**A concordance study between automated and manual HER2 FISH testing**

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The Leica HER2 FISH System is a fully automated, standardized system designed to provide accurate and consistent HER2 assessment. This study comprised a randomized, blinded concordance assessment between the automated Leica HER2 FISH system and two manual assays, the Abbott Molecular PathVysion DNA Probe Kit and the Kreatech Poseidon ERBB2 probe. Testing was performed on 100 breast cancer cases pre-characterized for protein and/or gene amplification status.

**INTRODUCTION**

The proper selection of the most appropriate therapy on the basis of biological markers is heavily dependent upon reliable and accurate laboratory testing and highlights the need for standardization of tissue handling, biomarker assay procedures and accurate interpretation of testing results.

Despite FISH assays being labour intensive and technically challenging they continue to remain the gold standard assay for HER2 assessment. Currently, FISH is performed manually and can be subject to operator variation and a lack of standardization. Fully automated FISH staining from section to DAPI counterstain now a reality.

The Leica HER2 FISH System is a fully automated, quantitative FISH assay for use on the Bond System to evaluate HER2 gene expression in FFPE breast cancer tissue specimens.

This study comprised a randomized, blinded concordance assessment between the automated Leica HER2 FISH System and two manual assays, the Abbott Molecular PathVysion DNA Probe Kit and the Kreatech Poseidon ERBB2 probe. Testing was performed on 100 breast cancer cases pre-characterized for protein and/or gene amplification status.

**METHOD**

100 cases of known HER2 protein and/or gene status were selected comprising:
- 25 IHC 0/1+
- 25 IHC 2+ with FISH ratio < 2.0
- 25 IHC 2+ with FISH ratio > 2.0
- 25 IHC 3+
- Of the 100 cases stained using the kreatech manual method, 6 were repeated due to weak signal intensity.
- Of the 100 cases stained using the Leica manual method, 2 were repeated due to weak staining.

Mixture of resections & core biopsies

Blocks serially sectioned
- Section 1 – H&E
- Section 2 – Manual HER2 (Kreatech HER2 probe)
- Section 3 – Automated HER2 (Leica HER2 FISH system)
- Section 4 – Manual HER2 (Abbott HER2 Probe)

Invasive tumour area marked on H&Es (Figure 1)

Same area assessed for both FISH assays using ratio method. The Leica and Kreatech tests were performed and scored at the RCSI in Dublin and the Abbott test was performed and scored at Birmingham Heartlands Hospital.

**RESULTS**

Of the 100 cases stained using the kreatech manual method, 6 were repeated due to weak signal intensity. Human error during the preparation of one of the reagents accounted for the weak staining.

100/100 cases stained successfully using the automated Leica method and the manual Abbott method.

The following results were achieved:

**KEYWORDS**

Fluorescent In Situ Hybridization (FISH); Human Epidermal Growth Factor Receptor 2 (HER2); Automation; Standardization.

**SUMMARY**

The significantly reduced labour time and excellent reliability shown by the HER2 FISH automated system provides a genuine alternative to manual HER2 FISH testing.

Full automation of the FISH assay provides:
- More consistent, standardized staining with reduced process variation
- Increased throughput
- Decreased TAT
- Enhanced ease of use
- Easy to use software

**KEY FINDINGS**

- Full automated FISH staining from section bake to DAPI counterstain now a reality
- Automation saves time and reduces variability
- Overall staining quality is equivalent to manual methods
- Excellent correlation with two long-standing manual assays

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