

HER2 Gene Amplification in Breast Cancer by Monoplex PCR

FOR RESOLVING DISCREPANCIES BETWEEN IHC AND FISH IN PREDICTING BREAST TUMORS THAT WILL RESPOND TO TRASTUZUMAB (HERCEPTIN[®]) THERAPY

Test Highlights

PCR analysis provides a sensitive and specific detection of *HER2* gene amplification, which is used to determine response to Trastuzumab (Herceptin[®]) therapy.

Disease Overview

- Breast cancer is the most common malignancy in women. Approximately 18–20 percent of breast cancers show amplification in the human epidermal growth factor receptor-2 gene (*HER2*). Amplification of this gene results in the increased membrane expression of the *HER2* protein. This increased amount leads to increased dimerization, which activates the *HER2* tyrosine kinase. This activation signal is transmitted to the cell nucleus. The cell responds by mitosis and cell replication.
- Antibodies directed against the extracellular portion of *HER2* (Trastuzumab) are able to inhibit *HER2*-overexpressing breast cancers. Trastuzumab has been shown to prolong the overall survival rate of patients with metastatic breast cancer whose tumors overexpress *HER2*. Due to high drug costs and cardiac toxicity, the clinical use of trastuzumab requires the identification of susceptible patient populations whose tumors amplify and overexpress *HER2*.
- Assessment of *HER2* status by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) is the standard of practice for the evaluation of newly diagnosed carcinomas of the breast. Immunohistochemistry measures the expression of the *HER2* protein on the surface of the tumor cell. FISH measures the amplification of the *HER2* gene. Concordance between the two methods has varied between studies with only 24 percent of 2+ IHC tumors showing gene amplification and some 3+ IHC tumors showing no amplification by FISH. *HER2* monoplex PCR is a test method to measure *HER2* gene copy number and can help resolve discrepancies between IHC and FISH.

Epidemiology

One million new breast cancer cases are diagnosed in the world each year. Almost one-third of all cancers diagnosed in women are breast cancers.

Pathophysiology

The exact cause of breast cancer is not known. It is known that most neoplasms result from acquired damage to DNA in the gene(s) of a cell, resulting in malignancy. *HER2* gene amplification can increase the rate of cell division and growth.

Indications for Ordering

- QA technique to confirm or validate *HER2* FISH results.
- To resolve discrepancies between FISH and IHC.

Contraindications

This test is not FDA-approved for clinical use. This test is not recommended for detection of minimal residual disease.

Additional Ordering Notes

The biopsy site, fixative used, and fixation time should be provided. The submitted sample should contain sufficient viable tumor.

Interpretation

Presence of *HER2* gene amplification predicts a favorable response to trastuzumab.

Limitations

Tissues fixed in alcohol-based or non-formalin fixatives have not been tested using this method.

Methodology

- Newer molecular methods, such as monoplex polymerase chain reaction, are being developed to quantitate *HER2* levels in breast cancer. These newer methods do not have the observer subjectively inherent in both IHC and FISH. They also yield quantitative estimates of *HER2* amplification levels.
- In monoplex polymerase chain reaction, *HER2* is quantified relative to the control gene, *IF2* (eukaryotic translation initiation factor). Since the individual *HER2* and *IF2* PCRs are performed in separate reactions with separate standard curves, the assay is referred to as a monoplex PCR. The control gene, *IF2*, is used since it is located near the centromere of chromosome 2, which is an area that is not deleted or amplified in breast cancer.
- DNA from a paraffin-embedded, formalin-fixed slide is isolated by standard proteinase K digestion. The sample is subjected to quantitative PCR with specific *HER2* primers and with specific *IF2* primers. The amount of *HER2* and *IF2* DNA in the sample is determined by comparing results with their respective standard curves. The *HER2* amplification in the sample is the ratio of *HER2* DNA nanograms to *IF2* DNA nanograms. Normal tissues and unamplified tumors show a *HER2/IF2* ratio of 1.1 ± 0.35. Samples of breast cancer showing a *HER2/IF2* ratio above 2.2 (three standard deviations above the mean of unamplified tissue) are *HER2* amplified.

References

1. Willmore C, Holden JA, Layfield LJ. Correlation of *HER2* gene amplification with immunohistochemistry in breast cancer as determined by a novel monoplex polymerase chain reaction assay. *Appl Immunohistochem Mol Morphol* 2005; 13(4):333–41.
2. Slamon DJ, et al. Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. *Science* 1987; 235:177–82.
3. Yarden Y. Biology of *HER2* and its importance in breast cancer. *Oncology* 2001; 61(Suppl 2):1–13.
4. Wolff AC, et al. American Society of Clinical Oncology/College of American Pathologist guideline recommendations for human epidermal growth factor receptor-2 testing in breast cancer. *J Clin Oncol* 2007; 25(1):118–45.

Test Information

0049390

***HER-2* Gene Amplification by Monoplex PCR, Paraffin**

For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.