Bright-Field Microscopy for HER2 Gene Assessment

Not Just DISH-ful Thinking?

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The human epidermal growth factor receptor gene ERBB2 (HER2) is located on chromosome 17 and amplified in approximately 20% of invasive breast carcinomas.1,2 Due to the availability of effective anti-HER2 therapies, the prognostic significance of HER2 gene amplification, and the predictive value of HER2 status with regard to response to chemotherapy, laboratory testing for protein overexpression and/or amplification is critical for clinical decision making in individual patients. Testing of HER2 on all newly diagnosed invasive breast carcinoma is standard of care. Although there is no gold standard for assessing HER2 status in breast cancer, immunohistochemistry (IHC) for protein expression and fluorescence in situ hybridization (FISH) for HER2 gene copy number are the preferred assays.3 Although no test is perfect, clinical utilization depends on a number of factors, including test accuracy but also consistency of results, as well as ease of performance and interpretation. Due to early standardization, consistency in reporting, and only a small number of equivocal results, the FISH assay became the preferred method for assessing HER2 gene status.5,5 FISH is also used as the reflex test in IHC equivocal (2+ score) cases. FISH on IHC equivocal cases can identify a small percentage of amplified cases but also aneuploidy and other difficult to assess abnormalities of chromosome 17. Despite its widespread use, the FISH assay does have some drawbacks. FISH is considered a morphology-based assay, but the fluorescent nature of the assay results in less than optimal histologic details (compared with bright-field microscopy), so the area of invasive tumor generally needs to be marked on a parallel H&E-stained section. The procedure itself takes 3 days to complete, and the signals fade over time. To overcome the delay in reporting FISH results and to have better histologic details present on tissue section, researchers developed bright-field in situ hybridization assays. Chromogenic in situ hybridization or CISH uses diaminobenzidine as the chromogen and therefore results in brown signals. Although some studies have shown excellent correlation between CISH and FISH,6,8 the HER2 signals on CISH slides are sometimes not very discrete and difficult to count due to background staining. Moreover, with the first-generation CISH assays, the centromeric probe for chromosome 17 (CEP17) and the HER2 probe had to be hybridized and visualized on different tissue sections, complicating assessment for aneuploidy and determination of the HER2/CEP17 ratio. The second-generation in situ hybridization assays use an enzyme-linked probe to deposit silver ions from the solution to the target site, which provides a dense, punctate, high-resolution black stain that is readily distinguished from other commonly used stains. This method is called silver in situ hybridization (SISH). This SISH methodology is combined with another probe for chromosome 17 in a test recently approved by the US Food and Drug Administration (FDA) called the INFORM dual in situ hybridization (DISH) assay from Ventana Medical Systems (Tucson, AZ). The availability of DISH, in which the in situ hybridization signals for both chromosome 17 and HER2 are visualized on a single slide, has made this technique a more practical alternative to FISH and offers several advantages. These include the ability to use a standard bright-field microscope, preservation of signals for archival review, and, perhaps most important, more reliable identification of invasive tumor cells for assessment. Bright-field techniques may also be superior in assessing heterogeneity (especially when a clustered area of amplification is present within a tumor), which is an increasingly important issue in HER2 testing. The test performs...
extremely well in unequivocally positive and unequivocally negative cases (cases at the extremes).\(^9\)\(^{-11}\) However, the data on other types of cases are quite limited.

In this issue of the journal, Mansfield et al\(^{12}\) studied invasive breast cancers exhibiting common and difficult to assess centromere 17 or HER2 anomalies. The study compared HER2 amplification status of 244 cases of invasive breast carcinoma by FISH using the PathVysion HER2 DNA Probe (Abbott Molecular, Abbott Park, IL) and by DISH using the INFORM HER2 Dual ISH DNA Probe Assay using the BenchMark XT Staining Platform (Ventana Medical Systems). Using the American Society of Clinical Oncology/College of American Pathologist criteria\(^3\) for determining HER2 gene status, there was 83% concordance between the testing modalities. Of the 25 cases characterized as amplified by FISH, 15 (60%) were also amplified by DISH, but 7 (28%) and 3 (12%) cases were classified as not amplified and equivocal, respectively. In contrast, of the 17 tumors characterized as amplified by DISH, 15 (88%) were also amplified by FISH, and 2 were classified as equivocal (12%). Overall, their results suggest a high false-negative rate for DISH when compared with FISH in a population of invasive breast carcinoma enriched for complex or problematic cases. Their results also suggest a bias toward fewer HER2 signals by DISH, although this appeared to be slight (0.12; 95% confidence interval, \(-1.23 \text{ to } 1.46\)). The tendency of signals to cluster in cases with high-level amplification may help to explain the authors’ observation that the agreement between FISH and DISH appeared to be worse at higher ratios. The authors’ findings are in stark contrast to some previously published reports.\(^9\)\(^{-11}\) However, the prior reports did not include the complex cases studied by Mansfield et al.\(^{12}\)

In our own personal experience, we agree that signals within the cells are more obvious on FISH compared to DISH, as cells are examined at \(\times 100\) under oil immersion lens with FISH. Furthermore, the ability to use filters for different fluorescent signals in FISH analysis can provide a more accurate signal count when signals are overlapping. But the average number of signals per cell observed on a DISH slide is generally similar to what is observed by FISH when the DISH slide is examined via a \(\times 60\) objective (high dry). Adjusting the focus can further make the hidden signals visible. It is critical that laboratories that have adopted DISH should count the DISH signals using a \(\times 60\) objective (not \(\times 40\)), especially for cases that have an IHC 2+ score. Overlapping signals can still create problems, but most cases are resolved using a \(\times 60\) objective and adjusting the focus. Although a dual-color probe provides an assessment about structural abnormalities of chromosome 17, the number of HER2 gene copies often drives the treatment choices in complex cases. Some clinical trials (such as the N9831 adjuvant trastuzumab trial) suggest that trastuzumab benefit is independent of HER2/centromere 17 ratio and chromosome 17 copy number.\(^{13,14}\) Therefore, if the black HER2 gene signals are completely hidden by the red chromosome 17 signals in a DISH slide, then one has no choice but to perform FISH to resolve the issue. Apart from overlapping signals, another factor that can result in lower sensitivity of DISH compared with FISH is counts performed by technologists on the DISH slide. The cyogenetic technologists are well trained and quite familiar with fluorescence microscopy to perform very accurate counts on FISH slides, but they have minimal or no experience with tissue histology on bright-field microscopy. At the current time, only pathologists should perform counts on DISH slides. It may be worthwhile to implement a certification program for technologists for counting signals on DISH slides. Another alternative is to use an automated image analysis system that can triage the DISH slides, but the universal availability of this technology may take slightly longer than expected.

The data presented by Mansfield et al\(^{12}\) are critical, as many laboratories have started performing DISH assays. However, it may not be enough to discount the DISH assay for HER2 assessment, given the clear advantages of bright-field microscopy in assessment of heterogeneity, preservation of morphology, and the permanent nature of the signals. For the majority of the cases, excellent concordance has been reported between DISH and FISH on unequivocally high-level amplified and normal cases. For cases with an IHC score of 2+, each laboratory should perform an internal quality assurance before adopting DISH for these cases. In addition, more studies are needed on difficult to score cases to evaluate the performance of DISH assay in routine clinical practice.

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References


