GenASIs HiPath as a recommended, cost-effective replacement product to the ACIS III imaging system, for HER2/neu IHC scoring in breast cancer

Applied Spectral Imaging
Bringing Details to Light

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SUMMARY

This white paper summarizes the results of scoring reproducibility of HER2/neu IHC expression, by two digital imaging platforms:
(1) GenASIs HiPath (Applied Spectral Imaging - ASI);
and (2) ACIS III (Dako-Agilent)

SUMMARY OF FINDINGS:
In the lower expression of HER2/neu, HiPath scored more accurate than ACIS. Out of the 32 cases that were scored equivocal by ACIS, only 23 produced an equivocal score with HiPath and nine were negative. Subsequent FISH analysis of these nine cases yielded a negative result, which confirms HiPath analysis

Concordance rate between ACIS and HiPath for the over-expressed (positive) cases was 92% (17 out of 18 cases); one case was reported by HiPath as equivocal

Overall, the number of cases that are reflexed to FISH analysis due to equivocal IHC score, is greater with ACIS than with HiPath, by approximately 28% (nine additional cases)

BACKGROUND AND GOALS

Pathologists assess HER2/neu IHC by evaluating membrane circumference, completeness and intensity. Scoring is based on current ASCO/CAP guidelines, summarized in Table 1. With the advent of computer-aided platforms, traditional manual counting and scoring is being replaced by automated image-analysis with statistical reporting. This white paper compares two computer-aided platforms, GenASIs HiPath (ASI) and ACIS III (Dako-Agilent), with regards to the analysis results of immunohistochemistry-stained HER2/neu paraffin embedded tissue-samples of breast-cancer.

Table 1: 2013 ASCO/CAP HER2/neu Test Guideline Recommendations (as of the date of this white paper)

SCORE	CRITERIA
Positive, IHC 3+	Circumferential membrane staining that is complete and intense, observed in a homogeneous and contiguous population and within >10% of the invasive tumor cells
Equivocal, IHC 2+	Circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of the invasive tumor cells; or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumor cells
Negative, IHC 0 or 1+	IHC 1+: incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells. IHC 0: no staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells

METHODOLOGY AND WORKFLOW

LOCATION:
The Pathology Laboratory; Lake Charles, Louisiana, USA

CONTROL GROUP:
63 slides stained with Dako AutostainerLink 48, using the polyclonal rabbit anti-human c-erB-2 Oncoprotein concentrate (DAKO). The 63 slides of the control group were analyzed with the ACIS system during 2015 and 2016 and were reanalyzed with the HiPath platform during 2016

EQUIPMENT:
- GenASIs HiPath platform: author's microscope (Nikon Labophot-2 with a halogen light source), camera adapter with a 1x C-Mount & ASI provided GigE high resolution, color CCD Camera with Image Analysis software (GenASIs HiPath)
- ACIS III Automated Cellular Imaging platform

WORKFLOW, ANALYSIS AND SCORING:
- GenASIs HiPath platform: selected frames from each slide of the control group were captured on author's microscope using 20x magnification. Each frame was analyzed automatically using GenASIs HiPath.
GenASIs HiPath analyses and classifies each tumor cell individually based on membrane morphology and intensity, in accordance with ASCO/CAP guidelines. Results are then presented as the percentage of cells in each class. Final score for HER2/neu is determined by the pathologist using the above ASCO/CAP guidelines. Average cell count per sample was 6,000.
- ACIS III system: slides from the control group were scanned using 10x magnification and selected tissue regions were scored. Staining intensity per slide was measured and scoring results were given automatically by the system, using the following criteria:
- Score less than 1.4   = Negative
- Score 1.4-2.2   = Equivocal
- Score larger than 2.2   = Positive

GenASIs HiPath functions as an adjunct tool to the microscope

ACIS III, stand-alone, automated Cellular Imaging System

RESULTS

ACIS scored 18 cases as over-expressed (positive) for HER2/neu, 32 as equivocal and 13 as negative. HiPath scored 17 cases as over-expressed (positive) for HER2/neu, 24 as equivocal and 22 as negative. Overall concordance between ACIS and HiPath for the 63 cases was 84% (53 out of 63 cases).

For all nine cases that were scored equivocal with ACIS and negative with HiPath, subsequent FISH analysis confirmed the HiPath score. The high concordance between HER2/neu IHC scoring by HiPath and FISH results is related with the per-cell classification performed by HiPath, which is less affected by staining artifacts such as non-specific staining.

Figure 1 is a bar-chart representation of the data for the 63 cases that were analyzed in this comparison study, using ACIS and HiPath platforms. It shows that ACIS, in general, tends to score low-staining samples as equivocal, whereas HiPath can more accurately differentiate between equivocal and negative cases. As a result, nine cases that were scored with ACIS as equivocal gave a negative result with HiPath. Figure 2 is a direct comparison between the two platforms for the 32 cases that were scored equivocal with ACIS. It shows that the nine cases that were scored as equivocal with ACIS and as negative with HiPath, were confirmed to be negative by subsequent FISH analysis.
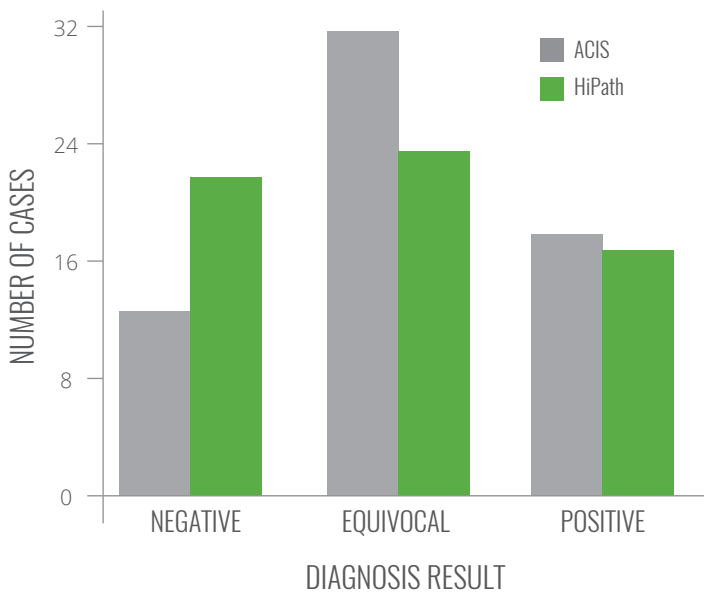

Figure 2: Comparison between ACIS and HiPath for the equivocal cases and relation to subsequent FISH analysis

Figure 1: Concordance between GenASIs HiPath (ASI) and ACIS III (DAKO) for routine diagnostic of HER2/neu in breast cancer
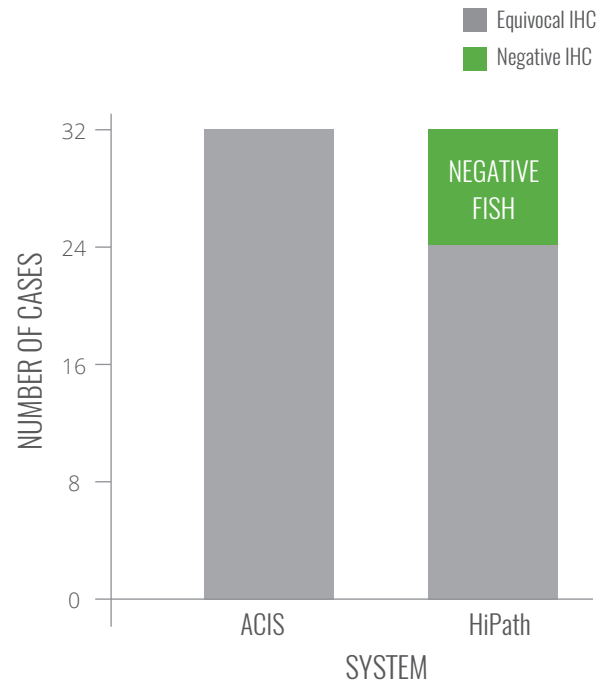
CONCLUSIONS

GenASIs HiPath, which is based on analysis of individual cells, performs statistical analysis for each frame, regions of interest within frames, as well as composite scoring for all frames. This allows rapid and accurate scoring, while preserving quality assurance and improved visibility of the image-analysis software.

We have found that GenASIs HiPath achieved high concordance with ACIS III for scoring of HER2/neu IHC biopsies.

Moreover, GenASIs HiPath was more accurate in differentiating between equivocal and negative HER2/neu IHC cases. This result was supported and confirmed by FISH analysis. This will result in a reduced volume of cases that require reflex to FISH, based on HER2/neu IHC, when using HiPath. In the current study, the overall number of cases that were reflexed to FISH analysis was reduced by 28% when using HiPath.