

FISH for *HER2* Gene Amplification

Background Information

Breast cancer in the United States is a devastating illness, affecting more than 200,000 newly diagnosed patients and resulting in more than 40,000 deaths annually.¹ Modern diagnostic procedures allow earlier detection, and excision of small tumors at an early clinical stage offers the best hope for cure.

Breast tumors differ in their biological behavior. One important challenge is the identification of tumors of low clinical and/or pathologic stage that display phenotypic or genotypic characteristics that indicate potential for aggressive malignant behavior (metastasis, recurrence, and early death).

Proteins expressed on tumor cells have become recognized as markers of biological behavior. Amplification and over-expression of the oncogene *HER2* occurs in 12-20% of human invasive breast carcinomas.^{2,3} *HER2* amplification in patients with invasive breast carcinoma is associated with a significant reduction in metastasis-free survival, and similarly staged patients without *HER2* gene amplification have a high likelihood of remaining cancer-free years after removal of the primary tumor.²⁻⁵ *HER2*-positive patients are more likely to respond to regimens containing adriamycin and less likely to respond to tamoxifen.^{6,7} Furthermore, patients with primary and metastatic breast carcinoma whose tumors have amplification of the *HER2* gene and the protein it encodes may benefit from therapy with the humanized monoclonal antibody trastuzumab (Herceptin®) in combination with chemotherapy.⁸⁻¹⁰

Cleveland Clinic Laboratories offers *HER2* gene amplification analysis of breast carcinoma by interphase fluorescence *in situ* hybridization (FISH) assay of paraffin-embedded tumor sections. Performance and interpretation of the assay are done in accordance with ASCO/CAP guidelines for *HER2* testing (reference below). If aneusomy of chromosome 17 is identified by the PathVysion assay, additional FISH testing using a laboratory-developed assay with a D17S122 reference probe is performed. This is done to either confirm the presence of aneusomy or correct the ratio for gains at the centromeric

reference probe locus. D17S122 reflex testing is also done for monosomy of chromosome 17.

Clinical Indications

HER2 gene amplification analysis is offered specifically as a prognostic and predictive molecular marker for invasive breast carcinoma and as an adjunctive test in the management of patients with advanced breast carcinoma for whom therapy with trastuzumab is contemplated. FISH also may be useful as a confirmatory reflex assay for breast carcinoma cases for which an equivocal score has been identified previously by immunohistochemistry.

Interpretation

HER2 gene amplification is reported as either amplified, not amplified, or equivocal. The *HER2* gene copy number is specified, and the *HER2*/chromosome 17 ratio is reported. Amplified cases demonstrate a ratio of 2.3 or greater and non-amplified cases demonstrate a ratio equal to or less than 1.7. Equivocal cases demonstrate a ratio equal to or greater than 1.8 but equal to or less than 2.2, and require reflex testing by immunohistochemistry per ASCO/CAP guidelines (reference below). Aneusomy and monosomy of chromosome 17, gains at the CEP17 centromeric locus, genomic heterogeneity, and allelic loss (usually monoallelic deletion) of *HER2* are also reported if identified.

Limitations of the Assay

The FISH assay performs well using paraffin sections of formalin-fixed tissue fixed and processed in accordance with ASCO/CAP guidelines. Time and duration of formalin fixation are required for testing to be performed.

Methodology

The assay consists of a modified interphase FISH probe assay (PathVysion, Abbott Molecular, Vysis, Des Plaines, Ill.) that quantifies *HER2* gene amplification in formalin-fixed, paraffin-embedded human breast carcinoma tissue.

References

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Test Overview

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| Test Name | <i>HER2</i> gene amplification analysis |
| Reference Range | <i>HER2</i> not amplified. <i>HER2</i> gene copy number is specified, and the <i>HER2</i> /chromosome 17 ratio is reported; a normal <i>HER2</i> /chromosome 17 ratio is equal to or less than 1.7 |
| Specimen Requirements | Collect: Paraffin block containing invasive breast carcinoma (duration of formalin fixation required) Transport: Room temperature |
| Billing Code | 80306 |
| CPT Code | 88368 (x2) |

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