UAMS Molecular Pathology/ Molecular Diagnostics Laboratory

HER-2/neu Amplification by Fluorescent *In Situ* Hybridization (FISH)

The test: The Abbott/Vysis PathVysion HER-2/neu DNA Probe Kit is designed to detect amplification of the HER-2/neu gene via fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens. The PathVysion Kit is FDA-approved for selecting breast cancer patients for Herceptin therapy. The PathVysion Kit is not intended for use to screen for or diagnose breast cancer.

Clinical Significance: The HER2 oncogene is located on chromosome 17 (17q11.2-q12) and encodes a 185 KD transmembrane glycoprotein receptor with intracellular tyrosine kinase activity. The HER2 receptor is a member of the epidermal growth factor receptor (EGFR) family of proteins and is important in the activation of intracellular signal transduction pathways controlling epithelial cell growth and differentiation. Her2 amplification has been observed in 20-30% of invasive breast carcinomas. Amplification of this gene has been correlated with a worse prognosis stage-for-stage. Those patients whose tumor has been shown to express amplified HER2 appear to benefit from treatment with the humanized monoclonal antibody Herceptin™.

A joint committee representing the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) has recommended that HER2 status should be determined for all invasive breast cancer. A set of guidelines that specifically address the technical and analytical aspects of HER2 testing has been published by this committee (1).

HER2 Nomenclature: A variety of nomenclatures has been used in the literature, including Her-2/neu, ERBB-2, HER-2, HER2, and c-erb-B2. Although ERBB-2 is the official name provided by the HUGO Gene Nomenclature Committee, HER2 is the most commonly used term.

Methodology: Interphase fluorescence *In situ* hybridization (FISH) is a technique that allows the visualization of specific nucleic acid sequences within a cellular preparation. In this assay, a fluorescently-labeled (SpectrumOrange™) DNA probe that specifically recognizes the HER2 locus (17q11.2-q12) is used in conjunction with a second fluorescently-labeled (SpectrumGreen™) DNA probe recognizing the centromeric region incubated with the tissue section. Hybridization of the probes to the tissue is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters allowing visualization of the intense orange and green fluorescent signals. Enumeration of the HER2 and CEP 17 signals is conducted by microscopic examination of the

nucleus, which yields a ratio of the HER2 gene to chromosome 17 copy number. It is this ration that is used to determine the amplification status of HER2.

Normal Range: HER2 is normally non-amplified.

Reportable Range:

Negative	A ratio <2.0 or <4.0 gene signals	HER2 not amplified
Borderline	A ratio <2.0 or >/= 4.0-6.0 gene signals	Borderline; results equivocal*
Positive	A ratio >2.0 or >/= 6.0 gene signals	HER2 amplified

** If the ratio falls close to or within the borderline range of 1.7 – 2.4, additional cells will be scored and the averages/ratio will be determined on the increased cell count.*

Specimen: Formalin-fixed, paraffin embedded tissue. Specimens should be fixed in 10% buffered formalin and processed for paraffin embedding between 6 and 24 hours for best results. Long term (>24 hours) storage in formalin may result in a reduction of the positive signal intensity. Use of other fixatives is not appropriate for this assay. Decalcified tissue can not be used for FISH assays. At least five sections and slides are required per paraffin block. One 3 micron-thick section should be placed on the bottom $^{1}/_{3}$ of each positively charged slide. Alternatively, a paraffin block can be submitted which will be returned upon completion of the test.

Note: Tissue must contain invasive or infiltration breast carcinoma. Carcinoma in situ is not acceptable.

Storage: Store unused slides or blocks at ambient temperature.

Assay Availability: Her2 by FISH is batched weekly, normally on Thursday.

Results Reported: 7 days

Rejection Criteria: Tissue which has been fixed in, or exposed to acids, strong bases or extreme heat will damage DNA. Tissues exposed to these conditions will not be tested. If it is determined by examination of the H & E slide that the case has an inadequate number of invasive or infiltrating malignant cells, HER2 analysis by FISH will not be performed. Sections should not be submitted for HER2 testing if they were cut greater than 6 weeks earlier.

CPT Coding: 88377x1

Sending specimens to the testing laboratory: All specimens must be accompanied by a completed requisition form available from the Anatomic Pathology Laboratory or download a **Consultation form**. All outside referrals should be accompanied by a copy of the surgical report.

Please call the Pathology lab at (501) 603-1963 for specimen delivery information.

Notes:

1) The Vysis PathVysion Kit is not intended for use to screen for or diagnose breast cancer. It is intended to be used as an adjunct to other prognostic factors currently used to predict disease-free and overall survival in patients with invasive breast cancer.

2) FISH assay results may not be informative if either the specimen quality and/or specimen slide preparation are inadequate.

3) Clearly label the slides or block with the patient's name, MP number and the surgical case number.

Laboratory Contact: For further information, please call the Molecular Diagnostics Laboratory at (501) 526-6439. Our fax number is (501) 686-7155.

References:

1) Wolff AC et al., American Society of Clinical Oncology/ College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. Journal of Clinical Oncology, 25:118-145, 2007.
2) Lester, Susan et al., Protocol for the Examination of Specimens From Patients with Invasive Carcinoma of the Breast. Arch Pathol Lab Medicine, Vol 133, October 2009.