in the collaborative approach to personalized patient care.
4. Encourage complete reporting according to guideline recommendations, as it supports collaboration between pathologists and oncologists when testing uncertainties or discrepancies occur.

Practice points: the multidisciplinary testing team

- HER2 testing begins when the tissue sample is removed from the patient.
- Ensures 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.
- Ensures 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.

Abbreviations: IHC, immunohistochemistry; ISH, in situ hybridization.
**Tissue sample-handling practices define specimen quality**

- 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.\(^1,2\)
  - Needle or excisional biopsy specimens are adequate for HER2 testing.\(^1\)
  - Proper tissue sample handling is important for obtaining reliable test results.\(^1,3\)
  - Some tissue-handling practices can introduce artifacts that may cause challenges for HER2 test result interpretation.\(^1,3\)

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**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.

- 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.\(^1\)

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**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. 

Excisional samples should be sliced at 5- to 10-mm intervals and placed in sufficient volume of 10% NBF. Time of placement in fixative should be recorded. 

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. 

According to ASCO/CAP, intact samples should not be fixed. 

10% NBF is the standard fixative for ER, PR, and HER2 testing. ASCO/CAP guidance regarding fixative type remains consistent with previous recommendations. 

* 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.\(^1,2\)

Sufficient fixation time ensures complete tissue preservation. 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.\(^1\)

**Fixation Rule Of Thumb**\(^9,10\)

<table>
<thead>
<tr>
<th>Penetration front</th>
<th>Fixation front</th>
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<tbody>
<tr>
<td>1 mm/hour</td>
<td>2-3 mm/24 hours</td>
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</table>

**Standardization promotes quality HER2 analysis**

- ASCO/CAP recommends that routine processing be performed according to standardized, analytically validated protocols.\(^1,3\)
- Any deviations from standardized protocols should be documented in the pathology report.\(^1\)

**Section size and age are integral to quality HER2 analysis**

For best results, ASCO/CAP recommends prompt use of slides prepared for HER2 testing.\(^1,3\)

- Prolonged storage of glass slides with cut sections of tissue should be avoided.
- Length of storage that does not compromise antigen preservation is variable depending on the fixation conditions.
- Sections cut more than 6 weeks prior should not be used for HER2 testing.
Good preanalytic practices foster specimen quality

1. Sample should be fixed for 6 to 72 hours.
2. Samples should be fixed in 10% NBF.

Ensure your lab is committed to quality testing

HER2 testing should be performed only in labs with demonstrated proficiency in the HER2 assays they perform.1

ASC0/CAP Recommendations for Quality Laboratory Practices

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Recommendation</th>
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<tbody>
<tr>
<td>Internal validation and concordance</td>
<td>• HER2 testing labs should follow all accreditation requirements, including those for initial test validation.</td>
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<td>• All HER2 assays (FDA-approved assays and LDTs) and changes to assays must be validated in the laboratory that will perform them.</td>
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<td>• Tissues used in concordance studies should include a broad representation of patients with breast cancer, with approximately 15% to 20% of whom will be observed to have HER2-positive status.</td>
</tr>
<tr>
<td>External proficiency testing</td>
<td>• External proficiency testing is mandatory for CAP-accredited laboratories.</td>
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<td>• External proficiency testing failure requires investigation and corrective action before the laboratory can continue to offer HER2 testing.</td>
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<tr>
<td>Internal competency assessment</td>
<td>• Ongoing education, training, and competency assessment should be part of internal QA programs.</td>
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<td></td>
<td>• Under CLIA, laboratory personnel competency must be addressed continuously.</td>
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<tr>
<td>Internal QA</td>
<td>• Internal quality assurance should be part of internal QA programs and include at least one test each year.</td>
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<td>• Internal QA should be developed and validated.</td>
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<tr>
<td>Scoring and interpretation</td>
<td>• Laboratory procedures should be standardized; deviations from standardized procedures should be documented.</td>
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<tr>
<td>Reporting</td>
<td>• CAP accreditation requires on-site inspection every other year and self-inspection yearly.</td>
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<td>• Consistently performing results that are worse than 95% for HER2 results with that method is not acceptable.</td>
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</table>

Practice points: the preanalytic phase

• 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

Evaluate HER2 status using FDA-approved IHC or ISH assays

HER2 IHC and ISH provide complementary information about the biology of the breast tumor sample.11,12

- ASCO/CAP preferentially recommends the use of FDA-approved IHC and ISH assays to evaluate HER2 status.1
- Standardized control materials must be used with every test run.1
- Standardized control materials must include cell lines or tumor blocks with well-defined results.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.11
- 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.
Labeled probes bind to the HER2 gene, allowing detection and quantification of the number of HER2 genes present in a cell. In dual-probe assays, the CEP17 probe is used to control for chromosome number and alterations to pericentromeric DNA.

HER2 ISH evaluates the number of HER2 genes present:

- HER2 amplification: CEP17:HER2 \( \geq 2 \)
- No HER2 amplification: CEP17:HER2 = 1

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

Tumor Sampling

Image Analysis

Fixation

Sample Incision

Sample Processing

Scoring and Interpretation

Reporting

Automation promotes consistency in analysis

According to ASCO/CAP, image analysis can be an effective tool for achieving consistent interpretation.

- Variation in visual acuity, light sources, and microscopes cannot be controlled in manual counting situations.
- As with all aspects of the HER2 testing process, image analysis procedures must be validated before implementation.

ASCOCAP encourages the use of quantitative image analysis for cases with weak membrane staining (1+ and 2+) to improve consistency of interpretation among pathologists.

Considerations for IHC analysis

- Score infiltrating ductal carcinoma only.
- For a positive result, more than 10% of the tumor must show circumferential membrane staining.
- Membrane staining must be intense and resemble chicken wire.
- Incomplete or pale membrane staining should be ignored.

When a HER2 test is rejected, testing should be repeated using the same or an alternate FDA-approved assay.
Considerations for ISH analysis

- A corresponding H&E and/or IHC slide should be used to localize the invasive component.6
- At least 20 nonoverlapping cells in 2 separate areas of invasive cancer (at least 10 cells per area) should be counted.6
- If HER2/CEP17 is between 1.8 and 2.2, have another person count an additional 20 nonoverlapping cells.6
- A pathologist must confirm the result and that only areas of invasive tumor were evaluated.6

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 interpretatio
- 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.1,3
  - IHC 3+ is defined as HER2-positive staining in greater than 10% of the invasive component.*
  - The HER2-equivocal (IHC 2+) category has been expanded to include samples that stain HER2-positive in 10% or less of the invasive component.1,3
  - Definitions for HER2-negative categories (IHC 1+ and IHC 0) have been updated.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.

Equivocal HER2 IHC results require a reflex or new test

- Equivocal result for HER2 IHC is 2+
  * Complete membrane staining that is nonuniform or weak in intensity with obvious circumferential distribution in at least 10% of cells
  * Circumferential membrane staining that is incomplete and/or weak/moderate 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)
- A new test must be ordered (new specimen, if available, either assay)
**ASCO/CAP ISH interpretation criteria have been updated**

- **ISH-positive** is defined as HER2/CEP17 ≥ 2.0 (dual-probe test) or an average HER2 copy number ≥ 6.0 signals/cell (single- and dual-probe tests).
- For dual-probe tests, ISH-equivocal and ISH-negative categories depend on both the HER2/CEP17 ratio and the average HER2 copy number per cell.
- If the ISH ratio is between 1.8 and 2.2, an additional person should count an additional 20 cells.

While it does not influence result interpretation, evaluating the average CEP17 signals/cell may help address concerns about aneusomy or alterations to pericentromeric DNA.

ASCO/CAP ISH interpretation criteria have been updated.* Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.

In cases of HER2 heterogeneity, ASCO/CAP now recommends that cases with both amplified and nonamplified areas be reported as positive for HER2.

**Tumor Sampling**

The Multidisciplinary Testing Team

- Sample Incision
- Fixation
- Sample Processing
- Transport to Diagnostics Lab
- Image Analysis
- Reporting

**PREANALYTIC**

**ANALYTIC**

**POSTANALYTIC**

**Scoring and Interpretation**

**APPLY ASCO/CAP**

**Histopathologic Discordance**

**Reflex Testing**

**References**

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**Equivocal HER2 ISH results require a reflex or new test**

### 2007 Guideline

- Equivocal result for HER2 ISH
  - FISH HER2CEP17 = 1.8 to 2.2 or
  - Average HER2 copy number = 4 to 6 HER2 signals/nucleus for test systems without an internal control

- ISH-equivocal test results must be reported as equivocal, and:
  - Reflex testing must be ordered (same specimen, FISH assay)
  - A new test must be ordered (new specimen, if available, either assay).

### 2013 Guideline

- Equivocal result for HER2 ISH
  - Single probe: Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell
  - Dual probe: HER2CEP17 < 2.0 and average HER2 copy number ≥ 4.0 and < 6.0 signals/cell

* Observed in a homogeneous and contiguous population.

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*This hybrid ISH algorithm combines the information from the single-probe (Figure 2) and dual-probe (Figure 3) ISH assay algorithms.‡

† This guideline is based on the American Society of Clinical Oncology/College of American Pathologists her2 and HER2/C EP17 ratio interpretation criteria.*

‡ Observed in a homogeneous and contiguous population.

†† Rare scenario.
Repeat testing for questionable HER2 test results

**Indications for Repeat HER2 Testing**

- 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)
- Apparent discordance between the HER2 test results and certain histopathologic features
  - A negative HER2 test result and limited invasive component in the core-biopsy sample (repeat test on excisional sample)
  - 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.

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**When to repeat test for histopathologic discordance**

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**Reporting elements for IHC**

Image used with permission.
Reporting elements for ISH

PREANALYTIC

Sample Incision
Fixation
Sample Processing
Transport to Diagnostics Lab

ANALYTIC

Image Analysis

POSTANALYTIC

Scoring and Interpretation
Reporting

APPLY ASCO/CAP
Histopathologic Discordance
Reflex Testing

Practice points: the postanalytic phase

- 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+: HER2/CEP17 ratio ≥ 2.0 or HER2 copy number ≥ 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).

HER2 testing in breast cancer

- About 15% to 20% of breast tumors are HER2-positive.
- HER2-positive breast cancer is aggressive and likely to metastasize.
- HER2 testing is the only means for determining eligibility for HER2-directed therapy.
- A coordinated, multidisciplinary approach to HER2 testing is essential for
  - Obtaining dependable HER2 test results
  - Establishing an accurate disease diagnosis
  - Personalizing patient care
**What would you do next?**

### Initial pathology results for core-needle biopsy sample

**Results summary**
- Stage IIIA breast cancer
- 5-cm primary tumor
- Positive lymph nodes
- Nottingham grade 3
- ER- and PR-negative
- HER2-negative

**HER2 testing details**
- Less than 10% of invasive cells exhibit complete membrane staining
- No homogeneous, dark, circumferential staining pattern
- Staining pattern is nonuniform
- HER2 IHC 1+

**Nottingham grade 3**

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Discordance between the HER2 result and histopathologic features

This case exhibits discordance.1

- Her2 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

Repeat testing is indicated due to discordance

Histopathologic Features Suggestive of Possible HER2 Test Discordance

<table>
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<tr>
<th>Feature</th>
<th>Interpretation</th>
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<tr>
<td>Faint or absent staining in a minority of invasive tumor cells</td>
<td>Repeat testing on an excisional specimen must be ordered.</td>
</tr>
<tr>
<td>HER2 1+ in &gt;10% of tumor cells</td>
<td>Repeat testing on an excisional specimen must be ordered.</td>
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</table>

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.2

References


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