HER2 (Human Epidermal Growth Factor Receptor 2) is overexpressed in 15-25% of human breast cancer, and is usually associated with concomitant HER2 gene amplification. HER2 overexpression/amplification is associated with cancer aggressiveness. HER2 is an independent predictor of reduced overall survival and disease-free survival in breast cancer patients, as well as to predict the response to trastuzumab, a humanized monoclonal antibody, which can be directed against the extracellular domain of the HER2 protein. HER2 assays are now becoming part of breast cancer diagnostics as well as hormone receptors. Therefore, there is an absolute need for accurate determination and reliable HER2 testing for the assessment of HER2 expression to identify patients that would benefit from trastuzumab therapy.

There are several studies of HER2 for detection of HER2 gene amplification and protein overexpression. Immunohistochemistry (IHC) and Western blot are used to detect protein overexpression. Southern blot, PCR (Polymerase Chain Reaction), FISH (fluorescence in situ hybridization) and CISH (chromogenic in situ hybridization) are used for gene amplification and Northern blot for mRNA. Some methods are not well suited for routine diagnostics and have been replaced by IHC/FISH/CISH. Currently the most widely used HER2 tests in the clinical setting are IHC, which detects HER2 protein overexpression on a cell membrane, FISH and CISH assays for HER2 gene amplification. All of these have been approved by the United States Food and Drug Administration (FDA). However, each of them has its advantages and pitfalls.

The vast majority of HER2 studies are done by using IHC which is available in most laboratories and is relatively quick, cheap and easy to perform. IHC-stained slides can be stored for years and can be reassessed as well as facilitating the assessment of cell morphology. However, IHC results are done on FFPE (formalin-fixed, paraffin-embedded) tissue which can cause some discrepancies or discordance in reports. Problems can come from technical artifacts, sensitivity differences between different antibodies, tissue pretreatments, differences in methodologies and interobserver variability. Therefore, it is necessary to strictly standardize and validate procedures.

FISH can be quantitative for the number of gene copies in the nucleus of cancer cells, and can be verified for its accuracy both in freshly frozen and FFPE tissue. FISH is reproducible, highly sensitive and specific compared with IHC. FISH is less affected by pre-analytical factors and handling. However, FISH need to use a fluorescent microscope which is not available in most routine diagnostic laboratories. This microscope is expensive and requires training and experience to use. Its signal can fade over time at room temperature as well as the morphology of invasive cancer is not easy to detect. These reasons make FISH not very practical for routine histopathological laboratories.

FISH is regarded as the gold standard method for detecting HER2 amplification, but CISH has recently been gaining in popularity. There are many advantages of CISH over FISH. CISH detects HER2 gene amplification on a cell-to-cell basis and uses chromogenic detection with an enzyme-labelled probe instead of a fluorescent dye. CISH methodology is more accessible and less costly than FISH. CISH uses a standard light microscope to identify HER2 positive cells.

The interpretation of CISH signals is easy and quick (Fig 1-5). The signal is permanent and does not...
diminish over time. CISH slides can be stored for future reference without degradation at room temperature. It is not necessary to photograph the results for documentation. The morphological details of hematoxylin-stained CISH slides can be correlated and verified with histopathological findings. The cost per case is still lower for CISH. The concordance rate between CISH and FISH ranges from 95%-100%.10

At Siriraj Hospital, we evaluated a new modification of in situ hybridization, CISH, which can detect HER2 gene copies with a peroxidase reaction, using FFPE tumor tissue sections. Gene copies can be visualized by CISH, using a conventional bright-field microscope for evaluation. This can be a useful alternative for determination of HER2 amplification, especially for confirming the IHC staining results.

Gene copies visualized by CISH can be easily distinguished with a x40 objective in hematoxylin-stained tissue sections. HER2 amplification typically appeared as dots/signals or small to large peroxidase-positive intranuclear gene copy clusters. The CISH signals appear as brown dots, with either single dots (each representing a single HER2 gene copy), small clusters or large clusters. In normal cells, two dots per nucleus will be recognized. A tumor with no amplification showed typically 1-2 dots/signal per nucleus (diploid) or 3-5 dots/signal in polysomy cases (Fig 1). Low-level amplification was defined as 6-10 signals per nucleus in more than 50% of cancer cells or a small gene copy cluster was found (Fig 2). High-level amplification of HER2 was defined as more than 10 separate gene copies or a large gene copy cluster was seen in more than 50% of cancer cells.11 (Fig 3-4)

In conclusion, most pathologists are not familiar with the fluorescent microscope, but are experienced in evaluating CISH-like peroxidase-based immunostainings (Fig 5). The time and effort needed for learning to evaluate CISH is much shorter than that of FISH. Therefore, CISH can be an alternative to FISH as a secondary test to clarify 2+ results or reconfirm 3+ results of IHC staining. Because of the feasibility and relative low cost, the combined use of IHC and CISH can be the simple and convenient method for evaluating gene alterations and their protein consequences in the primary tumor screening for HER2 oncogene status.

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REFERENCES


