

## ERBB2 (HER2/neu) (HercepTest) Testing

Both breast and gastric cancers are common causes of cancer-related deaths. Amplification of the *ERBB2* (*HER2*) gene occurs in 15-20% of breast cancers and approximately 7-38% of gastric cancers. Trastuzumab (Herceptin) may improve the overall survival rate in individuals with HER2-positive breast carcinoma or gastroesophageal adenocarcinoma. Laboratory testing can determine *ERBB2* status and aid in the prediction of response to HER2-directed therapy.

### Typical Testing Strategy

Standard practice for evaluating primary, recurrent, and metastatic breast carcinoma, and gastric or gastroesophageal adenocarcinoma:

#### Breast Carcinoma

- Assess *ERBB2* status by immunohistochemistry (IHC) or in situ hybridization (ISH)/fluorescence in situ hybridization (FISH)
  - Concordance between the methods can vary due to subjective interpretation
  - If IHC equivocal (2+), confirm by ISH/FISH
  - If ISH/FISH scores fall in Groups 2, 3, or 4 (formerly designated as equivocal), confirm by IHC with rescoring in area(s) of highest staining intensity

#### Gastric Carcinoma

IHC should be performed first, followed by FISH testing for equivocal results

### Disease Overview

#### Incidence

Breast cancer: ~268,600 cases diagnosed in the U.S.

Gastroesophageal cancers: ~27,510 cases diagnosed in the U.S.

#### Treatment Issues

Amplification of the *ERBB2* gene occurs in 15-20% of breast cancers and approximately 7-38% of gastroesophageal adenocarcinomas and predicts poor prognosis in invasive breast cancer.<sup>1,2</sup>

Trastuzumab therapy inhibits HER2-positive cancers by directing antibodies against the extracellular portion of the HER2 protein. Trastuzumab may improve the overall survival rate in individuals with HER2-positive tumors.

Trastuzumab has a potential for cardiac toxicity along with a high drug cost; therefore, tumors that demonstrate *ERBB2* (*HER2*) gene

### Tests to Consider

#### [ERBB2 \(HER2/neu\) Gene Amplification by FISH with Reflex, Tissue 2008603](#)

**Method:** Fluorescence in situ Hybridization (FISH)

- Aid in prediction of response to HER2-directed therapy [eg, trastuzumab (Herceptin)] in patients with breast carcinoma or gastroesophageal adenocarcinoma
- Confirm equivocal HercepTest (2+) IHC result

#### [ERBB2 \(HER2/neu\) \(HercepTest\) by Immunohistochemistry, Tissue with Reflex to FISH if 2+ 0049178](#)

**Method:** Immunohistochemistry

- Aid in prediction of response to HER2-directed therapy [eg, trastuzumab (Herceptin)] in patients with breast carcinoma or gastroesophageal adenocarcinoma
- Measure protein expression
- Reflex to FISH if IHC is 2+

#### [ERBB2 \(HER2/neu\) \(HercepTest\) with Interpretation by Immunohistochemistry, Tissue 0049174](#)

**Method:** Immunohistochemistry

- Aid in prediction of response to HER2-directed therapy [eg, trastuzumab (Herceptin)] in patients with breast carcinoma or gastroesophageal adenocarcinoma
- Confirm equivocal dual ISH or FISH result
- Measure protein expression

#### [ERBB2 \(HER2\) \(HercepTest\) by Immunohistochemistry 2007332](#)

**Method:** Immunohistochemistry

Measure protein expression

amplification or protein overexpression (3+ IHC result) must be identified prior to the initiation of therapy.

New therapies targeting HER2 include pertuzumab (Perjeta), T-DM1 (Kadcyla), and lapatinib (Tykerb); recent studies have shown that treatment with a combination of trastuzumab and pertuzumab is more effective than trastuzumab alone (in combination with docetaxel) in prolonging survival of breast cancer patients.



## Genetics

### Gene

*ERBB2*

### Function

Amplification of *ERBB2* gene

- Increases membrane expression and activation of the HER2 protein
- Stimulates cell proliferation

## Test Interpretation

### Gene Amplification

#### Breast

Result	Group	<i>ERBB2</i> /CEP17 Ratio	Average <i>ERBB2</i> Copy Number	Interpretation <sup>a</sup>
Positive	Group 1	≥2.0	≥4.0 signals/cell	Predicts favorable response to targeted therapy
Negative	Group 5	<2.0	<4.0 signals/cell	Predicts lack of response to targeted therapy
Indeterminate	Group 2	≥2.0	<4.0 signals/cell	Perform concomitant HER2 IHC review <ul style="list-style-type: none"><li>• IHC score of 3+ is considered positive and 0 or 1+ is considered negative</li><li>• For an IHC score of 2+, additional tumor nuclei are enumerated with FISH from area of highest IHC intensity by an individual blinded to the original results</li><li>• Repeat scoring consistent with groups 2 and 4 is considered negative while scoring consistent with group 3 is considered positive</li></ul>
	Group3	<2.0	≥6.0 signals/cell	
	Group3	<2.0	≥4.0 and <6.0 signals/cell	

<sup>a</sup>It is uncertain whether patients with ≥4.0 and <6.0 average HER2 signals/cell and *HER2*/CEP17 ratio <2.0 benefit from HER2 targeted therapy in the absence of protein overexpression (IHC 3+)

#### Gastric

- Positive: *ERBB2*/CEP17 ratio ≥2.0 or *ERBB2*/CEP17 ratio <2.0 and average *ERBB2* copy number ≥6.0 signals/cell
  - Predicts favorable response to targeted therapy
- Negative: *ERBB2*/CEP17 ratio <2.0 and average *ERBB2* copy number <4.0 signals/cell
  - Predicts lack of response to targeted therapy
- If results are indeterminate, consider further testing with an alternate control probe or analytic method or follow-up testing on the resection specimen

## Limitations

- Testing only validated for FFPE specimens; specimens fixed in other than 10% neutral buffered formalin have not been validated using this method
- Specimens placed in decal may have a false-negative result
- Assay is validated and FDA approved for invasive breast carcinoma and gastroesophageal adenocarcinoma only
- Testing is interpreted according to ASCO/CAP 2018 Updated Guidelines for breast cancer and ASCO/CAP 2017 Guidelines for *HER2* in gastroesophageal adenocarcinoma
- Repeat testing is recommended for discordant results



## Immunohistochemistry

### ASCO/CAP 2018 HER2 IHC Scoring Criteria Used in the Interpretation of the HercepTest for Breast Cancer

Score	Interpretation	Microscopic Finding
0	Negative	No staining or membrane staining that is incomplete, faint/barely perceptible and within ≤10% of the invasive tumor cells
1+	Negative	Incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells
2+	Equivocal <sup>a</sup>	Weak to moderate complete membrane staining observed in >10% of tumor cells
3+	Positive <sup>b</sup>	Circumferential membrane staining that is complete, intense and in >10% of invasive tumor cells

<sup>a</sup>Equivocal results (2+) should be confirmed by ISH testing

<sup>b</sup>Positive results (3+) indicate possible response to trastuzumab

### Biopsies of Gastric and Gastroesophageal Adenocarcinoma Using ERBB2 IHC Scoring

Score	Interpretation	Staining Pattern
0	Negative	No reactivity or no membranous reactivity in any tumor cell
1+	Negative	Tumor cell cluster (5 cells) with faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained
2+	Equivocal	Tumor cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained
3+	Positive	Tumor cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained

Hofmann, 2008<sup>3</sup>

### Resections of Gastric and Gastroesophageal Adenocarcinoma Using ERBB2 IHC Scoring

Score	Interpretation	Staining Pattern
0	Negative	No reactivity or membranous reactivity in <10% of tumor cells
1+	Negative	Faint/barely perceptible membranous reactivity in ≥ 10% of tumor cells. Cells are reactive only in part of their membrane
2+	Equivocal	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥ 10% of tumor cells
3+	Positive	Strong complete, basolateral or lateral membranous in ≥ 10% of tumor cells

Hofmann, 2008<sup>3</sup>



## References

1. Nitta H, Kelly BD, Allred C, et al. [The assessment of HER2 status in breast cancer: the past, the present, and the future](#). *Pathol Int*. 2016;66(6):313-324. PubMed
2. Bartley AN, Washington MKay, Colasacco C, et al. [HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology](#). *J Clin Oncol*. 2017;35(4):446-464. PubMed
3. Hofmann M, Stoss O, Shi D, et al. [Assessment of a HER2 scoring system for gastric cancer: results from a validation study](#). *Histopathology*. 2008;52(7):797-805. PubMed

## Additional Resources

American Cancer Society. [How common is breast cancer?](#) [Last revised: Jan 2019; Accessed: Apr 2019]

Baselga J, Cortés J, Kim SB, et al. [Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer](#). *N Engl J Med*. 2012;366(2):109-119. PubMed

Swain SM, Baselga J, Kim SB, et al. [Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer](#). *N Engl J Med*. 2015;372(8):724-734. PubMed

Wolff AC, Hammond EHale, Allison KH, et al. [Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update](#). *Arch Pathol Lab Med*. 2018;142(11):1364-1382. PubMed

## Related Information

### Breast Cancer Biomarkers

ARUP Laboratories is a nonprofit enterprise of the University of Utah and its Department of Pathology. 500 Chipeta Way, Salt Lake City, UT 84108  
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