

## **Receptor Testing in Breast Cancer as Performed in the Danbury Hospital Laboratory**

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### ***Background:***

The therapeutic markers that are currently recognized in breast cancer are estrogen receptor (ER), progesterone receptor (PgR) and HER2. These markers are also considered predictive markers or markers predictive of response to specific types of anti-estrogen therapy. ER/PgR positive breast cancers have better survival and longer time to recurrence than ER/PgR negative breast cancers. Breast cancers with amplification of the HER-2/neu gene, 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. Danbury Hospital laboratory tests all invasive breast cancers for the three markers, while ductal carcinoma in-situ (DCIS) is tested for ER and PgR alone. These tests are currently being performed on core needle biopsy specimens.

### ***ER/ PgR Testing:***

The ER and PgR are tested using the immunohistochemistry method using DAKO antibodies, clone 1D5 for ER and clone pgr636 for PgR. The material used for testing is formalin-fixed paraffin-embedded tissue. There are several preanalytical factors that affect testing, particularly ER and HER2. The most essential one is the formalin fixation time. The ASCO-CAP recommendations are to use neutral buffered 10% formalin to fix the tissue for at least 6-8 hours before processing and no longer than 48 hours. The other essential factor is the cold ischemic time (time between removal of tissue from patient and fixation). The ER and PgR IHC testing guidelines suggest that the cold ischemic time be kept to one hour or less. **Hence it is imperative that the time of removal of the tissue from the patient and the time the tissue went into fixative be documented on the accession slip that must be transferred to the final report.**

All breast cores that arrive in the laboratory before 3 pm are processed the same day and the slides are ready for review by 9 am next day. Since all breast core needle biopsies are treated as rush specimens, the RUSH pathologist reviews the slides the next morning, calls the clinician with positive results, and the receptors are ordered for invasive and in-situ carcinomas before noon that same day. Her2 FISH is also ordered simultaneously for all invasive carcinomas. The ER/PgR results are available the next day with an average TAT of 1-3 days. Specimens that are received late evening Thursday and Friday are treated with caution in order to accommodate the fixation time. The laboratory tissue processor is set in such a way so as not to exceed a fixation time of 48 hours altogether. It is important to follow the protocols to assist the laboratory in processing these specimens according to CAP and ASCO guidelines.

### ***HER2 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 Department of Pathology and Laboratory Medicine (DHL) utilizes only the FISH method for assessing the Her2 status.

DHL utilizes the PathVysion Her-2 DNA Probe Kit (Abbott Molecular, Inc.) for 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:

Note: The assay is run each Tuesday and Friday. Any orders received after 11:00 am Monday will be run on Friday; orders placed after 11:00 am Thursday will be run the following Tuesday:

- An appropriate block is chosen by a pathologist;
- 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). The EQUIVOCAL assays are evaluated by three technologists/director; non-equivocal cases are evaluated by two technologists. 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. As a quality assurance measure, HER2 FISH on cases with EQUIVOCAL results are repeated.

### ***Limitations to the Procedure:***

- Scoring difficulties found with FISH testing may be associated with the specific set of cells chosen to be included 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 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 fixation time is 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. These cases are being sent to an outside laboratory that has validated the test for above mentioned organs.

**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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