MAYO CLINIC LABORATORIES

Test Definition: H2BR

HER2, Breast Tumor, FISH, Tissue

Overview

Useful For

A predictive marker for patients with both node-positive or node-negative primary and metastatic breast cancer

Patients with *HER2* amplification that may be candidates for therapies targeting the human epidermal growth factor receptor 2 (HER2) protein (eg, trastuzumab [Herceptin], pertuzumab, lapatinib)

Confirming the presence of *HER2* amplification in cases with 2+ (low level) or 3+ (high level) HER2 overexpression by immunohistochemistry, and for certain histologic subtypes with aberrant patterns of HER2 expression seen by immunohistochemistry (eg, micropapillary carcinoma)

Testing Algorithm

Reflex testing will be performed using immunohistochemistry (IHC) when the fluorescence in situ hybridization (FISH) result falls within certain ranges as defined by the 2018 focused update to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines.(1) For FISH results in ASCO/CAP categories Group 2, 3, and 4 (formerly called "equivocal"), the IHC testing will be added, charged, and reported separately. An integrated interpretation of the IHC and FISH results will be provided (see Interpretation).

Initial results are typically completed within 6 days. If results of the initial probe set indicate IHC reflex testing is necessary based on ASCO/CAP guidelines, complete results will typically be available within 8 days.

A charge and CPT code is applied for each probe set hybridized, analyzed, and reported.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
HERBN	HER Breast IHC Automated NO Reflex	Yes	No

Method Name

Fluorescence In Situ Hybridization (FISH)

NY State Available

Yes

Specimen

Specimen Type
Tissue

Ordering Guidance
This test is only for primary or metastatic breast tumors.

-For urothelial tumors, order H2UR / *HER2* Amplification Associated with Urothelial Carcinoma, FISH, Tissue.

-For gastroesophageal tumors, order H2GE / *HER2* Amplification Associated with Gastroesophageal Cancer, FISH, Tissue.

-For all other tumor types, order H2MT / *HER2* Amplification, Miscellaneous Tumor, FISH, Tissue.

This test does not include a pathology consult. If a pathology consult is requested, PATHC / Pathology Consultation should be ordered and the appropriate fluorescence in situ hybridization test will be determined by the reviewing pathologist and testing performed at an additional charge.

Shipping Instructions
Advise Express Mail or equivalent if not on courier service.

Necessary Information
1. A pathology report is required in order for testing to be performed. Acceptable pathology reports include working drafts, preliminary pathology, or surgical pathology reports.

2. A reason for testing must be provided. If this information is not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.

3. The pathology report must include type and time of fixation, as well as the cold ischemia time.

Specimen Required
Note: In accordance to College of American Pathologists guidelines, place specimens for *HER2* (*ERBB2*) testing in fixative within one hour of biopsy or resection (cold ischemia time). Specimens should remain in 10% neutral buffered formalin for a minimum of six hours to a maximum of 72 hours (formalin fixation time). Do not use decalcification solutions with strong acids.(2)

Submit only 1 of the following specimens:

Specimen Type: Tissue

Preferred: Tissue block

Collection Instructions: Submit a formalin-fixed, paraffin-embedded (FFPE) tumor tissue block.

Acceptable: Slides

Collection Instructions: Four consecutive, unstained, 5 micron-thick sections placed on positively charged slides, and 1 hematoxylin and eosin-stained slide.

Forms

If not ordering electronically, complete, print, and send a Oncology Test Request (T729) with the specimen.

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Minimum Volume

Two consecutive, unstained, 5-micron-thick sections placed on positively charged slides, and 1 hematoxylin and eosin-stained slide.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Tissue	Ambient (preferred)		
	Refrigerated		

Clinical & Interpretive

Clinical Information

HER2 (ERBB2: c-erb-b2) is an oncogene on the long arm of chromosome 17 that is amplified in approximately 15% to 20% of breast cancers. Amplification or overexpression of *HER2* has been shown to be associated with shorter disease-free survival and poorer overall survival in breast cancers. Patients with *HER2* gene amplification or overexpression are candidates for treatment with the drugs that target the human epidermal growth factor receptor 2 (HER2) protein or its downstream pathways (eg, trastuzumab [Herceptin], pertuzumab).

Fluorescence in situ hybridization with labeled DNA probes to the pericentromeric region of chromosome 17 and to the *HER2* locus can be used to determine if a patient's breast cancer has *HER2* gene amplification. Immunohistochemical

analysis is used to determine if a tumor exhibits HER2 overexpression.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided. Results are interpreted utilizing the current American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines.(1)

Under the 2018 Focused Update to the ASCO/CAP Guidelines, reflex immunohistochemistry (IHC) is performed for certain categories of results, known as Groups 2, 3, and 4. These categories are shown in the table below (Group 4 is the category formerly referred to as fluorescence in situ hybridization (FISH) "equivocal"). If reflex IHC is performed and is either negative (0, 1+) or positive (3+), the result of the FISH assay is considered resolved by IHC as either negative or positive. If the IHC assay shows an equivocal (2+) result, then the FISH slide is re-scored within the areas showing the most intense membranous (2+) staining and the final FISH result is used to determine whether the result is negative or positive.

ASCO/CAP result category	HER2:D17Z1 ratio; average HER2 copies per cell	Reporting approach per 2018 ASCO/CAP guidelines
Group 1	HER2:D17Z1 =2.00; HER2/cell > or =4.0	Positive
Group 2	HER2:D17Z1 =2.00; HER2/cell <4.0	Reflex IHC; FISH re-analysis if 2+
Group 3	HER2:D17Z1 <2.00; HER2/cell > or =6.0	Reflex IHC; FISH re-analysis if 2+
Group 4	HER2:D17Z1 <2.00; HER2/cell > or =4.0 <6.0	Reflex IHC; FISH re-analysis if 2+
Group 5	HER2:D17Z1 <2.00; HER2/cell <4.0	Negative

The degree of *HER2* amplification varies in tumors. Some exhibit high levels of amplification (*HER2*:D17Z1 ratio >4.0), whereas others exhibit low-level amplification (*HER2*:D17Z1 ratio of 2.0-4.0). It is not currently known if patients with different levels of amplification have the same prognosis and response to therapy.

Reports also interpret the *HER2* copy number changes relative to chromosome 17 copy number (aneusomy) or potential structural genomic abnormalities that increase *HER2* copy number.

Rare cases may not show *HER2* amplification but still have human epidermal growth factor receptor 2 (HER2) protein overexpression demonstrated by immunohistochemistry. The clinical significance of HER2 protein overexpression in the absence of *HER2* gene amplification is unclear. However, these patients may have a worse prognosis and be candidates

for treatments that target the HER2 protein or its downstream pathways.

Cautions

Optimum fixation should be between 6 and 72 hours in 10% neutral buffered formalin. Other types of fixatives should not be used.

The prognostic information provided by the *HER2* status of a patient's tumor should not be interpreted in isolation because other prognostic features (eg, lymph node status, tumor size, estrogen/progesterone receptor status) may be of equal or greater importance in determining the patient's prognosis.

Supportive Data

The probe was independently validated in a blinded study on 1156 paraffin-embedded breast tissue samples. The results of the fluorescence in situ hybridization testing was correlated to the immunohistochemical analysis, which was interpreted on a scale ranging from 0 to 3+ according to FDA-approved guidelines.

Clinical Reference

1. Wolff AC, Hammond MEH, 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. J Clin Oncol. 2018 Jul 10;36(20):2105-2122 doi:10.1200/JCO.2018.77.8738

2. College of American Pathologists Cytogenetics Checklist item CYG.48932. 2019 Sep:25

3. Wolff AC, Hammond ME, Hicks DG, et al: Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society for Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Onc. 2013 Nov 1;31(31):3997-4013

4. Perez EA, Roche PC, Jenkins RB, et al: HER2 testing in patients with breast cancer: poor correlation between weak positively by immunohistochemistry and gene amplification by fluorescence in situ hybridization. Mayo Clin Proc. 2002 Feb;77(2):148-154

5. Romond EH, Perez EA, Bryant J, et al: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med. 2005 Oct 20;353(16):1673-1684

6. Perez EA, Romond EH, Suman VJ, et al: Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol. 2011 Sep 1;29(25):3366-3373

7. Blumenthal GM, Scher NS, Cortazar P, et al: First FDA approval of dual anti-HER2 regimen: pertuzumab in combination with trastuzumab and docetaxel for HER2-positive metastatic breast cancer. Clin Cancer Res. 2013 Sep 15;19(18):4911-4916

8. Robidoux A, Tang G, Rastogi P: Lapatinib as a component of neoadjuvant therapy for HER2-positive operable breast cancer (NSABP protocol B-41): an open-label, randomized phase 3 trial. Lancet Oncol. 2013 Nov;14(12):1183-1192

Performance

Method Description

The test is performed using the PathVysion HER2 DNA probe set (Abbott Molecular) with a *HER2* probe and a chromosome 17 centromere probe (D17Z1). Paraffin-embedded tissues are cut at 5 microns and mounted on positively charged glass slides. The selection of tissue and the identification of target areas on the hematoxylin and eosin (H and E)-stained slide are performed by a pathologist. Using the H and E-stained slide as a reference, target areas are etched with a diamond-tipped etcher on the back of the unstained slide to be assayed. The probe is hybridized to the appropriate target areas and 2 technologists each analyze 30 interphase nuclei (60 total) with the results expressed as a ratio *HER2:*D17Z1 signals. The results are interpreted based on the 2018 guidelines established by the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP).(1)

Reflex testing using immunohistochemistry will be performed when the HER2 FISH result is Group 2, Group 3, or Group 4 (based on 2018 Focused Update to ASCO/CAP guidelines).(Unpublished Mayo method)

PDF Report

No

Specimen Retention Time

Slides and H&E used for analysis are retained by the laboratory in accordance to CAP and NYS requirements. Client provided paraffin blocks and extra unstained slides (if provided) will be returned after testing is complete.

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

88377

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
H2BR	HER2, Breast Tumor, FISH, Tissue	96893-3

Result ID	Reporting Name	LOINC®
603074	Result Summary	50397-9
603075	Interpretation	69965-2
603076	Result	62356-1
GC028	Reason for Referral	42349-1
603077	Specimen	31208-2
603078	Source	85303-6
603079	Tissue ID	80398-1
603080	Fixative	8100-0
603081	Method	85069-3
603082	Additional Information	48767-8
603083	Disclaimer	62364-5
603084	Released By	18771-6