The new FDA-approved INFORM HER2 Dual ISH DNA Probe Cocktail assay is concordant to FISH and reproducible in determining HER2 gene status in invasive breast carcinoma

Isabell R Loftin, Abigail S. McElhinny, Rachel Miller, Carole Garcia, Isaac Bai, Jim Ranger-Moore, Jim Ostrem, Mary Padilla, Tom Grogan

Ventana Medical Systems, Inc. Tucson, AZ, USA

The HER2 gene, located on chromosome 17 (Chr17), is amplified in 15-25% of patients with invasive breast carcinoma. Amplification and/or HER2 overexpression is associated with poor clinical outcome for these patients; however, prognosis is improved if HER2 status indicates eligibility of patients for trastuzumab (Herceptin) therapy. Thus, accurate diagnosis of HER2 status through a companion diagnostic is essential.

Here we validated the INFORM HER2 Dual ISH DNA Probe Cocktail (Dual ISH) assay as an alternative to FISH, the current gold standard for HER2 testing. The Dual ISH assay is fully automated, achieving shorter time to result, and is scored using light microscopy.

Methodology:
A multi-site method comparison and inter-laboratory reproducibility study were performed. Five sites were used to compare Dual ISH results with Ysiss PathVision HER2 DNA Probe Kit (FISH) (Abbott).510 invasive breast carcinoma specimens were stained at three clinical sites, FISH staining was performed at a 4th site (central laboratory). IHC status was determined at a 5th site (2nd central laboratory). In addition, six cases were evaluated for inter-site (3 sites), inter-reader (6 readers), inter-run (15 runs) and intra-run (duplicate slides) reproducibility. All assay steps were fully automated on a VENTANA Benchmark XT automated stainer, using a HER2 repeat-reduced, digoxigenin-labeled probe targeting the HER2 gene, detected with silver metallographic detection, and a digoxigenin-labeled Chr 17 probe, detected by an alkaline phosphatase-driven red chromogenic detection. HER2 and CHR17 signals were enumerated using conventional light microscopy allowing interpretation within the morphological context of the specimen. HER2 status was determined as the ratio of HER2/Chr17, where a ratio <2 is non-amplified and a ratio ≥2 is amplified. In addition, an internal pilot study was performed to evaluate inter-observer reproducibility, agreement and the disamplified status success rate of HER2 Dual ISH and FISH.

Results and Conclusion:
The positive and negative agreement rates with FISH results (95% CI) were 96% (92.6-97.9) and 92.3% (88.6-94.8), respectively. The HER2 Dual ISH assay was also highly reproducible in determining HER2/Chr17 ratio across sites, days, readers and runs.

The fully automated, FDA-approved INFORM HER2 Dual ISH DNA Probe Cocktail assay is reproducible and concordant with the manual FISH assay in determining HER2 gene status in invasive breast carcinoma.

Data – Interlaboratory Reproducibility:
Nine cases total were stained, in duplicate, on five runs per site over 20 days for a total of 270 slides. Two readers per site evaluated the staining results. Six cases were used for analysis (180 slides), three were included as wildcards. (HER2/Chr17 ratio <2.0 is non-amplified; HER2/Chr17 ratio ≥2.0 is amplified)

The results indicate that cases 005 and 002 were classified as non-amplified 100% of the time. Cases 009, 004, and 001 (clustered for HER2) were classified as amplified in all but one read. Case 006, whose HER2/Chr17 ratio falls essentially at the amplification cut-off (mean of 2.088) should be noted. Case 006 was evaluated as non-amplified 48.3% of the time and amplified 51.7% of the time. This result is expected, since cases at a decision threshold will be classified randomly to either side of the threshold approximately 50% of the time. These cases represent a rare portion of breast carcinoma cases (1.6%). All other cases, non-amplified or amplified, were consistent in their clinical HER2 gene status.

Conclusion: The fully automated, FDA-approved INFORM HER2 Dual ISH DNA Probe Cocktail assay is reproducible and concordant with the manual FISH assay in determining HER2 gene status in invasive breast carcinoma.

References:
http://www.fda.gov/ (PMA – P100027)

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