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FISH Analysis – *HER2*

FISH Analysis for HER2 amplification for breast and gastric cancers

Clinical Background

- Breast cancer is the second most common cancer worldwide after lung cancerand one of the leading causes of cancer death in women in the US.
- Gastric cancer is the fifth most common cancer worldwide and 15th in the US with ~ 25,000 new cases annually.
- The human epidermal growth factor receptor 2 oncogene*HER2*(also known as *ERBB2*), located on chromosome 17q, is a tyrosine kinase that belongs to a family of transmembrane receptor proteins which include *EGFR/HER1, HER2, HER3, HER4.*
- The HER family of proteins are involved in regulation of cell growth, survival and differentiation of cells. Amplification and/or overexpression of *HER2* is reported in approximately 25-30 % of breast cancer patients and 20 – 33% of gastric cancers.
- Amplification of *HER2* is associated with aggressive disease andshorter disease-free survival in both breast and gastric cancer.
- Patients that demonstrate amplification of *HER2* may be candidates for treatment with chemotherapeutic agents that target the *HER2* protein.

Epidemiology

- *HER2* amplification is found in about 15 – 25% of breast cancers.
- *HER2* amplification is present in about 7 – 34% of gastric cancers.

Genetics

- Amplification of *HER2* is generally a new mutation.

Indications for Ordering

- Detect amplification of the *HER2* gene associated with breast and gastric cancer prognosis and treatment decisions.
- Confirm equivocal HercepTest (2+) or immunohistochemistry (IHC) result.

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Interpretation

- Negative for HER 2 Amplification –
  Dual-probe ratio (HER2/CEP17) <2.0 and average HER2 copy number <4.0 signals/cell
- Equivocal for HER2 Amplification –
  Dual-probe ratio (HER2/CEP17) <2.0 with an average HER2 copy number of ≥4.0 and ≤6.0 signals/cell
- Positive for HER2 Amplification –
  Dual-probe ratio ≥ 2.0 with an average HER2 copy number ≥4.0 signals/cell
  Dual-probe ratio ≥ 2.0 with an average HER2 copy number <4.0 signals/cell
  Dual-probe ratio <2.0 with an average HER2 copy number ≥ 6.0 signals/cell
- Indeterminate HER2 Test –
  No results obtained due to specimen quality, technical issues, etc.

Methodology

- Fluorescence *in situ* hybridization (FISH) is a molecular cytogenetic method that detects fluorescently-labeled DNA/RNA or oligonucleotide probes hybridized to metaphase or interphase cells. Fluorescent signals are detected with a fluorescence microscope.
- The PathVysion Kit® (Abbott/Vysis) is used for identifying and quantifying chromosome 17 (17p11.1-q11.1) and the *HER2* gene (17q11.2-q12) in interphase nuclei.
- The *HER2* probe is a 190kb DNA probe directly labeled with Spectrum Orange and the CEP 17 probe is a 5.4kb, directly labeled DNA probe, specific to alpha-satellite sequences on the centromere of chromosome 17.
- In general, sixty interphase cells are analyzed by two readers (30 cells/reader). In equivocal cases, a third reader counts an additional 30 cells.
- The high sensitivity and specificity, rapid turnaround time, capacity to analyze large numbers of cells, and ability to obtain adequate data from samples with a low mitotic index or terminally differentiated cells and cells in interphase are the main advantages of FISH. This method is most useful when the analysis is targeted toward those abnormalities that are known to be associated with a particular disease.
- FISH is suitable for use in both archived and fresh specimens and is considered the gold standard for *HER2* testing in breast cancer
- **Limitations:**Specimens fixed/processed in alternative fixatives (other than 10% formalin) are not optimal for testing by this method. De-calcified specimens may yield fail or yield false-negative results. Repeat testing may be necessary for discordant results.

References

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