MAYO CLINIC LABORATORIES

Test Definition: JHERF

HER2 Amplification Associated with Breast Cancer, FISH, Breast Primary, Tissue

Overview

Useful For

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

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 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," "Group 3," and "Group 4" (formerly called "equivocal"), immunohistochemistry (IHC) testing will be reviewed. *HER2* IHC will be performed on cases that do not have IHC done initially. An integrated interpretation of the IHC and FISH results will be performed (see Interpretation).

Method Name

Fluorescence In Situ Hybridization (FISH)

NY State Available

No

Specimen

Specimen Type

Tissue

Ordering Guidance

This test is only for primary or metastatic breast tumors. All other specimen types will be rejected and testing canceled.

Shipping Instructions

Advise Express Mail or equivalent if not on courier service.

Ship paraffin blocks on ice packs during warm months.

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 (CAP) 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

Collection Instructions: Submit a formalin-fixed, paraffin-embedded tumor tissue block. Blocks prepared with alternative fixation methods **will not** be accepted; provide fixation method used.

Acceptable

Specimen Type: Tissue slides

Slides: 1 Hematoxylin and eosin stained and 2 unstained

Collection Instructions: Submit 1 slide stained with hematoxylin and eosin and 2 consecutive, unstained, positively charged, unbaked slides with 4 to 5-micron-thick sections of the tumor tissue. Slides cut from blocks prepared with alternative fixation methods will not be accepted; provide fixation method used.

Specimen Minimum Volume

Slides: 1 Hematoxylin and eosin stained and 1 unstained

Reject Due To

Decalcified specimens	Reject
Non-Formalin Fixed, Paraffin embedded tissue specimens	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 cancer. 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 (FISH) 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.(1,2)

Under the current focused update to the ASCO/CAP guidelines, reflex immunohistochemistry 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 immunohistochemistry (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 rescored 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 > or =2.00 HER2/cell > or =4.0	Positive
Group 2	HER2:D17Z1 > or =2.00 HER2/cell <4.0	Reflex/review IHC; FISH reanalysis if 2+
Group 3	HER2:D17Z1 <2.00 HER2/cell > or =6.0	Reflex/review IHC; FISH reanalysis if 2+
Group 4	HER2:D17Z1 <2.00	Reflex/review IHC; FISH reanalysis if 2+

	HER2/cell > or =4.0 and <6.0	
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. Her2 fluorescence in situ hybridization (FISH) will not performed on tissues that have been decalcified. They will be sent to Mayo Clinic Laboratories in Rochester.

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 over 80 paraffin-embedded breast tissue samples. The results of the fluorescence in situ hybridization (FISH) testing were correlated to the Her2 FISH performed at another facility, which was interpreted according to College of American Pathologists/American Society of Clinical Oncology guidelines.

Clinical Reference

1. 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. American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. J Clin Oncol. 2023;41(22):3867-3872
3. CAP Accreditation Program. CYG.48932 Fixation - HER2 (ERBB2) Breast Predictive Marker Testing. Cytogenetics Checklist. College of American Pathologists. 08/20233.
4. 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 Oncol. 2013;31(31):3997-4013. doi:10.1200/JCO.2013.50.9984
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. doi:10.1056/NEJMoa052122
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. doi:10.1200/JCO.2011.35.0868
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. doi:10.1158/1078-0432.CCR-13-1212

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 4 to 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 or *HER2* immunohistochemistry (IHC)-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.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
2 to 8 days

Specimen Retention Time
Images are saved indefinitely. Extra unstained slides (if provided) and hematoxylin and eosin-stained slide will be sent to histology after testing is complete.

Performing Laboratory Location
Jacksonville

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 has been modified from the manufacturer's instructions. Its performance characteristics were 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
JHERF	HER2, Breast Tumor, FISH, Ts	96893-3

Result ID	Test Result Name	Result LOINC® Value
602815	Result Summary	85318-4
602816	Interpretation	69965-2
602817	Result	62356-1
602818	Reason for Referral	42349-1
602819	Specimen	31208-2
602820	Source	85303-6
602821	Tissue ID	80398-1
603062	Fixative	8100-0
602822	Method	85069-3
602823	Additional Information	48767-8
602824	Disclaimer	62364-5
602825	Released By	18771-6