Test Definition: H2MT

HER2 Amplification, Miscellaneous Tumor, FISH, Tissue

Overview

Useful For

Guiding cancer therapy, 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)

Confirming the presence of *HER2* amplification in cases with 2+ (low level) or 3+ (high level) HER2 protein overexpression by immunohistochemistry

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.

Note: In accordance with criteria outlined in the 2013 American Society of Clinical Oncology/College of American Pathologists guideline for breast cancer, reflex testing will not be performed using an alternative chromosome 17 probe when the FISH result is equivocal.(1)

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 tumors other than breast or gastroesophageal.

T**his test is not appropriate if the specimen is derived from primary or metastatic breast carcinoma**. See H2BR / *HER2* Amplification Associated with Breast Cancer, FISH, Tissue. If this test is ordered and the laboratory is informed that the specimen is a primary or metastatic breast carcinoma, it will be canceled and automatically reordered by the laboratory as H2BR.

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.

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

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
In much the same way as was demonstrated for *HER2*-positive breast cancer, the *HER2* gene status in some cancers can be used to determine treatment approaches. Amplification of the *HER2* gene and overexpression of the human epidermal growth factor receptor 2 (HER2) protein have been associated with a shorter disease-free survival and shorter overall survival in some cancers. Patients whose tumors demonstrate *HER2* amplification or overexpression may be candidates for treatment with the drugs that target the HER2 protein or its downstream pathways (eg, trastuzumab [Herceptin], pertuzumab).

Reference Values

An interpretative report will be provided.

Interpretation

An interpretive report will be provided.

Results for tumors of colorectal origin are interpreted with reference to the definition of *HER2* amplification in colorectal cancer in the HERACLES trial, a *HER2* / centromere ratio 2.0 or above is considered positive(2) as well as with reference to the MyPathway trial, a *HER2* / centromere ratio above 2.0 or average HER2 copy number above 6.0 is considered positive.(3)

Results for primary or metastatic endometrial serous carcinomas are interpreted according to expert recommendations(4) and according to updated American Society of Clinical Oncology/College of American Pathologists (ASCP/CAP) (2018) guidelines for breast cancer.(5)

All other tumors are interpreted as amplified if *HER2* / centromere ratio is greater than or equal to 2.0 or average HER2 copy number greater than or equal to 6.0 and according to according to updated ASCO/CAP (2013) guidelines for breast cancer.(1)

The degree of *HER2* amplification varies in tumors. Some exhibit a high level 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 a similar prognosis or response to therapy.

Rare cases may not show *HER2* amplification but 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 may 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) 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 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;31(31):3997-4013
2. Valtorta E, Martino C, Sartore-Bianchi A, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. Mod Pathol. 2015;28(11):1481-1491
3. Meric-Bernstam F, Hurwitz H, Raghav KPS, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol. 2019;20(4):518-530
4. Buza N. HER2 Testing and reporting in endometrial serous carcinoma: Practical recommendations for HER2 immunohistochemistry and fluorescent in situ hybridization: Proceedings of the ISGyP Companion Society Session at the 2020 USCAP Annual Meeting. Int J Gynecol Pathol. 2021;40(1):17-23
5. Wolff AC, Hammond ME, 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
6. Bolenz C, Shariat SF, Karakiewicz PI, et al. Human epidermal growth factor receptor 2 expression status provides independent prognostic information in patients with urothelial carcinoma of the urinary bladder. BJU Int. 2010;106(8):1216-1222
7. Lae M, Couturier J, Oudard S, et al. Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients. Ann Oncol. 2010;21(4):815-819

Performance

Method Description

The test is performed using the dual-color PathVysion *HER2* DNA probe set (Abbott Molecular) with a HER2 probe and a chromosome 17 centromere probe (CEP17; 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 of HER2:D17Z1 signals.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
6 to 8 days

Specimen Retention Time
Slides and H and E 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
H2MT	HER2, Misc. Tumor, FISH, Tissue	96893-3

Result ID	Test Result Name	Result LOINC® Value
603096	Result Summary	50397-9
603097	Interpretation	69965-2
603098	Result	62356-1
GC032	Reason for Referral	42349-1

603099	Specimen	31208-2
603100	Source	85298-8
603101	Tissue ID	80398-1
603102	Fixative	8100-0
603103	Method	85069-3
603104	Additional Information	48767-8
603105	Disclaimer	62364-5
603106	Released By	18771-6