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

Background

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.

Methods

A multi-site method comparison and inter-laboratory reproducibility study were performed. Five sites were used to compare Dual ISH results with Vysis PathVysion HER-2 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 slide stainer, using a HER2 repeat-reduced, dinitrophenyl-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 first pass staining success rates 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 also was 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 – Method Comparison

HER2 gene status: comparison between the HER2 Dual ISH assay and the FISH assay in breast carcinoma specimens (HER2/Chr 17 ratio<2.0 is non-amplified; HER2/Chr 17 ratio≥2.0 is amplified)

HER2 Dual ISH result	PathVision HER-2 FISH result		
	Amplified	Non-Amplified	Total
Amplified	216	22*	238
Non-Amplified	9*	263	272
Total	225	285	510

Percent overall, positive and negative agreement rates between the HER2 Dual ISH assay and the FISH assay for HER2 gene status in 510 breast carcinoma specimens. The score 95% confidence intervals are presented for each agreement rate.

Percent Positive Agreement (score 95% CI)	**96.0 %** (92.6-97.9)
Percent Negative Agreement (score 95% CI)	**92.3 %** (88.6-94.8)

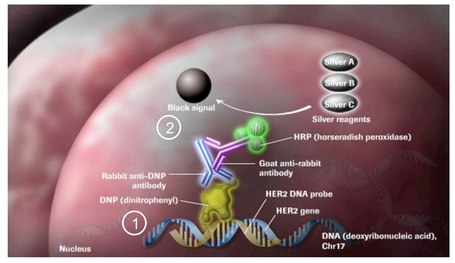
***Discrepancy Review:** 31 cases were found discrepant in this study. An independent review panel of three pathologists reviewed H&E, IHC, FISH and HER2 Dual ISH slide for each discrepant case. The review panel agreed with the results of HER2 Dual ISH on 22 cases, agreed with the results of FISH on 5 cases, could not reach a consensus on two cases, and excluded two cases for not having invasive carcinoma. When compared to HER2 IHC, 21 cases had the same final result as HER2 Dual ISH and six cases had the same result as FISH. The HER2 Dual ISH assay identified multiple cases as amplified, which were negative by FISH.

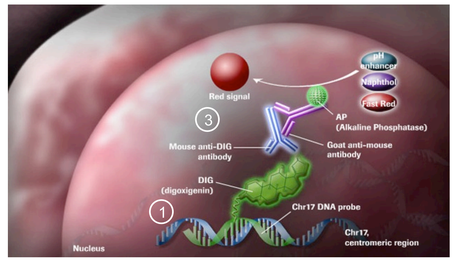
	Number of Discrepant Cases	
Total	31	
No consensus reached	2	
Excluded	2	
Total assessed by review panel	27	
	HER2 Dual ISH	**FISH**
Agreed with review panel n/N (%)	22/27 (81 %)	5/27 (19 %)
Agreed with IHC status n/N (%)	21/27 (77 %)	6/27 (23 %)

Initial and Final Staining Failure Rate: PathVysion HER-2 FISH 7.9% and 0.8%, respectively, INFORM HER2 Dual ISH 6.0% and 1.3%, respectively

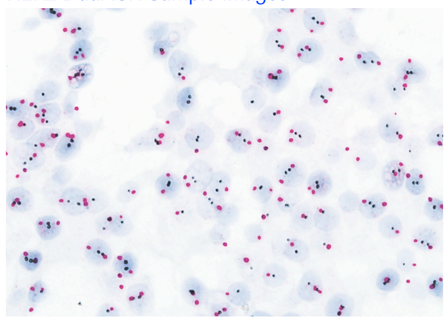
HER2 Dual ISH assay

1. HER2 and Chr 17 probes, labeled with DNP and DIG, respectively, are co-hybridized to their target sequences.
2. DNP-labeled HER2 probe is visualized through silver metallographic detection (SISH) as black signals.
3. DIG-labeled Chr 17 probe is visualized through red chromogenic detection (Red ISH) as Red signals.

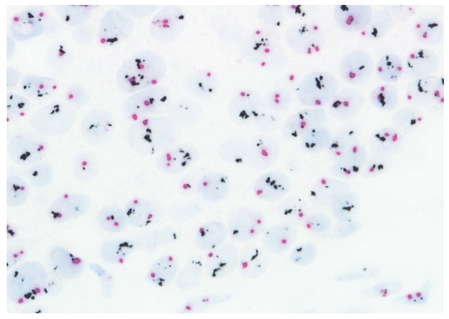




HER2 Dual ISH Sample Images



non-amplified breast carcinoma



amplified 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/Chr 17 ratio<2.0 is non-amplified; HER2/Chr 17 ratio≥2.0 is amplified)

Screening Status	HER2/Chr17 Ratio				Amplification Status	
	Case #	N	Mean	SD	Amplified n (%)	Non-Amplified n (%)
Non-Amp	005	57	1.058	0.097	0	57 (100.0)
Non-Amp	002	42	1.161	0.139	0	42 (100.0)
Low Amp	006	60	2.088	0.449	29 (48.3)	31 (51.7)
Low Amp	009	60	4.194	1.006	60 (100.0)	0
Amp	004	57	6.732	2.099	57 (100.0)	0
Amp	001	58	7.179	2.414	57 (98.3)	1 (1.7)

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 for one case. Case 006, whose HER2/Chr 17 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 as 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.

Agreement between Reader A and Reader B for HER2 Dual ISH staining results:
- Average Positive Agreement was 91.2%, 91.2% and 90.6% for Sites A, B and C respectively
- Average Negative Agreement was 86.4%, 86.4% and 85% for Sites A, B and C respectively

Data – Pilot Study

A cohort of invasive breast carcinoma cases was stained at Ventana, Medical Systems, Inc using INFORM HER2 Dual ISH and enumerated by two qualified readers. The same cohort was stained at an external laboratory using FISH and read by one qualified reader. For the HER2 Dual ISH assay, the first pass staining success rate was >93%, while the FISH staining rate was ~60%. Agreement rates for the cases which stained successfully with both assays were calculated.

	Agreement of FISH and Reader A's Dual ISH		Agreement of FISH and Reader B's Dual ISH		Agreement of Reader A and B for HER2 Dual ISH	
	n/N	%(95% Score CI)	n/N	%(95% Score CI)	n/N	%
% Positive Agreement	18/19	94.7 (75.4-99.1)	17/19	89.5 (68.6-97.1)	36/38	94.7
% Negative Agreement	40/42	95.2 (84.2-98.7)	41/42	97.6 (87.7-99.6)	82/84	97.6
%Overall Agreement	58/61	95.1 (86.5-98.3)	58/61	95.1 (86.5-98.3)		

References

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Slamon DJ, et al Science 1987;235:177-182.
http://www.fda.gov (PMA - P100027)

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.