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A Multi-Method Analysis of c-erbB2/HER2 in FFPE Tissue:

Comparison of a Novel Rapid *in situ* Hybridization Procedure (RISH™), HER2 CISH and IHC.

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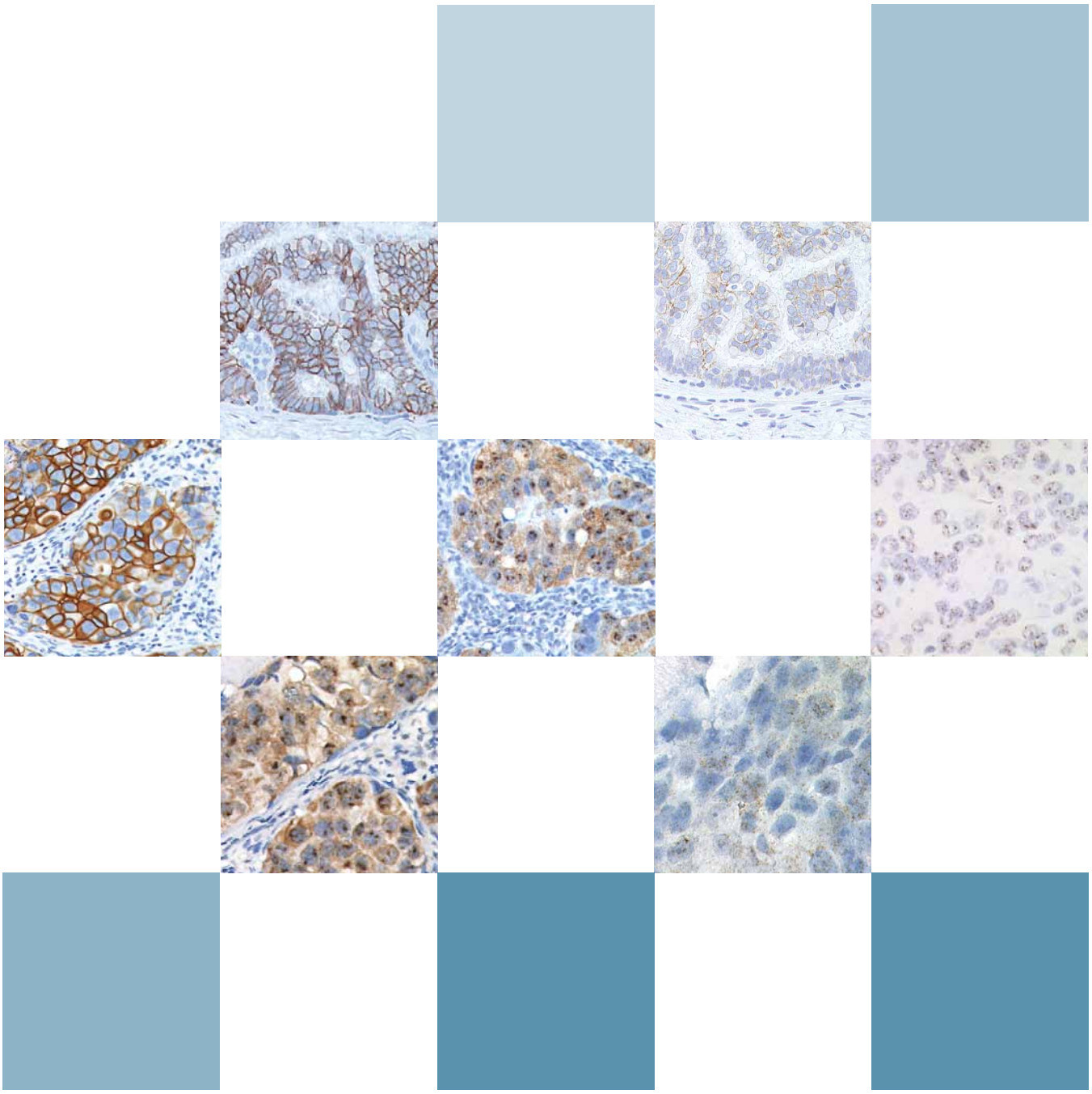
Biocare Medical LLC, Concord, CA; Universidade De Santiago De Compostela, Spain; POLUSA Medical Center, Spain; Clarient, Inc., Aliso Viejo USA.



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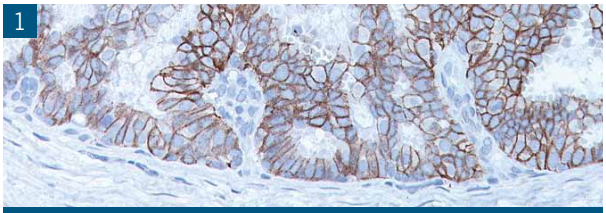

Rabbit Monoclonal c-erbB2 2+/3+ (IHC)

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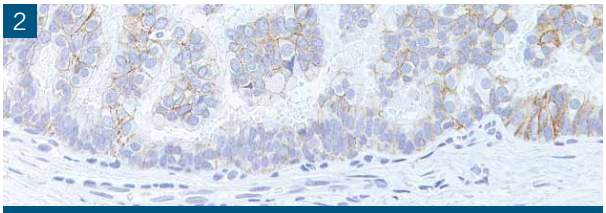

HerceptTest 1+/2+ (IHC)

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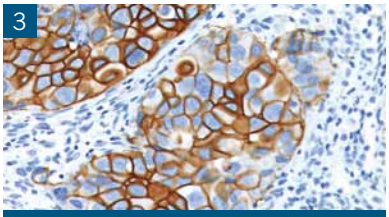

Rabbit Monoclonal c-erbB2 2+/3+ (IHC)

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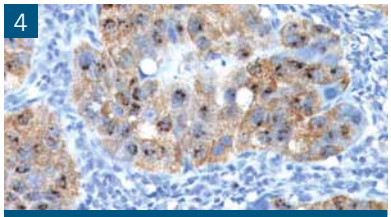

c-erbB2 Probe - High Expression (RISH™)

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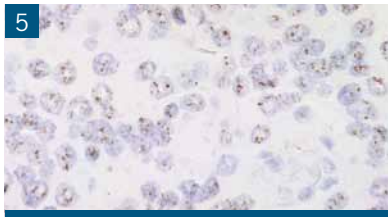

HER2 CISH Amplification

Background

c-erbB2 is denominated as such as it corresponds to the human homologue of a viral gene that causes leukemia erythroblastosis in birds. This gene was isolated in a neuroblastoma/glioblastoma rat cell colony and for this reason it is also known as HER2/*neu*. This gene codes for a tyrosine kinase receptor located in the cytoplasmic membrane and for whom the ligand is still unknown.

c-erbB2 protein belongs to a family of receptors whose principle member is EGFR (epidermal growth factor receptor) also known as c-erbB1. This family is made up of four members (c-erbB1, c-erbB2, c-erbB3 and c-erbB4) that share great internal homology. Due to this homology, antibodies targeted against this family of proteins can cross react with other members.

Over-expression of HER2/*neu* gene or protein is found in 20% to 30% of breast carcinomas and is predictive of response to treatment by Herceptin® (trastuzumab). Methods such as FISH or CISH determine gene amplification status, however, amplified genes may or may not be all transcribed at equivalent levels. Recently, a new c-erbB2 probe for a faster *in situ* hybridization (RISH™) has been developed. This probe consists of 2 fragments of single-stranded DNA with lengths of 672 and 1143 nucleotides that are targeted against mRNA sequences transcribed from the HER2/*neu* gene. The extended probe sequence confers increased specificity, sensitivity and speed of reaction with the target mRNA.

In this study, the correlation of the RISH™ method to FDA approved IHC and HER2 CISH methods and a method employing a rabbit monoclonal antibody is assessed. Equivocal IHC cases (IHC 2+) will also be validated.

Methods

Two TMA (98 cases: 2 cores/case) of FFPE cases of invasive breast carcinomas were used (cases were assessed for ER, PR and c-erbB2 IHC prior to this study). TMA tissues were deparaffinized and hydrated in the usual manner. In this study, IHC was assessed by Dako HercepTest® (test was performed by Clarient Inc.), a new rabbit monoclonal antibody [EP1045Y] (Biocare Medical), and two ISH methods: a c-erbB2 probe targeted against mRNA (Biocare Medical/HistoSonda), and a FDA approved HER2 CISH (Biocare Medical/Life Technologies). All cores were assessed for IHC 0, 1+, 2+ and 3+ staining and HER2 CISH was scored as amplified or non amplified according to the FDA approved interpretation scheme. RISH™ was scored as negative or positive and/or low and high expression.

Results

The new rabbit monoclonal c-erbB2 was observed to be superior to HercepTest® (Table 1). In one case the rabbit monoclonal c-erbB2 was graded as 2+/3+ but as 1+/2+ with HercepTest®; both were confirmed as HER2 CISH amplified. In a 98 case study, there was a 94% (92/98) concordance between HER2 CISH and IHC (rabbit monoclonal), 92% (90/98) concordance between RISH™ and both IHC methods. HER2 CISH and RISH™ showed a 98% (96/98) concordance. Morphology was superior with both IHC methods and RISH™ methods compared to morphology HER2 CISH (photo 3, 4, 5). In RISH™ cases, high and low expression (photo 6, 7) was observed. In all cases of RISH™ with low expression, HER2 CISH was amplified.

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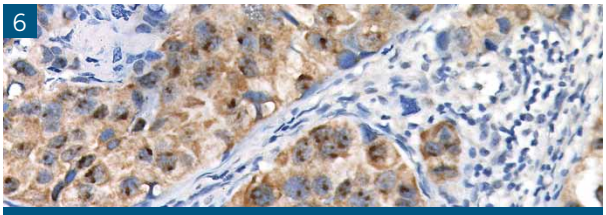

c-erbB2 High Expression (RISH™)

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c-erbB2 Low Expression (RISH™)

Table 1a

Test	Cases	0	1+	2+	3+
Rabbit Monoclonal c-erbB2	37	17	5	4	11
		0	0	1*	11
Dako HercepTest®	37	16	8	4	9
		0	0	3*	9

*Confirmed by HER2 CISH as HER2 Amplified

Table 1b

Test	Cases	0	Amp+
SPOT-Light® HER2 CISH	37	25	12

Table 2

Correlation of c-erbB2/HER2 by IHC, Gene Amplification (HER2 CISH) and mRNA (RISH™) on TMA

Score	0	1+	2+	3+
HercepTest® (%)	41.0%	20.5%	10.3%	28.2%
c-erbB2/HER2 RbMab (%)	39.8%	15.3%	11.2%	33.7%
c-erbB2/HER2 RISH Probe + (%)	1.0%	1.0%	1.0%	38.8%
HER2 CISH + (%)	0.0%	2.0%	1.0%	36.7%

Conclusion

c-erbB2 protein was assessed by IHC using HercepTest®. HER2 gene amplification was quantified using SPOT-Light® HER2 CISH. HER2 mRNA transcription was determined using a chromogenic single stranded DNA probe (RISH™). Concordance between HER2 CISH and RISH™ methods was 98%. The rabbit monoclonal antibody IHC method achieved a 94% correlation with HER2 CISH. Although only 37 cases were tested, the rabbit monoclonal c-erbB2 was shown to be superior to HerceptTest®. Other studies have shown a 95% correlation with HercepTest®. In conclusion, this study demonstrates the high concordance between RISH™ and the common methods (IHC, FISH/HER2 CISH) for assessment of c-erbB2 or HER2/neu status in breast cancer. Based upon these results, it may be proposed that RISH™ or HER2 CISH be used in the primary screening of HER2 status.

References

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