MAYO CLINIC LABORATORIES

Test Definition: H2BR

HER2 Amplification Associated with Breast Cancer, FISH, Tissue

Overview

Useful For

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

Guiding therapy for patients with primary or metastatic breast tumors, as patients with *HER2* amplification may be candidates for therapies that target the human epidermal growth factor receptor 2 (HER2) protein (eg, trastuzumab [Herceptin], pertuzumab, lapatinib)

Confirming the presence or absence 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)

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
HERBM	HER Breast Semi Quant IHC Manual	No	No
HERBN	HER Breast IHC Automated NO Reflex	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No

Testing Algorithm

This test includes a charge for the probe application, analysis, and professional interpretation of results for one probe set (2 individual fluorescence in situ hybridization [FISH] probes). No analysis charges will be incurred if an insufficient number of representative cells are available for analysis.

Reflex testing will be performed using immunohistochemistry (IHC) when the 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) FISH results in ASCO/CAP categories Group 2, 3, and 4 (formerly called "equivocal") will have IHC testing 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 initial results indicate IHC reflex testing is necessary based on ASCO/CAP guidelines, complete results will typically be available within 8 days.

Appropriate ancillary probes may be performed at consultant discretion to render comprehensive assessment. Any additional probes will have the results included within the final report and will be performed at an additional charge.

Method Name
Fluorescence In Situ Hybridization (FISH)

NY State Available
Yes

Specimen

Specimen Type
Tissue

Ordering Guidance
This test is only performed on specimens from patients with primary or metastatic breast tumors.

This test is not appropriate if the specimen is derived from primary or metastatic gastroesophageal carcinoma. See H2GE / *HER2* Amplification Associated with Gastroesophageal Cancer, FISH, Tissue. If this test is ordered and the laboratory is informed that the specimen is a primary or metastatic gastroesophageal carcinoma, it will be canceled and automatically reordered by the laboratory as H2GE.

For all other primary tumor types, order H2MT / *HER2* Amplification, Miscellaneous Tumor, FISH, Tissue. If this test is ordered and the laboratory is informed that the specimen is a primary or metastatic colorectal adenocarcinoma, endometrial serous carcinoma, urothelial carcinoma, or any other non-breast, non-gastroesophageal primary or metastatic tumor, it will be canceled and automatically reordered by the laboratory as H2MT.

This test does not include a pathology consult. If a pathology consultation is requested, order PATHC / Pathology Consultation, and the appropriate testing will be added at the discretion of the pathologist and performed at an additional charge.

Multiple oncology (cancer) gene panels are also available. For more information see Hematology and Oncology Test Selection Guide.

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

Necessary Information
1. A pathology report is required for testing to be performed. If not provided, appropriate testing and/or interpretation

may be compromised or delayed. Acceptable pathology reports include working drafts, preliminary pathology, or surgical pathology reports.

2. The following information must be included in the report provided:

-Patient name

-Block number **- must be on all blocks, slides, and paperwork**

-Date of collection

-Tissue source

-**Fixation used AND time in Fixation** (recommended: >6 hours and <72 hours).

3. 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.

Specimen Required

Note: In accordance with 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 6 hours to a maximum of 72 hours (formalin fixation time). Do not use decalcification solutions with strong acids.(CAP Accreditation Program. CYG.48932 Fixation - HER2 (ERBB2) Breast Predictive Marker Testing. Cytogenetics Checklist. College of American Pathologists. 08/2023)

Submit only 1 of the following specimens:

Preferred:

Specimen Type: Tissue block (fresh tissue is **not acceptable**)

Collection Instructions:

1. Submit a formalin-fixed, paraffin-embedded tumor tissue block.
2. Blocks prepared with alternative fixation methods (eg, Prefer, Bouin's) will be attempted but are less favorable for successful results. Provide fixation method used.

Additional Information:

1. Paraffin-embedded specimens can be from any anatomic location (skin, soft tissue, lymph node, etc).
2. Decalcified paraffin-embedded specimens will have testing attempted; however, the success rate is approximately 50%. **Testing may be canceled** if sufficient tumor tissue is not present.
3. **Submitted fresh tissue specimens will be canceled upon receipt**. If only fresh tissue is available, embed in paraffin prior to sending.

Acceptable:

Specimen Type: Tissue slides

Slides: 1 Hematoxylin and eosin-stained and 4 unstained

Collection Instructions: Submit 1 slide stained with hematoxylin and eosin and 4 consecutive unstained, positively charged, unbaked slides with 5 micron-thick sections of the tumor tissue.

Additional Information: Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

Forms

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

Specimen Minimum Volume

Slides: 1 Hematoxylin and eosin stained and 2 unstained

Reject Due To

Broken or cracked unstained slides	Reject
Broken or cracked stained slides that cannot be repaired	Reject

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 reanalyzed 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.

Table.

ASCO/CAP result category	**HER2:D17Z1 ratio; Average HER2 copies per cell**	**Reporting approach per 2018 ASCO/CAP guidelines**
Group 1	HER2:D17Z1 =2.0 HER2/cell > or =4.0	Positive
Group 2	HER2:D17Z1 =2.0 HER2/cell <4.0	Reflex IHC; FISH reanalysis if 2+
Group 3	HER2:D17Z1 <2.0 HER2/cell > or =6.0	Reflex IHC; FISH reanalysis if 2+
Group 4	HER2:D17Z1 <2.0 HER2/cel > or =4.0 and <6.0	Reflex IHC; FISH reanalysis if 2+
Group 5	HER2:D17Z1 <2.0 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.

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 fixation methods should not be used, but the specimen will not be rejected.

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.

This test is not approved by the US Food and Drug Administration, and it is best used as an adjunct to existing clinical and pathologic information.

This fluorescence in situ hybridization (FISH) assay does not rule out other chromosome abnormalities.

Paraffin-embedded tissues that have been decalcified may not be successful for fluorescence in situ hybridization (FISH) analysis. The success rate of FISH studies on decalcified tissue is approximately 50%, but FISH will be attempted if

sufficient tumor is present for analysis.

Fluorescence in situ hybridization studies will be attempted if sufficient tumor is present for analysis. The pathologist reviewing the hematoxylin and eosin-stained slide may find it necessary to cancel testing if insufficient tissue/tumor is available for testing.

If no FISH signals are observed post-hybridization, the case will be released indicating a lack of FISH results.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

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;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738
2. CAP Accreditation Program. CYG.48932 Fixation - HER2 (ERBB2) Breast Predictive Marker Testing. Cytogenetics Checklist. College of American Pathologists. 08/2023
3. 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 clinical practice guideline update. J Clin Oncol. 2013;31(31):3997-4013
4. Perez EA, Roche PC, Jenkins RB, et al. HER2 testing in patients with breast cancer: poor correlation between weak positivity by immunohistochemistry and gene amplification by fluorescence in situ hybridization. Mayo Clin Proc. 2002;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;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;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;19(18):4911-4916
8. Robidoux A, Tang G, Rastogi P, et al. Lapatinib as a component of neoadjuvant therapy for HER2-positive operable breast cancer (NSABP protocol B-41): an open-label, randomised phase 3 trial. Lancet Oncol. 2013;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 engraving tool 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.

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[1]).(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
6 to 8 days

Specimen Retention Time
Slides and hematoxylin and eosin used for analysis are retained by the laboratory in accordance with regulatory requirements. Client provided paraffin blocks and extra unstained slides will be returned after testing is complete.

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It 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	Test Result Name	Result LOINC® Value

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