

Overview of HER-2/neu Testing in Breast Cancer as Performed in the Danbury Hospital Laboratory

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Background:

The human epidermal growth factor receptor 2 (HER-2/neu) gene, (chromosome 17q12), encodes a trans-membrane tyrosine kinase growth factor receptor that sends messages to the cell to divide more frequently. In normal (non-dividing) cells, there are two copies of the HER2 gene. Breast cancers with amplification of the HER-2/neu genes, are considered HER2-positive, are more aggressive, and are found in about 15 - 20% of women with breast cancer. The drug trastuzumab is effective in the treatment of HER2-positive breast cancer. Due to the effectiveness, side effects, and cost of trastuzumab, it is very important to have tests that accurately determine HER2 tumor status.

Testing:

There are two methods of testing for HER2 tumor status: immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). If appropriate quality control procedures are in place for a laboratory, either IHC or FISH methods may be utilized. Since FISH is a gold standard for testing HER-2/neu status in women with invasive breast cancers, Danbury Hospital Laboratory (DHL) utilizes only the FISH method for assessing the Her2 status.

The DHL utilizes the PathVysion Her-2 DNA Probe Kit (Abbott Molecular, Inc.) FISH testing. *Please note:* This assay is FDA approved **only** for stage II, node-positive invasive carcinoma on human breast tissue.

Fluorescence in situ Hybridization (FISH):

FISH is a gene-based test used to determine the ratio of the average HER2 gene copy number/cell to average Chromosome 17 copy number/cell from invasive tumor cells. The DHL follows the process outlined below:

Hormone receptors (ER and PR) are ordered by the assigned pathologist on the appropriate block on all invasive carcinomas. At the same time, a request for Her2 testing by FISH is also generated via an email to Cytogenetics specifying the appropriate block and fixation time.

The physician places an order for HER2 testing by FISH. Note: The assay is performed each Tuesday and Friday. Any orders received after 11:00 am Monday will be tested on Friday; orders placed after 11:00 am Thursday will be tested the following Tuesday.

- An appropriate block is chosen by a pathologist (whenever possible, an excisional biopsy specimen block will be chosen; appropriate fixation parameters for needle core biopsies have not been established);
- Sections are cut and aged overnight;
- Slides are then assayed in the Cytogenetics lab (2-day process);
- Two trained cytogenetic technologists and/or pathologist read the prepared slide;
- The final report is generated by the Cytogenetics laboratory.

Turnaround Time: 4-7 calendar days.

Reported Results:

HER2 FISH results are reported as AMPLIFIED (ratio >2.2), NEGATIVE (ratio <1.8), or EQUIVOCAL (ratio 1.8-2.2). EQUIVOCAL assays are evaluated by three technologists/director (regular cases are evaluated by two technologists). EQUIVOCAL results should be interpreted with caution; at this time, no high-level evidence or agreement is available on how results in the equivocal range should be interpreted or confirmed. *As a quality assurance measure, HER2 FISH on cases with EQUIVOCAL results will be repeated.*

Limitations to the Procedure:

- Scoring difficulties found with FISH testing may be associated with the specific set of cells chosen to include in the determination of tissue processing.
- False positive or false negative (especially with prolonged fixation times, > 48 hours) HER2 test results can occur.
- CAP/ASCO exclusion criteria to perform or interpret a Her2 FISH include:
 - Samples with only limited invasive cancer difficult to define under UV light
 - Tissue fixed in fixatives other than buffered formalin
 - Tissue fixed for prolonged intervals in formalin (greater than 48 hours)†
 - Controls with unexpected results
 - FISH signals are non-uniform (< 75% identifiable)
 - Background obscures signal (> 10% of signals over cytoplasm)
 - Non-optimal enzymatic digestion (poor nuclear resolution, persistent autofluorescence)

†This is not an absolute exclusion criterion, but if tissue is known to be fixed longer than 48 hours or unknown, the report should qualify any negative result with this information.

Note: In addition to breast tissue, Her2 FISH testing is sometimes requested on carcinomas arising from other organs including stomach, esophagus, lung and some gynecologic organs, as well as breast cancer metastasis to organs such as liver and lymph node. The DHL is currently in the process of validating the PathVysion Kit on some of the above mentioned specimens. More information will follow upon completion. Implementation dates are to be determined.

References:

Antonio C. Wolff, M. Elizabeth H, et al. ASCO/CAP Guideline Recommendations for Human Epidermal Growth Factor Receptor 2. Testing in Breast Cancer Guideline for HER2 Testing in Breast Cancer. Arch Pathol Lab Med Vol 131, January 2007.

Abbott Molecular, Inc. PathVysion HER-2 DNA Probe Kit (Order # 30-161060) instructions.

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