DANBURY HOSPITAL
A Higher Level of Care

Technically Speaking

C.S. Guidess, Editor

Department of Pathology
& Laboratory Medicine

MAY 2009: VOL 3 N0. 4

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

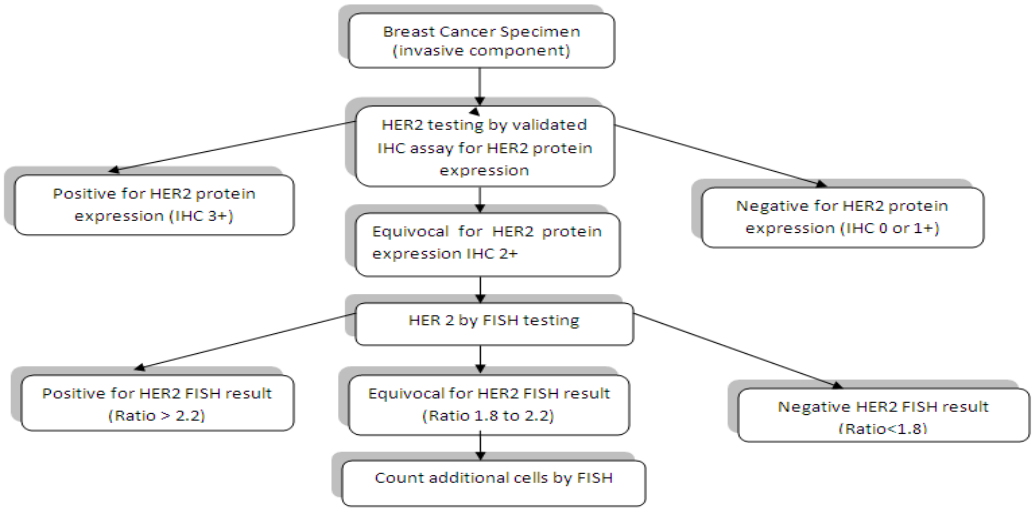
Rina Siddiqui, M.D., FACP, FACMG, Ramapriya Vidhun, MD, FACP and Julia DeLayo, B.S., CLSp(CG)

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. The Danbury Hospital Laboratory (DHL) utilizes both testing methods and adheres to the following testing algorithm, set according to the standards of CAP (College of American Pathologists):



The DHL lab utilizes the Dako HERCEPtest IHC kit, with reflex FISH testing, for all 2+ cases, to the PathVysion Her-2 DNA Probe Kit (Abbott Molecular, Inc). Please note: the FISH test is FDA approved only for stage II, node-positive invasive carcinoma.

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:

- The physician places an order for HER2 testing by FISH. **Note:** The assay is run each Friday. The deadline for orders to be received by the Cytogenetics Lab is Thursday at 2:00 pm. Orders may be faxed to 739-6471 or phoned in to 739-7688. Verbal orders must be followed up with a written request. Any orders received after 2:00 pm Thursday will be run the following week;
- An appropriate block is chosen by a pathologist (whenever possible, an excisional biopsy specimen block will be chosen);
- Sections are cut and aged overnight;
- Slides are then assayed in the Cytogenetics lab, beginning Friday a.m. (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:
The TAT for Her-2 testing by FISH is 10 calendar days. Reports are available M-F, 8:00 am – 4:30 pm.

Reported Results:
HER2 FISH results are reported as AMPLIFIED (ratio >2.2), NEGATIVE (ratio <1.8), or EQUIVOCAL (ratio 1.8-2.2). EQUIVOCAL results should be interpreted with caution as, at this time, no high-level evidence or agreement is available on how results in the equivocal range should be interpreted or confirmed.

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.
- Following are the CAP/ASCO exclusion criteria to perform or interpret a Her2 FISH:
    - 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 nonuniform (< 75% identifiable)
    - Background obscures signal (> 10% of signals over cytoplasm)
    - Nonoptimal enzymatic digestion (poor nuclear resolution, persistent autofluorescence)

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

Chromogenic in Situ Hybridization (CISH) testing under consideration at DHL
Chromogenic in situ hybridization (CISH) which enables detection of the gene copies through an immunoperoxidase reaction offers some advantages over FISH in terms of equipment, ability to view the CISH signal and tissue morphology simultaneously, ease of implementation and preservation of results. This test is being considered to replace the current FISH assay.

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.

G. H. Raab, B. Hoegel, J. Biebl , et al. CISH for HER2 assessment is highly concordant with FISH in core cut biopsies of primary T2 breast cancers. Journal of Clinical Oncology, 2004 22, No 14S, 2004:569

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