SUMMARY OF SAFETY and EFFECTIVENESS (SSED)

I. General Information

Device Generic Name:	*In Vitro* Diagnostic Test Kit for HER2 gene amplification in formalin-fixed, paraffin-embedded (FFPE) tissue sections using Chromogenic *In Situ* Hybridization (CISH)
Device Trade Name:	SPOT-Light® HER2 CISH Kit
Applicant's Name and Address:	Invitrogen Corporation 5791 Van Allen Way Carlsbad, CA 92008
Date of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P050040
Date of FDA Notice of Approval:	July 1, 2008

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II. Indications for use

For *In Vitro* Diagnostic Use

The SPOT-Light® HER2 CISH Kit is intended to quantitatively determine *HER2* gene amplification in formalin-fixed, paraffin-embedded (FFPE) breast carcinoma tissue sections using Chromogenic *In Situ* Hybridization (CISH) and brightfield microscopy. This test should be performed in a histopathology laboratory.

The SPOT-Light® HER2 CISH Kit is indicated as an aid in the assessment of patients for whom Herceptin® (trastuzumab) treatment is being considered. The assay results are intended for use as an adjunct to the clinicopathological information currently being used as part of the management of breast cancer patients. Interpretation of test results must be made within the context of the patient's clinical history by a qualified pathologist.

III. CONTRAINDICATIONS

None known

IV. WARNINGS AND PRECAUTIONS

Refer to product labeling for a list of warnings and precautions.

V. DEVICE DESCRIPTION

Invitrogen's SPOT-Light® HER2 CISH Kit is intended for the quantitative detection of HER2 gene amplification in FFPE breast cancer tissue sections by Chromogenic *In Situ* Hybridization (CISH). CISH detects hybridization of labeled nucleic acid probes *in situ* to specific sections of complementary nucleic acid in the sample using conventional peroxidase-DAB reactions, which can be viewed under the brightfield microscope. This allows pathologists to view tissue morphology and gene aberrations simultaneously. The HER2 DNA probe in the SPOT-Light® HER2 CISH Kit is generated using Subtraction Probe Technology™ (SPT™), which creates specific probes by significantly reducing the repetitive sequences (e.g., Alu and LINE elements) found in human nucleic acids. Consequently, SPT™ probes are inherently specific and do not require repetitive sequence blocking, as required for traditional cytogenetic DNA probes.

Tumors with HER2 gene amplification typically appear as large DAB intra-nuclear clusters, as multiple DAB single dots (>5 single dots), or as a mixture of clusters and multiple dots. Tumors without HER2 gene amplification typically exhibit ≤5 single dots per nucleus.

The SPOT-Light® HER2 CISH Kit is a standardized kit containing 20 tests that includes all necessary reagents and buffers in a convenient ready-to-use format. The kit also includes a FFPE control slide with both HER2 non-amplified (negative) and amplified (positive)

cancer cell lines on each slide. A 4-color HER2 CISH Test Interpretation Guide is included to assist in the interpretation of HER2 CISH results.

The SPOT-Light® HER2 Probe (Reagent C) is a double-stranded DNA probe that has been labeled with digoxigenin. It is supplied in a liquid format in hybridization buffer. It has been demonstrated to contain the HER2 gene by PCR, and to bind specifically to the HER2 gene locus on chromosome 17q11.2-21 by metaphase FISH in normal lymphocytes. Repetitive nucleic acid sequences have been quantitatively removed from the probe by SPT™.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several HER2 fluorescence *in situ* hybridization (FISH) test kits commercially available for the determination of gene amplification in breast tissue. Additional procedures for detection of gene product overexpression in human breast tissue include immunohistochemical (IHC) and polymerase chain reaction (PCR) techniques.

VII. MARKETING HISTORY

The SPOT-Light® HER2 CISH Kit was launched internationally in July 2004 and is CE marked for the EU. The product is available in the following countries:

SPOT-Light® HER2 CISH Kit Marketing History

Argentina	Egypt	Italy	South Africa
Australia	Finland	Japan	Serbia
Austria	France	Korea	Singapore
Benelux	Germany	Kuwait	Spain
Brazil	Greece/Cyprus	Lithuania	Sweden
Canada	Hong Kong	Mexico	Taiwan
China	Ireland	Norway	Thailand
Denmark	Israel	Portugal	United Kingdom

The SPOT-Light® HER2 CISH Kit has not been withdrawn from any of these markets for any reason.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

As with any *in vitro* diagnostic test, the potential risks are associated with incorrect result interpretations. A false positive test result would likely assign patients to receive a more aggressive adjuvant therapy regimen than needed, possibly exposing the patient to serious side effects and, in rare cases, death. Alternatively, a false negative test result may exclude a patient who might benefit from therapy, potentially resulting in a poor outcome.

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IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies :

Key to Abbreviation for All Tables:
NAM = Non-amplified
AM = Amplified
BC = Big Clusters
BL = Borderline
ACC = Acceptable
STD = Standard Deviation
%CV = Percent Coefficient of Variation
' = Minute
" = Second
ND = Not done
1+ through 4+ refer to staining intensities
1+: Difficult to see under 40X objective lens
2+: Difficult to see under 20X objective lens, but easy under 40X
3+: Difficult to see under 10X objective lens, but easy to see under 20X
4+: Easy to see under 10X objective lens

1. Non-Clinical Studies - Internal

a. <u>Analytical Sensitivity</u>

The analytical *sensitivity* study objective was to evaluate the hybridization efficiency and sensitivity of the SPOT-Light® HER2 CISH Kit when testing formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue sections of non-amplified and amplified HER2 status as well as the FFPE cell lines used in the control slides.

The samples under evaluation for the analytical *sensitivity* study included slides from two breast cancer tissue blocks: HER2 non-amplification (normal HER2), and HER2 amplification and four cell line blocks (two with HER2 non-amplification, and two with HER2 amplification). These cell lines are the cell lines used in the SPOT-Light® HER2 CISH Kit control slide.

The HER2 gene was detected as a single dot on the tissue section and cell block section with normal HER2 gene (Tissue 1, Cell Line 3, and Cell Line 4). The data for hybridization efficiency are shown in Table 1.

Table 1. Hybridization efficiency

Sample Type	HER2 gene status	Total sections analyzed	Total cells analyzed	Total cells with CISH signal	Hybridization Efficiency
Tissue 1	NAM	10	300	284	94.7%
Tissue 2	AM	10	300	300	100%
Cell Line 1	AM	1	100	100	100%
Cell Line 2	AM	1	100	100	100%
Cell Line 3	NAM	1	300	285	95%
Cell Line 4	NAM	1	300	289	96.3%

b. **Analytical Specificity**

The analytical *specificity* study objective was to evaluate the specificity of the HER2 probe when testing metaphase spreads from normal lymphocytes by fluorescence *in situ* hybridization (FISH), when testing polymerase chain reaction (PCR) with HER2 gene specific primer pair, and when sequencing both ends of the DNA probes.

Analytical Specificity Results by FISH

The sample under evaluation for the analytical *specificity* study by FISH consisted of eight cytogenetically prepared slides with metaphase spreads from a normal lymphocyte cell line. There were a total of 132 metaphases in the eight cytogenetically prepared slides. All 132 metaphases showed that the green signal (HER2) and the red signal (chromosome 17 centromere) are co-localized on the same chromosome, while the green signal is located on the long arm of Chromosome 17 band 11.2-21. No cross-hybridization to other chromosome loci was observed in any of 132 metaphase examples and hybridization was limited to the intended target regions of the two probes. Results demonstrated that the DNA probe was bound specifically to the HER2 gene locus on chromosome band 17q11.2-21. In addition, chromosome localization was confirmed by metaphase FISH in normal lymphocytes.

Analytical Specificity Results by PCR

The sample under evaluation for the analytical specificity study by PCR included DNA from the BAC clones, which were used to prepare the HER2 DNA probe. The HER2 SPT™ DNA template has been shown to contain the HER2 gene sequence by PCR with HER2 specific primers (Schneeberger et al., 1996. Anticancer Res, 16:849-852).

PCR Primers:

HER2-1F: *5' GAT GTA TTT GAT GGT GAC CT 3'*

HER2-2R: *5' ATC TGG CTG GTT CAC ATA TT 3'*

PCR Condition and Electrophoresis

The PCR reaction was performed in a volume of 25 µl containing HER2 DNA template, 20 pmols of each primer, 1 x KlenTag DNA polymerase, and 200 µm of each dNTPs. The PCR was performed for 30 cycles at 94°C for 5 seconds, 50°C for 30 seconds and 72°C for 45 seconds. The 1% gel electrophoresis PCR product showed correct size for HER2 specific primers.

Analytical Specificity Result by DNA Sequencing

After sequencing both ends of the HER2 BAC clones, the HER2 probe size is 180 kb.

Analytical Sensitivity and Specificity Results and Conclusions

i. All CISH slides showed strong CISH signal, and good morphology.
ii. Hybridization efficiency: 95 - 100%.
iii. A single HER2 gene copy in a FFPE control cell block or in a breast cancer tissue section can be detected; the analytical sensitivity is 1 dot.
iv. The HER2 DNA probe is located on the long arm of chromosome 17q11.2-21 and contains the HER2 gene sequence.

c. Repeatability and Reproducibility Studies on Consecutive Tissue Sections and Various Tissue Thickness

The repeatability and reproducibility studies were conducted to evaluate the SPOT-Light® HER2 CISH Kit repeatability and reproducibility when testing consecutive non-amplified, borderline, and amplified breast cancer tissues of varying thicknesses.

The samples under evaluation included slides from three breast cancer tissue blocks: HER2 non-amplification (normal), HER2 borderline amplification, and HER2 amplification. Ten samples per block of consecutive sections of 4 µm thickness were processed and tested according to standard procedures (control condition), and samples of different thicknesses (range 2-8 µm) from each block were similarly processed and tested in duplicate according to standard procedures.

Results are shown in Tables 2-10.

Table 2. Average HER2 CISH dots per cell in consecutive sections at 4 μm (breast cancer with *normal* HER2)

	Section Number									
	1	2	3	4	5	6	7	8	9	10
Average HER2 CISH dots/nucleus	1.8	2.0	1.9	2.0	1.6	1.7	1.7	1.6	1.7	2.0

Average HER2 1.8
STD 0.16
%CV 8.9

Table 3. Average HER2 CISH dots per cell in consecutive sections at 4 μm (breast cancer with *borderline amplification* of HER2)

	Section Number									
	1	2	3	4	5	6	7	8	9	10
Average HER2 CISH dots/nucleus	5.4	5.5	5.3	5.8	5.2	6.2	5.7	5.4	5.5	5.8

Average HER2 5.6
STD 0.30
%CV 5.4

Table 4. Average HER2 CISH dots per cell in consecutive sections at 4 μm (breast cancer with *amplified* HER2)

	Section Number									
	1	2	3	4	5	6	7	8	9	10
Average HER2 CISH dots/nucleus	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC

Table 5. Average HER2 CISH dots per cell in consecutive sections of different thickness (breast cancer with *normal* HER2)

	Thickness of Section (microns)							
	2	2	4	4	6	6	8	8
Average HER2 CISH dots/nucleus	1.5	1.4	1.7	1.6	2	2	2.1	2.2

Data from 8 consecutive tissue sections from 2-8 μm thickness:
Average HER2 1.8
STD 0.30
%CV 17.0

Data from 6 consecutive tissue sections from 4-8 μm thickness:
Average HER2 1.9
STD 0.23
%CV 12.0

Data from 6 consecutive tissue sections from 2-6 μm thickness:
Average HER2 1.7
STD 0.25
%CV 14.7

Data from 4 consecutive tissue sections from 4-6 μm thickness:
Average HER2 1.8
STD 0.21
%CV 12.0

Table 6. Average HER2 CISH dots per cell in consecutive sections of different thickness (breast cancer with *borderline amplification* of HER2)

	Thickness of Section (microns)							
	2	2	4	4	6	6	8	8
Average HER2 CISH dots /nucleus	4.5	4.0	5.2	5.6	6.3	6.7	7.8	8.0

Data from 8 consecutive tissue sections from 2-8 µm thickness:

Average HER2	6.0
STD	1.46
%CV	24.0

Data from 6 consecutive tissue sections from 4-8 µm thickness:

Average HER2	6.6
STD	1.14
%CV	17.0

Data from 6 consecutive tissue sections from 2-6 µm thickness:

Average HER2	5.4
STD	1.03
%CV	19.1

Data 4 consecutive tissue sections from 4-6 µm thickness

Average HER2	6.0
STD	0.68
%CV	11.0

Table 7. Average HER2 CISH dots per cell in consecutive sections of different thicknesses (breast cancer with *amplification* of HER2)

	Thickness of Section (microns)							
	2	2	4	4	6	6	8	8
Average HER2 CISH dots/nucleus	BC	BC	BC	BC	BC	BC	BC	BC

Data from 8 consecutive tissue sections from 2-8 µm thickness: amplified with Big Clusters

Table 8. Average HER2 CISH dots per cell in consecutive sections of different thickness (breast cancer with *normal* HER2)

	Thickness of Section (microns)					
	3	3	4	4	5	5
Average HER2 CISH dots/nucleus	1.7	1.7	1.8	1.7	2.1	2.0

Data from 6 consecutive tissue sections of 3-5 µm thickness:

Average HER2	1.8
STD	0.18
%CV	10.0

Data from 4 consecutive tissue sections of 4-5 µm thickness:

Average HER2	1.9
STD	0.18
%CV	9.4

Table 9. Average HER2 CISH dots per cell in consecutive sections of different thickness (breast cancer with *borderline amplification* of HER2)

	Thickness of Section (microns)					
	3	3	4	4	5	5
Average HER2 CISH dots/nucleus	4.8	4.3	5.7	5.3	6.2	6.8

Data from 6 consecutive tissue sections of 3-5 μm thickness:

Average HER2	5.5
STD	0.92
%CV	17.0

Data from 4 consecutive tissue sections of 2-4 μm thickness:

Average HER2	5.0
STD	0.61
%CV	12.2

Data from 4 consecutive tissue sections of 4-5 μm thickness:

Average HER2	6.0
STD	0.65
%CV	10.8

Table 10. Average HER2 CISH dots per cell in consecutive sections of different thickness (breast cancer with *amplification* of HER2)

	Thickness of Section (microns)					
	3	3	4	4	5	5
Average HER2 CISH dots/nucleus	BC	BC	BC	BC	BC	BC

Data from 6 consecutive tissue sections of 3-5 μm thickness: amplified with Big Clusters

Repeatability and Reproducibility Results and Conclusions

i. The %CV for the control condition of 4 μm with the non-amplification sample is 8.9%, and the %CV for the borderline amplification sample was 5.4%.
ii. There was no misclassification for HER2 amplification and HER2 non-amplification.
iii. Misclassification occurred for the borderline amplification when the tissue section was <4 μm (2 and 3 μm) thickness.
iv. Misclassification did not occur for HER2 amplification and non-amplification when the tissue section was ≥4 μm.
v. %CV for the borderline amplification was >15% (17%) when the tissue section was 4-8 μm. %CV for the low amplification was <15% (11%) when the tissue section was 4-6 μm.

The results from the repeatability testing with consecutive tissue sections with the same thicknesses of breast cancers with normal HER2 gene, HER2 gene borderline amplification, and HER2 gene amplification indicated an acceptable degree of repeatability of the HER2 CISH assay.

The results from reproducibility testing with different thicknesses (2-8 μm) of breast cancers with normal HER2 gene, HER2 gene borderline amplification, and HER2 gene amplification indicated an acceptable degree of reproducibility of the HER2 CISH assay when using tissue sections with thicknesses between 4-6 μm.

The 4-5 μm thickness is the recommendation in the product labeling, with 6 μm as the outside limit.

d. Reproducibility Studies

i. Day-to-Day Reproducibility

The day-to-day reproducibility of the SPOT-Light® HER2 CISH Kit was evaluated with slides from three breast cancer tissue blocks: HER2 non-amplification (normal), HER2 borderline amplification, and HER2 amplification, and the kit control slide that includes positive and negative cell lines. The samples were processed and tested in duplicate for four days according to standard procedure.

The normal HER2 sample and the amplified HER2 sample demonstrated the expected "<5 dots" and "big clusters" interpretations, respectively, 100% of the time (8/8 cases per sample). The borderline HER2 amplification sample demonstrated the correct borderline quantities, with an average dot count of 5.4, a range of 5.1 to 5.9, and a %CV of 5.7%. The control samples also demonstrated the expected results 100% of the time. Results from the testing of the clinical specimens are found in Table 11, and results from the control testing are found in Table 12.

Table 11. Day-to-day reproducibility for breast cancer tissue sections (over a single lot/technologist/and reader)

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status							
	Day 1		Day 2		Day 3		Day 4	
	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2
Tissue 1 (NAM)	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM
Tissue 2 (borderline)	3+ 5.2 AM	3+ 5.4 AM	3+ 5.8 AM	3+ 5.1 AM	3+ 5.3 AM	3+ 5.1 AM	3+ 5.9 AM	3+ 5.6 AM
Tissue 3 (AM)	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM

Table 12. Day-to-day reproducibility on control cell line block sections

Cell Line Name	CISH Signal, Average HER2 CISH dots/cell, and HER2 Gene Status							
	Day 1		Day 2		Day 3		Day 4	
	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2
Cell Line 1 (AM)	3+ BC AM	3+ BC AM	3+ BC AM	3+ BC AM	3+ BC AM	3+ BC AM	3+ BC AM	3+ BC AM
Cell Line 2 (NAM)	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM

On eight consecutive tissue sections from one breast cancer specimen with borderline amplification, the day-to-day data (4 different days) showed:

Average HER2	5.4
STD	0.31
%CV	5.7

ii. Lot-to-Lot Reproducibility

The lot-to-lot reproducibility of the SPOT-Light® HER2 CISH Kit was shown with slides from three breast cancer tissue blocks: HER2 non-amplification (normal), HER2 borderline amplification, and HER2 amplification, and two sets of the kit control slide that includes positive and negative cell lines. The samples were processed and tested in duplicate with three distinct lots, plus a fourth lot that included a different HER2 DNA probe. All testing was done according to standard procedure.

The normal HER2 sample and the amplified HER2 sample demonstrated the expected "<5 dots" and "big clusters" interpretations, respectively, 100% of the time (16 slides, 2 slides per sample, per lot). The borderline HER2 amplification sample demonstrated the correct borderline results, with an average dot count of 5.7, a range of 5.1 to 6.2, and a %CV of 7.7%. The control samples also demonstrated the expected results 100% of the time. Results from the testing of the clinical specimens are found in Table 13, and results from the control testing are found in Table 14.

Table 13. Lot-to-lot reproducibility of three breast cancer tissue specimens

Tissue Name	CISH Signal, Average HER2 CISH dots/cell, and HER2 Gene Status							
	Lot 1		Lot 2		Lot 3		Lot 4	
	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2
Tissue 1 (NAM)	3+ <5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM
Tissue 2 (borderline)	3+ 5.1 AM	3+ 6.0 AM	3+ 5.9 AM	3+ 5.2 AM	3+ 6.1 AM	3+ 5.9 AM	3+ 6.2 AM	3+ 5.2 AM
Tissue 3 (AM)	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM

Table 14. Lot-to-lot reproducibility of two sets of control cell line block sections

Cell Line Name	CISH Signal, Average HER2 CISH dots/cell, and HER2 Gene Status							
	Lot 1		Lot 2		Lot 3		Lot 4	
	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2
Cell Line 1 (AM)	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM
Cell Line 2 (NAM)	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM
Cell Line 3 (AM)	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM
Cell Line 4 (NAM)	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM

On eight consecutive tissue sections from the HER2 borderline amplification breast cancer specimen, the lot-to-lot data showed:

Average HER2	5.7
STD	0.44
% CV	7.7%

iii. Inter-run, Day-to-day

The inter-run, day-to-day reproducibility of the SPOT-Light® HER2 CISH Kit was shown using archived breast cancer tissue blocks: HER2 non-amplified (1-2 dots per cell), HER2 non-amplified with polysomy (3-5 dots per cell), and HER2 amplified (>5 dots per cell). The polysomy specimens were verified as polysomy by assessing the copy number of chromosome 17 centromere. The samples were processed and tested in triplicate over three separate days.

All slides demonstrated a strong CISH signal and good morphology. The inter-day data represents the average, standard deviation, and correlation variance determined for the pair of slides tested on each day. There is no significant difference in the variability with the HER2 status of the specimen. The average %CV for HER2 non-amplified, HER2 non-amplified with polysomy, and amplified HER2 specimens is 3%, 4%, and 8%, respectively.

The inter-day %CV and standard deviation are listed in Tables 15, 16, and 17 for each specimen for each day. Average, standard deviation, and %CV is calculated for each specimen for three different days. Intra-day average, standard deviation, and %CV are also provided. The overall inter-day %CV for all specimens tested was 5%.

Table 15. Inter-run, day-to-day reproducibility for breast cancer tissue sections for HER2 Non-amplified breast cancer archived specimens

	Breast Cancer HER2 Non-amplified								
	Specimen 1			Specimen 2			Specimen 3		
	AVG	STD	%CV	AVG	STD	%CV	AVG	STD	%CV
Day 1	1.90	0.05	2%	1.83	0.00	0%	1.75	0.02	1%
Day 2	1.77	0.05	3%	1.78	0.16	9%	1.77	0.05	3%
Day 3	1.85	0.07	4%	1.75	0.02	1%	1.73	0.00	0%
	Inter-day, Summary								
AVG	1.84			1.79			1.75		
STD	0.07			0.04			0.02		
%CV	4%			2%			1%		
Average % CV	3%								

Table 16. Inter-run, day-to-day reproducibility for breast cancer tissue sections for HER2 Non-amplified polysomy breast cancer archived specimens

	Breast Cancer HER2 Non-amplified, Polysomy								
	Specimen 1			Specimen 2			Specimen 3		
	AVG	STDV	%CV	AVG	STDV	%CV	AVG	STDV	%CV
Day 1	3.27	0.28	9%	3.83	0.05	1%	3.1	0.05	2%
Day 2	3.4	0.24	7%	3.95	0.05	1%	3.13	0.00	0%
Day 3	3.63	0.8	10%	3.7	0.09	3%	3.03	0.0	0%
	Inter-day, Summary								
AVG	3.43			3.83			3.09		
STDV	0.19			0.13			0.05		
%CV	5%			3%			2%		
Average % CV	4%								

Table 17. Inter-run, day-to-day reproducibility for breast cancer tissue sections for HER2amplified breast cancer archived specimens

	Breast Cancer HER2 Amplified								
	Specimen 1			Specimen 2			Specimen 3		
	AVG	STDV	%CV	AVG	STDV	%CV	AVG	STDV	%CV
Day 1	25.65	0.68	3%	17.68	0.5	2%	15.88	1.11	7%
Day 2	24.60	0.42	2%	18.77	0.19	1%	15.93	0.5	5%
Day 3	27.37	6.51	24%	19.97	4.1	21%	16.13	1.37	8%
	Inter-day, Summary								
AVG	25.87			18.81			15.98		
STDV	1.40			1.14			0.13		
%CV	5%			6%			1%		
Average % CV	8%								

e. Assay Robustness Studies

i. Pretreatment conditions

The study objective was to determine the tolerance limits of the pretreatment condition of the SPOT-Light® HER2 CISH Kit when testing breast cancer tissue sections of normal (non-amplified), borderline, and amplified HER2 status.

The samples under evaluation included slides from four breast cancer tissue blocks (HER2 non-amplification, borderline, and two HER2 amplification specimens).

Samples were processed and tested in duplicate for different heat pretreatment times (5, 10, 15, 20, and 30 minutes) at 100°C (Table 18), different heat pretreatment temperatures (93°C, 97°C, 98°C, 99°C, and 100°C) for 15 minutes (Table 19), and different pepsin incubation times (4, 7, 8, 10, 12, 13, and 16 minutes) at room temperature (Table 20), according to standard procedure.

Table 18. CISH results on three breast cancer tissue using different heat pretreatment times at 100°C

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status				
	5 Minute	10 Minute	15 Minute	20 Minute	30 Minute
	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2
Tissue 1 (NAM)	2+, local 3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+* < 5 NAM
Tissue 2 (borderline)	†	3+ 6.2, 5.8 AM	3+ 5.8, 5.2 AM	3+ 5.5, 5.3 AM	†
Tissue 3 (AM)	3+, local 4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+* BC AM

* Tissue partly damaged

† Results showed weak staining or tissue damage

Table 19. CISH results on four breast cancer tissue using different heat pretreatment temperatures for 15 minutes

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status				
	93°C	97°C	98°C	99°C	100°C
	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2
Tissue 1 (NAM)	2+, some 3+ < 5 NAM	3+ < 5 NAM	2-3+	3+ <5 NAM	3+ < 5 NAM
Tissue 2 (borderline)	ND	2-3+	2-3+	3+ 5.4, 6.0 AM	ND
Tissue 3 (AM)	3+ BC AM	4+ BC AM	ND	NA	4+ BC AM
Tissue 4 (AM)	ND	ND	3-4	4+ BC AM	ND

Table 20. CISH results on four breast cancer tissue specimens using different pepsin pretreatment times at room temperature

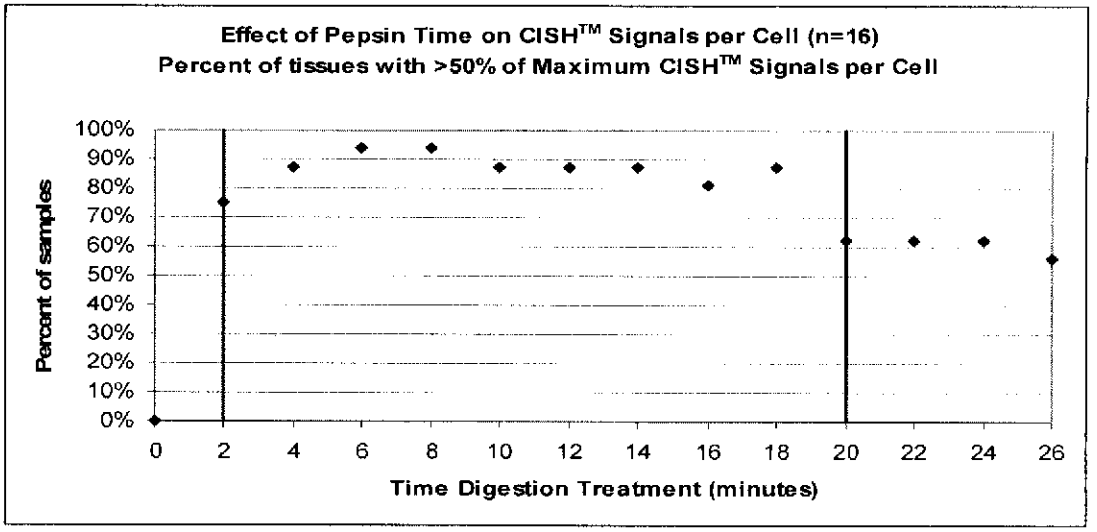
Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status						
	4 Min	7 Min	8 Min	10 Min	12 Min	13 Min	16 Min
Tissue 1 (NAM)	1-2+ < 5 NAM	2-3+ < 5 NAM	3+ <5 NAM	3+ < 5 NAM	3+ <5 NAM	2+ < 5 NAM	2, local 3+ < 5 NAM
Tissue 2 (borderline)	ND	ND	3+ 5.4, 6.0 AM	3+ 5.4, 6.0 AM	3+ 5.4, 6.0 AM	ND	ND
Tissue 3 (AM)	3+, local 4+ BC AM	3+, local 4+ BC AM	ND	4+ BC AM	ND	3+, local 4+ BC AM	3+, local 4+ BC AM
Tissue 4 (AM)	ND	ND	4+ BC AM	4+ BC AM	4+ BC AM	ND	ND

Additional Pepsin Digestion Studies

Additional pepsin digestion studies were performed to determine the suitable pepsin digestion time for a set of archival tissue samples. Tissue sections from three types of HER2 gene status were selected based on gene copy number: HER2 non-amplified (1-2 dots per cell), HER2 non-amplified with polysomy (3-5 dots per cell), and HER2 amplified (> 5 dots per cell). The polysomy cases were confirmed using a Cep-17 probe prior to the study. For each type of tissue samples, the maximum number of average CISH signals per cell was determined for the entire digestion series. The percent of total slides with at least 50% of the maximum number of CISH signals per cell was also determined for each digestion time. The predominant result used to determine the optimal pepsin digestion time was the average CISH signal per cell, although the cellular morphology and CISH signal intensity were also considered. The correlation of pepsin digestion time with average CISH signal per cell is summarized in Figure 1.

There is no difference between the suitable pepsin digestion time for all three categories of HER2 gene status. Pepsin digestion for any length of time between 2 and 20 minutes resulted in greater than 80% of specimens having at least 50% of the maximum number of CISH signals per cell for any set of tissues. About 90% of the samples demonstrated at least 50% of the maximum CISH signals per cell when digested for 4 to 6 minutes. For all samples that failed the criterion pepsin digestion for 10 minutes, resulted in at least 50% of the maximum CISH signals per cell.

Figure 1. Effect of Pepsin Digestion Time on CISH™ Signals per Cell.

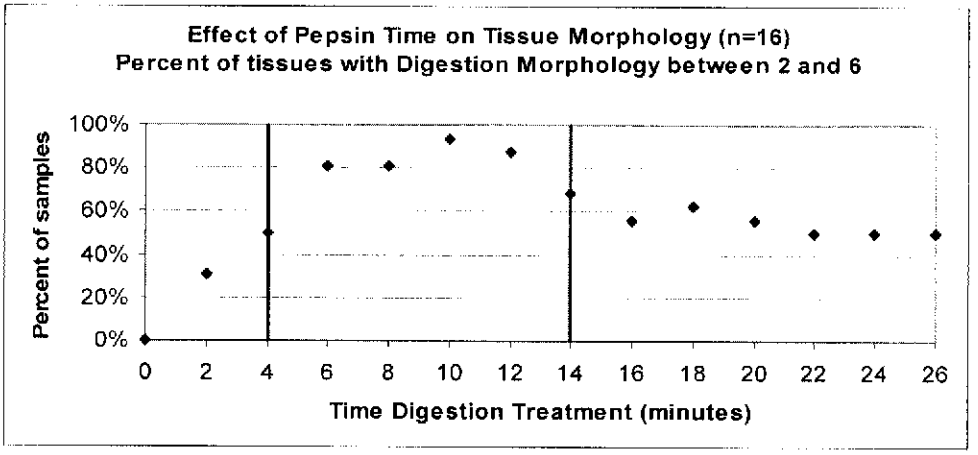


For each tissue, the maximum number of average CISH signals per cell was determined for each tissue of the entire digestion series. The percent of total slides with at least 50% of the maximum number of CISH signals per cell was determined for each digestion time.

The correlation between pepsin digestion time with cellular morphology is summarized in Figure 2. For the purposes of this study, a numerical system for quantifying cellular morphology was adopted to reduce observer variability. The cellular morphology score ranged from 0 to 8. A score of 0 was defined as "dark nuclear counterstaining with most cells lacking chromogenic signals" in which the CISH signal was significantly obscured by the nuclear counterstaining. The maximum score of 8 was defined as "nearly complete loss of CISH signals from many cells or "severe loss of nuclear morphology and staining." The percent of total samples, which have pepsin cellular morphology numerical values between 2 and 6, was determined for each pepsin digestion time.

Pepsin digestion times between 4 and 14 minutes (Figure 2) resulted in at least 80% of the tissue samples having optimal pepsin cellular morphology (cellular morphology score between 2 and 6). Based on these results, the same range of pepsin digestion time can be applied across all three categories of HER2 gene status.

Figure 2. The Effect of Pepsin Digestion Time on the Percent of samples with digestion morphology numerical values between 2 and 6.



An initial pepsin digestion time of 5 minutes is recommended. If 5 minutes of pepsin digestion is inadequate, the test should be repeated using a 10 minute digestion time. Based on this study, 100% of the samples will demonstrate at least 50% of the maximum CISH signals per cell using this approach.

Pretreatment Condition Results and Conclusions

(a) The heat pretreatment temperature range is >98.0-100°C for 15 minutes.
(b) The heat pretreatment time at 100°C is 10-20 minutes.
(c) The pepsin pretreatment time is 5 minutes.
(d) Under the pretreatment conditions described above, all slides from each specimen demonstrated the strong (≥3+) CISH signal, and good morphology.
(e) The normal HER2 sample and the amplified HER2 sample demonstrated the correct "<5" and "big clusters" interpretations, respectively, in all sections tested.
(f) The borderline sample demonstrated X-bar of HER2 copies 5.67, STD 0.33, and %CV 5.8% (<15%).
(g) There was no misclassification for HER2 amplification, borderline, or HER2 non-amplification.

ii. Denaturization conditions

The denaturization stringency study was conducted to determine the tolerance limits for denaturization using the SPOT-Light® HER2 CISH Kit when testing breast cancer tissue section of normal, borderline, and amplified HER2 status.

The samples under evaluation included slides from four breast cancer tissue blocks (HER2 non-amplification, borderline, and two HER2 amplification specimens). Samples were processed and tested in duplicate at different denaturing temperatures (75°C, 85°C, 90°C, 93°C, 95°C, and 98°C) and different times (2, 5, and 8 minutes) using PCR themalcycler according to standard procedure. The results from this testing appear in Tables 21 and 22.

Table 21. CISH results on two breast cancer tissue using different denaturing temperatures for 2-8 minutes

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status								
	75°C			85°C			90°C		
	2min	5min	8min	2min	5min	8min	2min	5min	8min
Tissue 1 (NAM)	2+ <5 NAM	2+ <5 NAM	2+ <5 NAM	2-3+ <5 NAM	2-3+ <5 NAM	2-3+ <5 NAM	2-3+ <5 NAM	2-3+ <5 NAM	2-3+ <5 NAM
Tissue 3 (AM)	3+ BC AM	3+ BC AM	3+ BC AM	3+ BC AM	3-4+ BC AM	3-4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM

Table 22. CISH results on four breast cancer tissue using different denaturing temperatures for 2-8 minutes

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status								
	93°C			95°C			98°C		
	2min	5min	8min	2min	5min	8min	2min	5min	8min
Tissue 1 (NAM)	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM
Tissue 2 (borderline)	3+ 5.6, 5.5 AM	3+ 5.3, 5.7 AM	3+ 6.1, 5.9 AM	3+ 5.8, 5.6 AM	3+ 5.3, 5.3 AM	3+ 5.4, 5.5 AM	3+ 5.6, 6.4 AM	3+ 5.3, 6.0 AM	3+ 5.7, 6.0 AM
Tissue 3 (AM)	ND	ND	ND	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM
Tissue 4 (AM)	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM

Denaturization Condition Results and Conclusions

(a) The recommended temperature and time range for denaturing when using the PCR thermalcycler is 95 (± 1°C) for 5 min., followed by overnight incubation (10-18 hrs) at 37°C (± 1°C).

(b) If using a heating block and humidity chamber with 37°C incubator: Denature at 95°C (± 1°C) for 5 min., followed by overnight incubation (10-18 hrs) at 37°C (± 1°C).

(c) Under the pretreatment conditions described above, all slides from each specimen demonstrated a strong (≥3+) CISH signal, and good morphology.

(d) The normal HER2 sample and the amplified HER2 sample demonstrated the correct "<5" and "big clusters" interpretations, respectively, in all sections tested.
(e) The borderline sample demonstrated x-bar of HER2 copies 5.61, STD 0.32, and %CV 5.7% (<15%).
(f) There was no misclassification for HER2 amplification, borderline, or HER2 non-amplification.
(g) All sample duplicate slides showed similar staining intensity.

iii. Hybridization conditions

The hybridization stringency study evaluated the hybridization tolerance limits of the SPOT-Light® HER2 CISH Kit when testing breast cancer tissue sections of HER2 non-amplified, borderline, and amplified HER2 status.

Samples under evaluation included slides from four breast cancer tissue blocks (HER2 non-amplification, borderline, and two HER2 amplification specimens). Samples were processed and tested in duplicate at different hybridization temperatures (27°C, 32°C, 37°C, 39 °C, 40 °C, and 42°C) for 15 hours, and different hybridization times (10 hours, 14 hours, 18 hours, and 22 hours) at 37°C according to standard procedure. The results from this study are presented in Tables 23 and 24.

Table 23. CISH results on two breast cancer tissues using different hybridization temperatures for 15 hours

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status											
	27°C		32°C		37°C		39°C		40°C		42°C	
	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2
Tissue 1 (NAM)	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ <5 NAM	3+ <5 NAM	2-3+	2-3+	2+ < 5 NAM	2+ < 5 NAM
Tissue 2 (borderline)	ND	ND	ND	ND	ND	ND	3+ 5.5 AM	3+ 5.2 AM	2-3+	2-3+	NA	NA
Tissue 3 (AM)	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	ND	ND	ND	ND	3-4+ BC AM	3+ BC AM
Tissue 4 (AM)	ND	ND	ND	ND	ND	ND	4+ BC AM	4+ BC AM	3-4+	3-4+	ND	ND

Table 24. CISH results on two breast cancer tissue specimens using different hybridization times at 37°C

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status			
	10 Hours	14 Hours	18 Hours	22 Hours
	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2	Slide 1 and 2
Tissue 1 (NAM)	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM
Tissue 3 (AM)	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+* BC AM

*some cytoplasmic background staining

Additionally, the test for hybridization temperature (27°C and 39°C) was performed for different hybridization times (10, 15, and 18 hours) according to standard procedure. Samples under evaluation included slides from three breast cancer tissue blocks (HER2 non-amplification, borderline, and HER2 amplification specimens). The results from this study are presented in Tables 25 and 26.

Table 25. CISH results on three breast cancer tissues using hybridization temperatures 27°C for different time

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status					
	10 hours		15 hours		18 hours	
	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2
Tissue 1 (NAM)	ND	ND	ND	ND	ND	ND
Tissue 2 (borderline)	2-3+	2-3+	3+ 5.6 AM	3+ 5.7 AM	3+ 5.1 AM	3+ 5.5 AM
Tissue 4 (AM)	ND	ND	ND	ND	ND	ND

Table 26. CISH results on three breast cancer tissues using hybridization temperatures 39°C for different time

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status		
	10 hours	15 hours	18 hours
	Slide 1, 2	Slide 1, 2	Slide 1, 2
Tissue 1 (NAM)	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM
Tissue 2 (borderline)	3+ 4.9, 4.8 NAM	3+ 5.4, 5.3 AM	3+ 4.9, 5.1 NAM, AM
Tissue 4 (AM)	4+ BC AM	4+ BC AM	4+ BC AM

Hybridization Condition Results and Conclusions

(a) The CISH denaturization conditions are 30-39°C and 10-18 hours with a recommendation of 37°C (±1°C) over 10-18 hours as a start.
(b) Under the pretreatment conditions described above, all slides from each specimen demonstrated a strong (≥3+) CISH signal, and good morphology.
(c) The normal (non-amplified) HER2 sample and the amplified HER2 sample demonstrated the correct "<5" and "big clusters" interpretations, respectively, in all sections tested.
(d) The borderline sample demonstrated X-bar of HER2 copies 5.29, STD 0.30 and %CV 5.6% (<15%).
(e) There was no misclassification for HER2 amplification, borderline, or HER2 non-amplification.

iv. Stringent wash

The stringent wash study identified the tolerance limits of the stringent wash conditions for the SPOT-Light® HER2 CISH kit when testing breast cancer tissue sections of normal and amplified HER2 status.

The samples under evaluation included slides from three breast cancer tissue blocks (HER2 non-amplification, borderline, and HER2 amplification). Samples were processed and tested in duplicate at different stringent wash temperatures for 5 minutes and three different temperatures for different times according to standard operating procedures (Table 27 and 28). Samples were also processed and tested at different stringent wash temperatures for 5 minutes (Table 29).

Table 27. CISH results using different stringent wash temperature and time on three breast cancer tissue

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status												
	66°C	69°C	72°C	75°C	78°C			80°C			83°C		
	5min	5min	5min	5min	2 min	5min	8min	2 min	5 min	8min	2min	5min	8min
Tissue 1 (NAM)	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	ND	3+ < 5 NAM	ND	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	2-3+ < 5 NAM	2+ < 5 NAM	2+ < 5 NAM
Tissue 2 (borderline)	ND	ND	ND	ND	3+ 5.6, 5.3 AM	ND	3+ 4.8, 5.3 NAM, AM	2-3+	3+	3+	ND	ND	ND
Tissue 3 (AM)	4+ BC AM	4+ BC AM	4+ BC AM	4+ BC AM	ND	4+ BC AM	ND	4+ BC AM	4+ BC AM	4+ BC AM	3-4+ BC AM	3-4+ BC AM	3+ BC AM

Table 28. CISH results using different stringent wash temperatures and constant time on three breast cancer tissue

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status					
	37°C	45°C	55°C	60°C		
	5min	5min	5min	2min	5min	8min
Tissue 1 (NAM)	3+* < 5 NAM	3+* < 5 NAM	3+* < 5 NAM	ND	3+ < 5 NAM	ND
Tissue 2 (borderline)	ND	ND	ND	3+ 5.4, 5.4 AM	3+ 5.1, 5.9 AM	3+ 5.3, 5.7 AM
Tissue 3 (AM)	4+* BC AM	4+* BC AM	4+* BC AM	ND	4+ BC AM	ND

*some background staining

Table 29. CISH results using three different stringent wash temperatures and constant time (5 minutes) on eight breast cancer tissue, one normal breast and two FFPE cell line blocks

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status		
	55°C*	65°C	75°C
Normal breast 1 (NAM)	3+ 2 NAM	3+ 2 NAM	3+ 2 NAM
Breast cancer 1 (AM)	4+ BC AM	3-4+ BC AM	3-4+ BC AM
Breast cancer 2 (AM)	4+ BC AM	4+ BC AM	4+ BC AM
Breast cancer 3 (NAM)	3+ 2 NAM	3+ BC NAM	3+ BC NAM
Breast cancer 4 (NAM)	3+ 3-5 NAM	3+ 3-5 NAM	3+ 3-5 NAM
Breast cancer 5 (NAM)	3+ 2 NAM	3+ 2 NAM	3+ 2 NAM
Breast cancer 6 (NAM)	3+ 2 NAM	3+ 2 NAM	3+ 2 NAM
Breast cancer 7 (NAM)	3+ 2 NAM	3+ 2 NAM	3+ 2 NAM
Breast cancer 8 (NAM)	3-4+ 2 NAM	3-4+ 2 NAM	3-4+ 2 NAM
FFPE Cell line block 1 (NAM)	3+ 2 NAM	3+ 2 NAM	3+ 2 NAM
FFPE Cell line block 2 (AM)	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM

*some background staining

Stringent Wash Conclusions

(a) The recommended time and temperature are 60-78°C for 2 – 8 minutes, with a recommended stringent wash at 70°C at 5 minutes.

(b) The non-amplified HER2 sample and the amplified HER2 sample demonstrated the correct "<5" and "big clusters" interpretations, respectively, in all sections tested.

(c) The borderline sample demonstrated x-bar of HER2 copies 5.39, STD 0.30, and %CV 5.6% (<15%).

(d) There was no misclassification for HER2 amplification, borderline, or HER2 non-amplification.

(e) All sample duplicate slides showed similar staining intensity.

v. Immunodetection

The immunodetection study was conducted to determine an acceptable range of incubation times for the major components in the SPOT-Light® HER2 CISH Kit detection step when testing breast cancer tissue sections of normal, borderline, and amplified HER2 status.

Samples under evaluation included slides from four breast cancer tissue blocks: HER2 non-amplification (normal), borderline, and two HER2 amplification specimens. Samples were processed and tested in duplicate using different incubation times for the major components in the detection step (mouse anti-DIG, Polymer HRP conjugated goat anti-mouse), (15, 20, 25, 30, and 60 minutes), and different incubation times for DAB, (15, 30, 45, and 60 minutes), according to standard procedures. Results are shown in Tables 30 and 31 below.

Table 30. Result of CISH performance using different incubation times of mouse-anti DIG, polymer HRP conjugate goat anti-mouse, and DAB chromogen on two breast cancer tissue

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status									
	15 minute		20 minute		25 minute		30 minute		60 minute	
	Slide1	Slide 2	Slide1	Slide 2	Slide1	Slide 2	Slide1	Slide 2	Slide 1	Slide 2
Tissue 1 (NAM)	2 -3+ < 5 NAM	2 -3+ < 5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ <5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM
Tissue 2 (borderline)	ND	ND	3+	3+	3+ 5.2 AM	3+ 5.3 AM	ND	ND	ND	ND
Tissue 3 (AM)	3+ BC AM	3+ BC AM	ND	ND	ND	ND	4+ BC AM	* BC AM	4+ BC AM	4+ BC AM
Tissue 4 (AM)	ND	ND	3-4+ BC AM	3-4+ BC AM	4+ BC AM	4+ BC AM	ND	ND	ND	ND

* one of the steps was omitted; therefore no signal was recorded.

Table 31. Result of CISH performance using different incubation times for DAB on three breast cancer tissue

Tissue Name	CISH Signal, Average HER2 CISH dots/cell and HER2 Gene Status							
	15 minute		30 minute		45 minute		60 minute	
	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2	Slide 1	Slide 2
Tissue 1 (NAM)	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM	3+ < 5 NAM
Tissue 2 (borderline)	3+ 5.4, 5.1 AM	3+ 5.1, 5.1 AM	ND	ND	ND	ND	3+ 5.1 AM	3+ 5.1 AM
Tissue 3 (AM)	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM	3-4+ BC AM

Immunodetection Conclusions

(a) The incubation time range for mouse anti-DIG, Polymer HRP conjugated goat anti-mouse in the SPOT-Light® HER2 CISH Kit was 15 to 60 minutes. There were no differences seen in the range of 25 to 60 minutes, while the 20 minute incubation time showed weaker staining intensity (Table 30).

(b) The incubation range of DAB in the SPOT-Light® HER2 CISH Kit was from 15 to 60 minutes. There were no differences in the range of 15 to 60 minutes (Table 31). The non-amplified sample and the amplified HER2 sample demonstrated the correct <5 and big clusters interpretations, respectively, in all sections tested. The borderline sample demonstrated X-bar of HER2 copies 5.18, STD 0.15, and %CV 2.9% (<15%).

(c) The results from the immunodetection stringency study indicated that the recommended incubation time for mouse anti-DIG, Polymer HRP conjugated goat anti-mouse, and for DAB, is 30 minutes.

vi. Hematoxylin counterstaining conditions

The hematoxylin counterstaining stringency study was conducted to evaluate the counterstaining conditions and tolerance limits when testing breast cancer tissue sections of normal (non-amplified), borderline, and amplified HER2 status with the SPOT-Light® HER2 CISH Kit.

The samples under evaluation included slides from seven breast cancer tissue blocks (three with HER2 non-amplification, three with HER2 amplification, and one borderline), and one cell block containing one cell line with HER2 amplification and one cell line with HER2 non-amplification. Samples were processed and tested at different hematoxylin counterstaining times (5, 10, 20, 30, and 40 seconds) according to standard procedure. The results from this testing appear in Table 32.

Table 32. CISH results on seven breast cancer tissue and one cell block section using different hematoxylin counterstaining times

Tissue or cell block name	HER2 CISH Signal					Hematoxylin Counterstaining				
	5sec	10sec	20sec	30sec	40sec	5sec	10sec	20sec	30sec	40sec
Tissue 1 (AM)	4+ AM	4+ AM	4+ AM	4+ AM	4+ AM	AM light	ACC	ACC	ACC	ACC
Tissue 2 (AM)	4+ AM	4+ AM	4+ AM	3-4+ AM	3-4+ AM	AM light	ACC	ACC	ACC	ACC
Tissue 3 (NAM)	3+ NAM	3+ NAM	3+ NAM	3+ NAM	3+ NAM	AM light	ACC	ACC	ACC	ACC
Tissue 4 (NAM)	3+ NAM	3+ NAM	3+ NAM	3+ NAM	3+ NAM	AM light	ACC	ACC	ACC	ACC
Tissue 5 (NAM)	3+ NAM	3+ NAM	3+ NAM	3+ NAM	3+ NAM	AM light	ACC	ACC	ACC	ACC
Tissue 6 (NAM)	3+ NAM	3+ NAM	3+ NAM	3+ NAM	3+ NAM	AM light	ACC	ACC	NAM too dark	NAM too dark
Tissue 7 (borderline)	ND	3+ 5.5, 5.7 AM	3+ 5.6, 4.9 AM	3+ 5.3, 6.0 AM	ND	ND	ND	ND	ND	ND
FFPE Cell line 1 (AM)	3-4+ AM	3-4+ AM	3-4+ AM	3-4+ AM	3-4+ AM	AM light	ACC	ACC	ACC	ACC
FFPE Cell line 2 (NAM)	3+ NAM	3+ NAM	3+ NAM	3+ NAM	3+ NAM	AM light	ACC	ACC	ACC	ACC

Hematoxylin Counterstaining Condition Results/Conclusion

(a) The hematoxylin counterstaining time ranges from 5-30 seconds in this study. In this range, the counterstaining was not too light for tissue morphology, nor too dark to obscure positive staining signals. The final counterstaining depends on the pathologist's preferences. To avoid any accidental over-counterstaining that may obscure CISH signal, 3-5 seconds of counterstaining time is recommended as a start. Additional 3-5 seconds or more may be added to adjust the intensity of the counterstaining based on the individual preference.

(b) Under these hematoxylin counterstaining times, all slides from each specimen demonstrated a high (≥3+) CISH signal, and good morphology.

(c) The HER2 non-amplification sample and the HER2 amplified sample demonstrated the correct "<5" and "big clusters" interpretations, respectively, in all sections tested. The borderline sample demonstrated X-bar of HER2 copies 5.5, STD 0.37, and %CV 6.8% (<15%).

(d) There was no misclassification for HER2 amplification, borderline, or HER2 non-amplification.

f. Kit Stability

Kits Manufactured at the South San Francisco, CA Site

Real time stability testing was done for each component separately prior to the HER2 CISH Kit configuration. The stability of the SPOT-Light® HER2 CISH™ Kit was demonstrated via both accelerated stability testing at an elevated temperature, and real-time stability testing at 2-8°C.

i. Real time stability testing using one lot indicated that DIG-labeled HER2 DNA probe (ready-to-use) is stable at -20°C for at least 4.5 years. Real time stability testing using one lot indicated that DIG-labeled HER2 DNA probe is stable at 2-8° for 2 years.
ii. The QC data from different lots of ready-to-use DIG-labeled DNA probe made from two distinct concentrated DIG-labeled DNA probe demonstrated that the concentrated format of the probe is stable for at least 2 years.
iii. Real time stability testing indicated that the CISH Polymer Detection Kit is stable at 2-8° for at least 1 year.

The accelerated model consisted of incubations at 37°C for the following time points: Weeks 1, 2, 3, and 4 (one lot), and additionally Weeks 5, 6, 7, and 8 for the second lot. At each time point, retained material was removed from the stress condition, and tested for conformance to the release specifications of the controlled product. The results from this testing showed that the testing probe had a strong CISH signal, and that the signal intensity was equal to that of the control probe through Week 7. At Week 8, the testing probe showed a little weaker staining than that of the control probe (see Table 33). Based on experience with the accelerated model, it is estimated that one week at 37°C corresponds to approximately six months at 2-8°C, and therefore the real time stability should be just under four years. The product now in use is labeled with one-year stability from the data of manufacture.

Table 33. Summarized data from accelerated stability testing Kits Manufactured at South San Francisco, CA Manufacturing Site

Testing Material	Lot Number	Note	Result
84-0100, SPOT-Light® HER2 DNA Probe	01062294	Accelerated stability 1-4 weeks at 37°C	Pass
84-0100, SPOT-Light® HER2 DNA Probe	30778586	Accelerated stability 5-8 weeks at 37°C	Pass 5-7 weeks Fail 8th week

To support the one-year labeling claim, stability data from three CISH Kit lots manufactured at the Zymed South San Francisco site were generated. Results from samples retained at 2-8°C for 1-year have passed, and thus support the 1-year expiry dating. These results are contained in Table 34.

Table 34. Summarized Kit Stability Results for Kits Manufactured at South San Francisco, CA Manufacturing Site

Lot Designation	Testing Material	Lot Number	Condition	Result
1st	SPOT-Light® HER2 CISH™ Kit 84-0146	41281186	Real time stability 1 year at 2-8°C	Pass
2nd	SPOT-Light® HER2 CISH™ Kit 84-0146	50481599	Real time stability 1 year at 2-8°C	Pass
3rd	SPOT-Light® HER2 CISH™ Kit 84-0146	50681715	Real time stability 1 year at 2-8°C	Pass

Kits Manufactured at the Camarillo, CA Manufacturing Site

In support of the CISH manufacturing site transfer from the South San Francisco, CA site to the Camarillo, CA site, additional stability data have been generated on three CISH Kit lots manufactured at the Camarillo, CA manufacturing site. To date, stability data is available for time periods up to and including twelve months, on three lots of product manufactured at the Camarillo facility using the same analytical methods as the initial stability program. The stability test results for three lots manufactured at Camarillo, CA are included in the following Table 35.

Table 35: Kit Stability Results for Kits Manufactured at the Invitrogen Camarillo, CA Manufacturing Site

Lot Designation	Testing Material	Lot Number	Condition	Result
1st	SPOT-Light® HER2 CISH™ Kit 84-0146	1402569	Real time stability 3-month at 2-8°C	Pass
			Real time stability 6-month at 2-8°C	Pass
			Real time stability 9-month at 2-8°C	Pass
			Real time stability 12-month at 2-8°C	Pass
2nd	SPOT-Light® HER2 CISH™ Kit 84-0146	1402570	Real time stability 3-month at 2-8°C	Pass
			Real time stability 6-month at 2-8°C	Pass
			Real time stability 9-month at 2-8°C	Pass
			Real time stability 12-month at 2-8°C	Pass
3rd	SPOT-Light® HER2 CISH™ Kit 84-0146	1413291	Real time stability 3-month at 2-8°C	Pass
			Real time stability 6-month at 2-8°C	Pass
			Real time stability 9-month at 2-8°C	Pass
			Real time stability 12-month at 2-8°C	Pass

The 12-month stability results for the SPOT-Light HER2 CISH Kit continue to support the previously established one-year expiry dating when stored according to instructions for use.

2. Non-Clinical Studies- External

a. Site-to Site Reproducibility

The objective of the site-to-site reproducibility study was to additionally evaluate the reproducibility of the SPOT-Light® HER2 CISH Kit results at different sites in the U.S. with different personnel performing the assay. Trained histotechnologists performed the CISH assay, and trained pathologists performed the interpretations.

The samples used in this study included slides from three breast cancer tissue blocks (non-amplified, amplified, and borderline) and one cell block containing a positive cell line (amplified) and a negative cell line (not amplified). All samples were identified only by an ID number, and therefore the histotechnologists were blinded to the correct results. The 14 slides (12 samples and 2 control cell line slides from the kit) were stained at each external site, and after the samples were stained, they were given to the pathologists. The results appear in Tables 36-39.

Table 36. Site-to-site reproducibility

Slide	Cell line or Tissue	Site 1	Site 2	Site 3
1	Cell line A (NAM) Cell line B (AM)	<5 dots, NAM >5 dots, AM	*Not Evaluable* *Not Evaluable*	<5 dots, NAM >5 dots, AM
2	Cell line A (NAM) Cell line B (AM)	<5 dots, NAM >5 dots, AM	*Not Evaluable* *Not Evaluable*	<5 dots, NAM >5 dots, AM
3	Tissue 1 (NAM)	NAM (not counted)	NAM (not counted)	NAM (not counted)
4	Tissue 1 (NAM)	NAM (not counted)	NAM (not counted)	NAM (not counted)
5	Tissue 1: NAM	NAM (not counted)	NAM (not counted)	NAM (not counted)
6	Tissue 2: AM	AM Mixture multiple dots & large clusters	AM Mixture multiple dots & large clusters	AM Mixture multiple dots & large clusters
7	Tissue 2: AM	AM Mixture multiple dots & large clusters	AM Mixture multiple dots & large clusters	AM Mixture multiple dots & large clusters
8	Tissue 2: AM	AM Mixture multiple dots & large clusters	AM Mixture multiple dots & large clusters	AM Mixture multiple dots & large clusters
9	Tissue 3: BL	AM 5.1 (counted 60 cells)	NAM 3.6 (counted 30 cells)	AM 5.6 (counted 30 cells)
10	Tissue 3: BL	AM 6.1 (counted 30 cells)	NAM 3.4 (counted 30 cells)	NAM 4.6 (counted 30 cells)
11	Tissue 3: BL	AM 6.0 (counted 30 cells)	NAM 1-5 (not counted)	AM 5.0 (counted 60 cells)
12	Cell line L Cell line R	AM Mixture multiple dots and large clusters NAM (not counted)	*Not Evaluable*	AM Mixture multiple dots and large clusters NAM (not counted)
13	Cell line L Cell line R	AM Mixture multiple dots and large clusters NAM (not counted)	*Not Evaluable*	AM Mixture multiple dots and large clusters NAM (not counted)
14	Cell line L Cell line R	AM Mixture multiple dots and large clusters NAM (not counted)	*Not Evaluable*	AM Mixture multiple dots and large clusters NAM (not counted)

Table 37. Results from Sites 1 and 3 (*not including borderline cases*)

Number of Samples Tested For Each Site	Number Agree		Number Discordant	% Agreement
	Amplified	Non-Amplified		
16	8	8	0	100%

Table 38. Samples with Evaluable Results from All Three Sites (*not including borderline cases*)

Number of Samples Tested For Each Site	Number Agree		Number Discordant	% Agreement
	Amplified	Non-Amplified		
6	3	3	0	100%

Table 39. Borderline Cases

Number of Samples Tested For Each Site	Number Site 1 and 3 agree Amplified, Site 2 disagree	Number Site 2 and 3 agree Non-Amplified, Site 1 disagrees
3	2	1

Due to a technician error at Site 2, non-evaluable results were recorded. After further investigation, it was determined that the cells were excessively digested during the pepsin digestion step.

b. Observer-to-Observer Reproducibility

The objective of the observer-to-observer reproducibility study was to evaluate the reproducibility of the SPOT-Light® HER2 CISH Kit results at different sites with different personnel interpreting the slide results. The slides (n=8) were all stained at Invitrogen, and were represented by: two non-amplified cases, two amplified cases, and four non-amplified cases with polysomy.

A total of three pathologists at three different sites interpreted and reported the results of a set of stained slides. Each pathologist reported the results in the Sample Reporting Worksheet and returned them to Invitrogen. The results appear in Table 40.

Table 40. Observer-to-observer reproducibility

Slide ID	Slide #	CISH Signal, Average HER2 CISH dots/cell, and HER2 Gene Status		
		Observer 1	Observer 2	Observer 3
1	Sample 1 (NAM)	2 dots NAM	1-5 dots NAM	1-2 NAM
2	Sample 2 (AM)	Large Cluster + multiple dots AM	Large Cluster + dots AM	>10 AM
3	Sample 3 (NAM)	2-5 NAM	1-5 NAM	3-5 NAM
4	Sample 4 (NAM)	2-4 NAM	2.8 NAM	3-5 NAM
5	Sample 5 (NAM)	2 NAM	1-5 NAM	1-2 NAM
6	Sample 6 (NAM)	2-5 NAM	1-5 NAM	4 NAM
7	Sample 7 (AM)	Large cluster + multiple dots AM	Large cluster AM	Large cluster + multiple dots AM
8	Sample 8 (NAM)	2-5 NAM	3.4 NAM	4.3 NAM

The correct interpretations were achieved 100% of the time.

B. Animal Studies: None

C. Additional Studies: None

X. Summary of clinical studies

A. Study design

The safety and effectiveness of the SPOT-Light® HER2 CISH™ Kit has been evaluated in the Invitrogen sponsored pivotal clinical study (Clinical Report 30266CA: SPOT-Light® HER2 CISH™ Kit for the Evaluation of HER-2/neu Gene Status in Human Breast Tissue). This study provides comparative data between the SPOT-Light® HER2 CISH™ Kit method and Pathvysion™ HER-2 DNA Probe Kit, an FDA-approved, commercially-available fluorescence *in-situ* hybridization (FISH) method (P980024), along with data from DAKO Herceptest™ a standardized and FDA-approved (P980018) immunohistochemistry (IHC) method for the detection HER2 protein expressed on the surface of tumor cells (P980018). This Invitrogen

sponsored clinical study provides the pivotal clinical data for assessing the safety and effectiveness of the SPOT- Light® HER2 CISH™ Kit.

Invitrogen Pivotal Clinical Study (Clinical Report 30266CA): *SPOT-Light® HER2 CISH™ Kit for the Evaluation of HER-2/neu Gene Status in Human Breast Tissue*

The primary study objective was to evaluate the concordance of the SPOT-Light® HER2 CISH™ Kit to PathVysion™ HER-2 DNA Probe kit for the detection of HER2/neu gene amplification status in human breast tissue. The secondary objective was to evaluate concordance of the SPOT-Light® HER2 CISH™ Kit to HercepTest™, across three IHC classes of protein expression (0, 1+: negative HER2 expression, 2+: weakly positive, and 3+: strongly positive), with a detailed analysis in Equivocal Cases of IHC2+ staining. The study was conducted at three study sites: Department of Pathology at MD Anderson Cancer Center, Jilab, Inc. Tampere University, and Invitrogen Corporation.

1. **Clinical Inclusion and Exclusion Criteria**
   a. The Inclusion Criteria for cases selected for the clinical study included the following:
      i. Confirmed pathology diagnosis of primary invasive breast cancer
      ii. Adequate tissue sample for the entire study.

   b. The Exclusion Criteria for cases selected for the clinical study included any one or more of the following:
      i. The case is missing the clinically relevant data such as pathology description of the tumor.
      ii. The case has inadequate or no existing tissue sample for the entire clinical study or the samples are not readily available at the Study Site. Such situations may include the referral cases where the existing tissue samples will be difficult to obtain, or samples obtained through biopsy and/or fine needle aspirate where such samples are inherently inadequate for further analysis.
      iii. The case has missing tissue sample or medical records.
      iv. The tissue for the case is from a core biopsy.
      v. A case may be excluded from some or all of the analysis, if the case has adequate tissue sample but it fails CISH, FISH, or IHC staining after two attempts or after no additional slides are available to complete staining.

2. **Follow-up schedule – Not applicable**

3. **Clinical endpoints**
   The primary endpoint was the total agreement rate between CISH and FISH. The positive (amplified) and negative (not amplified) CISH and FISH outcomes were defined as follows:

FISH: PathVysion™ (Vysis Inc., Downer's Grove, IL)

Not amplified: HER2:CEP17 ratio < 2.0

Amplification: HER2:CEP17 ratio ≥ 2.0

CISH: SPOT-Light® HER2 CISH Kit, (Invitrogen Corporation, Camarillo, CA)

Not amplified: 1-5 signals (dots)/nucleus in tumor cells

Amplification: > 5 signal/nucleus, or clusters of amplified spots/nucleus in 30 tumor cells

The percentage of cases with agreed CISH and FISH outcomes (i.e. amplified from both tests or non-amplified from both tests) was calculated and the corresponding 95% confidence interval was calculated using normal distribution approximation. Cohen's kappa was also used to evaluate the agreement between CISH and FISH and between CISH and IHC.

B. Study population demographics, baseline parameters, PMA cohort accountability, Safety and Effectiveness Results

This study included two sets of cases, from Consecutive Cases and Supplemental Cases that had met the inclusion and exclusion criteria as stated in the Clinical Study Protocol. The Consecutive Cases were selected in reverse chronological order, starting with Consecutive Cases identified in December 2006, and working backward in time until the desired number of Consecutive Cases was selected at both the MD Anderson and Tampere sites. Table 41 shows the distribution of the specimen characteristics for Consecutive Cases from the MD Anderson and Tampere sites as reported in the pathology reports.

Table 41: Specimen Accountability Consecutive Cases

	MD Anderson	Tampere	Total
Total Cases[1]	N = 110	N = 116	N = 226
Primary	103	116	219
Secondary	7	0	7
Fisher's Exact Test	0.006		
Ductal	86	89	175
Lobular	6	20	26
Other histologic type	18	7	25
Fisher's Exact Test	0.002		
# of Positive Nodes			
N[2]	106	See Table 42	106
Mean (SD)	1.41 (4.06)	See Table 42	1.41 (4.06)
Range	0.00, 25.0	See Table 42	0.00, 25.0
Tumor Size			
N[2]	110	See Table 42	110

Mean (SD)	1.75 (1.56)	See Table 42	1.75 (1.56)
Range	0.15, 10.5	See Table 42	0.15, 10.5

1 Number of consecutive cases provided by the corresponding Study Sites.
2 Smaller total number of subjects for each parameter indicates missing values.

Table 42: Specimen Accountability Consecutive Cases; supplemental information from Tampere

Summary of Tumor Size			Summary of Nodal Status		
No data available	1	0.86%	No data available	3	2.59%
T1	78	67.24%	0	69	59.48%
T2	27	23.28%	1	33	28.45%
T3	5	4.31%	2	8	6.90%
T4	5	4.31%	3	3	2.59%

Table 42 shows one of the 116 (0.86%) consecutive cases from the Tampere supplemental information had no data on tumor size. Three of the 116 cases (2.59%) have no nodal status in the reports. One of these 3 cases (B-010) was reported to have distant metastasis.

The MD Anderson Cancer Center also selected 60 Supplemental Cases that showed an IHC2+ score for IHC testing (antibody AB8, Neomarkers) during patient care at the MD Anderson Cancer Center. A summary of specimen characteristics for the Supplemental Cases for this study are shown in Table 43.

Table 43: Specimen Accountability Supplemental Cases from MD Anderson

	MD Anderson
Total Cases[1]	N = 60
Primary	56
Secondary	4
Ductal	52
Lobular	4
Other histologic type	4
# of Positive Nodes	
N[2]	58
Mean (SD)	0.81 (2.57)
Range	0.00, 18.00
Tumor Size	
N[2]	59
Mean (SD)	1.75 (1.53)
Range	0.15, 7.50

1 Number of supplemental cases provided by the corresponding Study Site.
2 Smaller total number of subjects for each parameter indicates missing values.

1. Consecutive Cases

Table 44, summarizes the staining distribution of all consecutive cases for the concordance analyses. The invalid cases were the result of failed staining twice during test sample preparation or no invasive tumor in the tissue section.

Table 44: Accountability of IHC, FISH, and CISH Tests Consecutive Cases Examined by the Study Sites

	Consecutive Case Series	
	# Cases	Percent
IHC test successfully completed	221	97.8%
IHC test invalid	5	2.2%
Test Not Available	0	0.0%
Total (N)	226	100.0%
FISH test successfully completed	220	97.3%
FISH test invalid	6	2.7%
Test Not Available	0	0.0%
Total (N)	226	100.0%
CISH test successfully completed	209	92.5%
CISH test invalid	17	7.5%
Test Not Available	0	0.0%
Total (N)	226	100.0%

A total of 226 cases were available for IHC, FISH, and CISH tests. IHC was successfully completed for 221 (97.8%), FISH for 220 (97.3%), and CISH for 209 (92.5%) of 226 cases. A summary of the reasons the tests were invalid are provided in Table 45.

Table 45: Reasons for Invalid IHC, FISH, and CISH Tests

	# IHC	# FISH	# CISH
Failed staining twice	0	2	14
No invasive tumor	4	4	3
Morphology, signal intensity, or nuclear morphology is poor	1	0	0
Missing cases, no slides	0	0	0

A summary of the distribution of CISH and FISH test results in relation to the HercepTest™ scores, based on the manufacturers' guidelines, are included in Table 46.

Table 46: Results of IHC, FISH, and CISH

Protein Expression		**0**	**1**	**2**	**3**	**Total**
IHC, HercepTest™ Score	(N)	141	19	21	40	221
	(%)[1]	63.8%	8.6%	9.5%	18.1%	100%
Gene Ratio with FISH HER2 status						
Number of valid FISH cases[2]		140	19	21	38	218
Amplified (n,%)[3]		1 (0.5)	0 (0.0)	5 (2.3)	31 (14.2)	37
Non-amplified (n,%)[3]		139 (63.8)	19 (8.7)	16 (7.3)	7 (3.2)	181

Gene Copies with CISH HER2 status					
Number of valid CISH cases[2]	132	17	19	38	206
Amplified (n,%)[3]	1 (0.5)	0 (0.0)	3 (1.5)	32 (15.5)	36
Non-amplified (n,%)[3]	131 (63.6)	17 (8.3)	16 (7.8)	6 (2.9)	170

[1] % = N ÷ Total N × 100%

[2] Number of valid FISH (or CISH) cases for valid IHC cases with the corresponding IHC grade.

[3] % = n ÷ Total number of valid FISH (or CISH) cases × 100%

a. Comparison study with the HercepTest™

Table 47: Agreement between CISH and IHC

CISH Result	IHC Result		Total
	Positive (3+)	Negative (<3+)	
Amplified	32	4	36
Non-amplified	6	164	170
Total	38	168	206

20 cases were reported with either missing or invalid IHC or CISH test outcomes and were excluded from the table.

Positive agreement	=	**32/38**	**= 84.2%**	**(95% CI: 68.8%, 94.0%)**
Negative agreement	=	**164/168**	**= 97.6%**	**(95% CI: 94.0%, 99.4%)**
Total percentage agreement	=	**(32+164)/206**	**= 95.1%**	**(95% CI: 91.3%, 97.7%)**

b. Comparison study with the PathVysion™ HER2 DNA Probe Kit

Table 48: Agreement between FISH and IHC

FISH Result	IHC Result		Total
	Positive (3+)	Negative (<3+)	
Amplified	31	6	37
Non-amplified	7	174	181
Total	38	180	218

8 cases were reported with either missing or invalid IHC or FISH test outcomes and were excluded from the table.

Positive agreement	=	**31/38**	**= 81.6%**	**(95% CI: 65.7%, 92.3%)**
Negative agreement	=	**174/180**	**= 96.7%**	**(95% CI: 92.9%, 98.8%)**
Total percentage agreement	=	**(31+174)/218**	**= 94.0%**	**(95% CI: 90.0%, 96.8%)**

Table 49 presents the agreement of CISH and FISH using HercepTest™ 3+ as a reference. The overall concordance was 99.0% (95%CI: 96.5%-99.9%). Additionally, the positive and negative agreements were 94.4% and 100%, respectively.

Table 49: Agreement between CISH and FISH

CISH Result	FISH Result		Total
	Amplified	Non-amplified	
Amplified	34	0	34
Non-amplified	2	169	171
Total	36	169	205

21 cases were reported with either missing or invalid FISH or CISH test outcomes and were

excluded from the table.

Positive agreement	=	**34/36**	**= 94.4%**	**(95% CI: 81.3%, 99.3%)**
Negative agreement	=	**169/169**	**= 100.0%**	**(95% CI: 97.8%, 100.0%)**
Total percentage agreement	=	**(34+169)/205**	**= 99.0%**	**(95% CI: 96.5%, 99.9%)**

Table 50 summarizes the two discordance cases between CISH and FISH. CISH and FISH data are presented as mean and range, and the IHC result is represented by the HercepTest™ score.

Table 50: Discordance Cases between CISH and FISH

HER2 CISH (Pos)/FISH (Neg)				HER2 CISH (Neg)/FISH (Pos)			
Case #	**CISH**	**FISH**	**IHC**	**Case #**	**CISH**	**FISH**	**IHC**
				(2, 0.98%)[1]			
				A-095-01	4.07 (1.00 - 10.00)	2.81 (1.67 - 5.00)	2+
				B-008	2.77 (2.00 - 4.00)	2.65 (1.00 - 6.00)	2+

1 % = number of discordance cases divided by total number of cases with valid FISH and CISH from the corresponding site × 100%

The discordance rate was low at 0.98%. The 2 discordance cases were from the IHC2+ cases. CISH scoring for both of these cases indicated HER2 gene non-amplification, whereas, the FISH results showed HER2 gene amplification.

2. Supplemental Cases

Concordances for CISH vs. FISH were examined at each study site: MD Anderson, Tampere, and Invitrogen. Tables 51 and 52 show the correlation of PathVysion™ HER2 DNA probe Kit test to the SPOT-Light® HER2 CISH™ Kit test results for the stated number of samples at each site, the scoring methods are based on the respective manufacturers' guidelines.

CISH and FISH testing for the MD Anderson site as presented in Table 51 indicate an overall concordance level of 96.3% (95%CI:87.3% -99.6%). Additionally, the positive and negative agreements were 75.0% and 100%, respectively

Table 51: Agreement between CISH and FISH at MD Anderson on IHC2+ Cases

CISH Results	FISH Results		Total
	Amplified	Non-amplified	
Amplified	6	0	6
Non-amplified	2	46	48
Total	8	46	54

6 cases were reported with either missing or invalid results and were not included in the table.

Positive agreement	=	**6/8**	**= 75.0%**	**(95% CI: 34.9%, 96.8%)**
Negative agreement	=	**46/46**	**= 100.0%**	**(95% CI: 92.3%, 100.0%)**
Total percentage agreement	=	**(6+46)/54**	**= 96.3%**	**(95% CI: 87.3%, 99.6%)**

The results in Table 52, for the supplemental cases tested by the Tampere site, showed a overall concordance level of 92.9% (95%CI:82.7%-98.0%). Additionally, the positive and negative agreements were 77.8% and 95.7%, respectively

Table 52: Agreement between CISH and FISH at Tampere IHC2+ Cases

CISH Results	FISH Results		
	Amplified	Non-amplified	Total
Amplified	7	2	9
Non-amplified	2	45	47
Total	9	47	56

4 cases were reported with either missing or invalid results and were not included in the table.

Positive agreement	=	**7/9**	**= 77.8%**	**(95% CI: 40.0%, 97.2%)**
Negative agreement	=	**45/47**	**= 95.7%**	**(95% CI: 85.5%, 99.5%)**
Total percentage agreement	=	**(7+45)/56**	**= 92.9%**	**(95% CI: 82.7%, 98.0%)**

Table 53 summarizes the 6 discordance cases between CISH and FISH. The CISH and FISH data are presented as mean and range, and the IHC data shows the HercepTest™ score based on retested results with the HercepTest™ Kit.

Table 53: Discordance IHC2+ Cases between CISH and FISH

HER2 CISH (Pos)/FISH (Neg)				HER2 CISH (Neg)/FISH (Pos)			
Case #	CISH	FISH	IHC	Case #	CISH	FISH	IHC
MD Anderson (2, 3.70%)[1]							
				S-156-01	2.77 (1.00 - 5.00)	2.48 (0.50 - 5.00)	2+
				S-173-01	3.67 (1.00 - 7.00)	2.34 (0.50 - 8.00)	2+
Tampere (4, 7.14%)[1]							
S-171	5.20 (2.00 - 8.00)	1.08 (0.50 - 2.00)	3+	S-156	5.00 (1.00 - 9.00)	2.23 (1.00 - 5.00)	3+
S-178	20.00 (20.00 - 20.00)	1.25 (0.50 - 2.00)	0	S-183	4.13 (3.00 - 6.00)	2.73 (1.00 - 6.00)	0

1 % = number of discordance cases divided by total number of cases with valid FISH and CISH from the corresponding site × 100%

The discordance cases from the MD Anderson site showed IHC2+, and HER2 gene amplification for FISH, whereas, CISH tests showed HER2 gene non-amplification. There were 4 discordance cases from the Tampere site for the supplemental cases. Two of the 4 cases were HER2 positive (IHC3+) for IHC test, one case was HER2 gene amplified for CISH, but non-amplified for FISH, and the other case was HER2 gene non-amplified for CISH, but amplified for FISH. The other 2 cases were HER2 negative (IHC 0) with the IHC tests, one was HER2 non-amplified for CISH, but HER2 amplified for FISH, and the other was HER2 amplified for CISH, but HER2 non-amplified for FISH

2. Subgroup Analysis

a. IHC2+ Cases

The IHC2+ supplemental cases were combined with the IHC2+ consecutive cases for analysis, and this group was designated as IHC2+. The results were analyzed and presented for each study site.

The results of the IHC2+ cases for the MD Anderson site are presented in Table 54. The overall concordance rate was 94.5% (95%CI: 86.6% - 98.5%). Additionally, the positive and negative agreements were 69.2% and 100.0%, respectively

Table 54: Agreement between CISH and FISH at MD Anderson IHC2+ Cases

CISH Results	FISH Results		Total
	Amplified	Non-amplified	
Amplified	9	0	9
Non-amplified	4	60	64
Total	13	60	73

8 cases were reported with either missing or invalid FISH or CISH test outcomes and were excluded from the table.

Positive agreement	=	**9/13**	**= 69.2%**	**(95% CI: 38.6%, 90.9%)**
Negative agreement	=	**60/60**	**= 100.0%**	**(95% CI: 94.0%, 100.0%)**
Total percentage agreement	=	**(9+60)/73**	**= 94.5%**	**(95% CI: 86.6%, 98.5%)**

The results in Table 55, for the Tampere site, the overall agreement between the CISH and FISH methods was 92.1% (95%CI:83.6%-97.1%) Additionally, the positive and negative agreements were 76.9% and 95.2%, respectively

Table 55: Agreement between CISH and FISH at Tampere IHC2+ Cases

CISH Results	FISH Results		
	Amplified	Non-amplified	Total
Amplified	10	3	13
Non-amplified	3	60	63
Total	13	63	76

5 cases were reported with either missing or invalid FISH or CISH test outcomes and were excluded from the table.

Positive agreement	=	**10/13**	**= 76.9%**	**(95% CI: 46.2%, 95.0%)**
Negative agreement	=	**60/63**	**= 95.2%**	**(95% CI: 86.7%, 99.0%)**
Total percentage agreement	=	**(10+60)/76**	**= 92.1%**	**(95% CI: 83.6%, 97.1%)**

Table 56: Discordance IHC2+Cases between CISH and FISH

HER2 CISH (Pos)/FISH (Neg)				HER2 CISH (Neg)/FISH (Pos)			
Case #	CISH	FISH	IHC	Case #	CISH	FISH	IHC
MD Anderson (4, 5.48%)[1]							
				A-095-01	4.07 (1.00 - 10.00)	2.81 (1.67 - 5.00)	2+
				B-008-08	1.77 (1.00 - 4.00)	2.45 (1.00 - 5.00)	2+
				S-156-01	2.77 (1.00 - 5.00)	2.48 (0.50 - 5.00)	2+
				S-173-01	3.67 (1.00 - 7.00)	2.34 (0.50 - 8.00)	2+
Tampere (6, 7.89%)[1]							
A-023	5.20 (2.00 - 8.00)	1.49 (0.67 - 3.50)	2+	B-008	2.77 (2.00 - 4.00)	2.65 (1.00 - 6.00)	2+
S-171	5.20 (2.00 - 8.00)	1.08 (0.50 - 2.00)	3+	S-156	5.00 (1.00 - 9.00)	2.23 (1.00 - 5.00)	3+
S-178	20.00 (20.00 - 20.00)	1.25 (0.50 - 2.00)	0	S-183	4.13 (3.00 - 6.00)	2.73 (1.00 - 6.00)	0

1 % = number of discordance cases divided by total number of cases with valid FISH and CISH from the corresponding site × 100%

There were 6 discordance cases with IHC2+: 4 from the MD Anderson site and 2 from the Tampere site. Five of the 6 IHC2+ cases were HER2 gene non-amplified with CISH test, but were HER2 gene-amplified with FISH test; the remaining 1 case was HER2 amplified with CISH, but was HER2 non-amplified with FISH. There were 2 cases with IHC3+: one case was HER2 gene non-amplified with CISH, but was HER2 amplified with FISH, and the other case was HER2 gene amplified with CISH, but HER2 non-amplified with FISH. There were two IHC 0 cases: one was HER2 gene non-amplified with CISH and HER2 amplified with FISH; and the other was HER2 gene-amplified with CISH and HER2 non-amplified with FISH.

b. Equivocal Cases

The supplemental cases that were IHC2+ based on the re-tested results, using the HercepTest™ Kit, were combined with the consecutive cases that were IHC 2+. These results are summarized in Table 57 and 58.

Table 57: Agreement between CISH and FISH on HercepTest 2+ Cases at MD Anderson

CISH Results	FISH Results		Total
	Amplified	Non-amplified	
Amplified	3	0	3
Non-amplified	4	27	31
Total	7	27	34

2 cases were reported with either missing or invalid FISH or CISH test outcomes and were excluded from the table.

Positive agreement	=	**3/7**	**= 42.9%**	**(95% CI: 9.9%, 81.6%)**
Negative agreement	=	**27/27**	**= 100.0%**	**(95% CI: 87.2%, 100.0%)**
Total percentage agreement	=	**(3+27)/34**	**= 88.2%**	**(95% CI: 72.6%, 96.7%)**

The concordance level for the MD Anderson site presented in Table 57. The overall agreement was 88.2% (95% CI:72.6% -96.7%). In addition,

the results in Table 58, for the Tampere site, showed an overall agreement between the CISH and FISH methods with a concordance of 95.1% (95% CI:83.5-99.4%).

Table 58: Agreement between CISH and FISH on HercepTest 2+ Cases at Tampere

CISH Results	FISH Results		Total
	Amplified	Non-amplified	
Amplified	4	1	5
Non-amplified	1	35	36
Total	5	36	41

2 cases were reported with either missing or invalid FISH or test outcomes and were excluded from the table.

Positive agreement	=	**4/5**	**= 80.0%**	**(95% CI: 28.4%, 99.5%)**
Negative agreement	=	**35/36**	**= 97.2%**	**(95% CI: 85.5%, 99.9%)**
Total percentage agreement	=	**(4+35)/41**	**= 95.1%**	**(95% CI: 83.5%, 99.4%)**

Table 59 summarizes the 6 discordance cases between CISH and FISH. CISH and FISH data are presented as the mean and range, and IHC as the HercepTest™ score.

Table 59: HercepTest 2+ Discordance Cases between CISH and FISH

HER2 CISH (Pos)/FISH (Neg)				HER2 CISH (Neg)/FISH (Pos)			
Case #	CISH	FISH	IHC	Case #	CISH	FISH	IHC
MD Anderson (4, 11.76%)[1]							
				A-095-01	4.07 (1.00 - 10.00)	2.81 (1.67 - 5.00)	2+
				B-008-08	1.77 (1.00 - 4.00)	2.45 (1.00 - 5.00)	2+
				S-156-01	2.77 (1.00 - 5.00)	2.48 (0.50 - 5.00)	2+
				S-173-01	3.67 (1.00 - 7.00)	2.34 (0.50 - 8.00)	2+
Tampere (2, 4.88%)[1]							
A-023	5.20 (2.00 - 8.00)	1.49 (0.67 - 3.50)	2+	B-008	2.77 (2.00 - 4.00)	2.65 (1.00 - 6.00)	2+

1 % = number of discordance cases divided by total number of cases with valid FISH and CISH from the corresponding site × 100%

For the 6 discordances cases, the CISH test results showed HER2 gene non-amplification in 5 cases and HER2 gene amplification in 1 case, whereas, the FISH test results showed HER2 gene amplification for 5 cases and HER2 gene non-amplification in 1 case. Thus, the results suggest that the SPOT-Light® HER2 CISH™ Kit test and HercepTest™ tend to yield similar agreement in these cases.

c. **Polysomy Cases**

In addition, to providing comparative data for CISH vs. FISH and CISH vs. IHC, this study examined the CISH performance on tumors that showed polysomy on chromosome 17 with FISH as the additional endpoint. Since the criteria for polysomy has never been clearly stated by the scientific community, the study sites evaluated the polysomy cases based on the respective institutional clinical practices. As such, at the MD Anderson

site, polysomy on chromosome 17 was defined as the presence of ≥3 CEP17 signals in at least 10% of the tumor cells, whereas, the criteria at the Tampere site, was the presence of ≥3 CEP17 signals in at least 30% of the tumor cells. Table 60 summarizes the distribution of polysomy cases at both clinical sites.

Table 60: Number of Polysomy Cases Stratified by the Study Site

Polysomy Cases	MD Anderson		Tampere	
	# Cases[1]	Percent	# Cases[1]	Percent
Consecutive Case	47/218	21.56%	17/221	7.69%
Supplemental Case	4/ 55	7.27%	5/ 57	8.77%
Total	**51/273**	**18.68%**	**22/278**	**7.91%**

1 Numerator = # of Polysomy cases. Denominator = # of consecutive cases (or supplemental cases) with valid FISH results

The frequency of tumors with polysomy on chromosome 17 was 18.68% at the MD Anderson site and 7.91% at the Tampere site. The detection rate of polysomy on the same tumor set varied substantially between the two test sites. This is due to the differences in the definition of polysomy at the respective clinical sites.

When Tampere cases were re-analyzed using the definition of the presence of ≥3 CEP17 signals in at least 10% of the tumor cells, the recalculated polysomy tumor rate at Tampere was 26.98% (75/278). The re-analyzed rate is provided as a reference.

The concordance analysis as detailed below used the original definition at each site and the results are presented for each site separately.

Table 61: Agreement between CISH and FISH Polysomy Cases at MD Anderson

CISH Results	FISH Results		Total
	Amplified	Non-amplified	
Amplified	12	0	12
Non-amplified	2	34	36
Total	14	34	48

3 cases were reported with either missing or invalid FISH or CISH test outcomes and were excluded from the table.

Positive agreement	=	**12/14**	**= 85.7%**	**(95% CI: 57.2%, 98.2%)**
Negative agreement	=	**34/34**	**= 100.0%**	**(95% CI: 89.7%, 100.0%)**
Total percentage agreement	=	**(12+34)/48**	**= 95.8%**	**(95% CI: 85.8%, 99.5%)**

Table 61 represents the polysomy cases from the MD Anderson site. The overall concordance of 95.8% (95% CI: 85.8%-99.5%). The results of polysomy cases tested at the Tampere site, shown in Table 62, indicate an overall agreement of 95.5% (95% CI: 77.2%-99.9%). This data demonstrates a strong agreement between the two test methods.

Table 62: Agreement between CISH and FISH Polysomy Cases at Tampere

CISH Results	FISH Results		Total
	Amplified	Non-amplified	
Amplified	12	1	13
Non-amplified	0	9	9
Total	12	10	22

0 cases were reported with either missing or invalid FISH or CISH test outcomes and were excluded from the table.

Positive agreement	**= 12/12**	**= 100.0%**	**(95% CI: 73.5%, 100.0%)**
Negative agreement	**= 9/10**	**= 90.0%**	**(95% CI: 55.5%, 99.8%)**
Total percentage agreement	**= (12+9)/22**	**= 95.5%**	**(95% CI: 77.2%, 99.9%)**

The discordance cases between the CISH and FISH test methods for the polysomy cases are summarized in Table 63. CISH and FISH data are presented as the mean and range, and IHC shows the HercepTest™ score.

Table 63: Discordance Polysomy Cases between CISH and FISH

HER2 CISH (Pos)/FISH (Neg)				HER2 CISH (Neg)/FISH (Pos)			
Case #	CISH	FISH	IHC	Case #	CISH	FISH	IHC
MD Anderson (2, 4.17%)[1]							
				A-095-01	4.07 (1.00 - 10.00)	2.81 (1.67 - 5.00)	2+
				S-156-01	2.77 (1.00 - 5.00)	2.48 (0.50 - 5.00)	2+
Tampere (1, 4.55%)[1]							
A-023	5.20 (2.00 - 8.00)	1.49 (0.67 - 3.50)	2+				

1 % = number of discordance cases divided by total number of cases with valid FISH and CISH from the corresponding site × 100%

The three polysomy tumors were IHC2+. Two cases were HER2 gene non-amplified with the CISH test, but were HER2 gene amplified with FISH. The remaining case was CISH HER2 gene amplified, but HER2 gene non-amplified with FISH.

d. CISH Reproducibility Study

The site-to-site CISH reproducibility study used the consecutive cases and was performed by all three test sites. The consecutive cases consisted of 226 cases: 110 cases from the MD Anderson site and 116 cases from the Tampere site. The specimen accountability for CISH reproducibility testing is summarized in Table 64.

Table 64: CISH Reproducibility Accountability for All Consecutive Cases Stratified by Study Site

	MD Anderson	Tampere	Invitrogen
CISH test successfully completed	207 (91.6%)	223 (98.7%)	224 (99.1%)
CISH test invalid	19 (8.4%)	3 (1.3%)	2 (0.9%)
Total	226 (100.0%)	226 (100.0%)	226 (100.0%)

For the CISH reproducibility study, the cases with successful staining for CISH at both sites were used for comparison. The results are shown in Tables 65-67.

Table 65: CISH Reproducibility for All Consecutive Cases Examined by MD Anderson and Tampere

MD Anderson CISH Results	Tampere: CISH Results		Total
	Amplified	Non-amplified	
Amplified	34	0	34
Non-amplified	2	170	172
Total	36	170	206

20 cases were reported with either missing or invalid CISH test outcomes and were excluded from the table.

Total percentage agreement = (34+170)/206 = 99.0% (95% CI: 96.5%, 99.9%)

Table 66: CISH Reproducibility for All Consecutive Cases Examined by Invitrogen and Tampere

Invitrogen CISH Results	Tampere: CISH Results		Total
	Amplified	Non-amplified	
Amplified	35	0	35
Non-amplified	3	184	187
Total	38	184	222

4 cases were reported with either missing or invalid CISH test outcomes and were excluded from the table.

Total percentage agreement = (35+184)/222 = 98.6% (95% CI: 96.1%, 99.7%)

Table 67: CISH Reproducibility for All Consecutive Cases Examined by Invitrogen and MD Anderson

Invitrogen CISH Results	MD Anderson: CISH Results		Total
	Amplified	Non-amplified	
Amplified	32	1	33
Non-amplified	3	171	174
Total	35	172	207

19 cases were reported with either missing or invalid CISH test outcomes and were excluded from the table.

Total percentage agreement = (32+171)/207 = 98.1% (95% CI: 95.1%, 99.5%)

Total agreements for CISH reproducibility across three comparison sets were 99.0%, 98.6%, and 98.1% indicating strong reproducibility for the SPOT-Light® HER2 CISH™ Kit test.

A summary of the SPOT-Light® HER2 CISH Kit and the PathVysion™ HER2 DNA Probe kit cases are included in Table 68.

Table 68: Summary of SPOT-Light® HER2 CISH Kit and the PathVysion™ HER2 DNA Probe kit.

Type of Cases	Consecutive Case	Supplemental Case		IHC2+ Cases		EquivocalCases	
		MD Anderson	Tampere	MD Anderson	Tampere	MD Anderson	Tampere
Number of Samples	205	54	56	73	76	34	41
Positive % agreement	94.4%	75.0%	77.8%	69.2%	76.9%	42.9%	80.0%
Negative % agreement	100.0%	100.0%	95.7%	100.0%	95.2%	100.0%	97.2%
Total % agreement	99.0%	96.3%	92.9%	94.5%	92.1%	88.2%	95.1%

Summary:

The results from the Invitrogen Clinical Study demonstrated the following:

A. CISH vs. FISH

There is strong agreement between the SPOT-Light® HER2 CISH™ Kit test and the PathVysion™ HER2 test as evidenced by the agreement rate of 99.0% for the Consecutive Cases.

The SPOT-Light® HER2 CISH™ Kit test, as supported by the results from the Supplemental Cases, show the resulting concordances for CISH and FISH at the two study sites were 96.3% and 92.9%, respectively, demonstrating a strong agreement between PathVysion™ HER2 test and SPOT-Light® HER2 CISH™ Kit test.

There was a high level of concordance between the CISH and FISH test methods. This was evidenced by the strong agreement from the analysis of the IHC2+ Cases, with overall agreement of 94.5% and 92.1%, respectively. The concordances at each study site also indicate a strong agreement between CISH and FISH at each study site.

In the Equivocal Cases (IHC2+ based on HercepTest™), the results obtained indicate a good to strong agreement between CISH and FISH on the IHC confirmed equivocal cases. Total agreements were 88.2% and 95.1%, respectively. The analysis of discordance cases indicate that the CISH and IHC test methods tend to yield similar agreement in these borderline cases; that is, HER2 gene non-amplification and negative HER2 protein overexpression of IHC2+.

The concordances for CISH and FISH at both sites for the polysomy cases indicate a strong agreement between the two test methods. This is based on polysomy tumors evaluated independently at both the MD Anderson and Tampere sites. The resulting concordances for FISH and CISH are 95.8% and 95.5%, respectively. This indicates a high overall concordance between the two methods.

B. CISH vs. IHC

The SPOT-Light® HER2 CISH™ Kit test is in strong agreement to HerceptTest™, which is supported by the high concordance level of 95.1%. Additionally, the same conclusion was reached independently by analyzing the results at each study site.

C. Site-to-Site Reproducibility

The SPOT-Light® HER2 CISH™ Kit test has a very high site-to-site reproducibility rate with an overall site-to-site reproducibility agreement of 98.6% based on the reproducibility study. The total agreements for CISH reproducibility across three comparison sets were 99.0%, 98.6%, and 98.1%. The results indicate a strong agreement between the two study sites in the comparison series. When compared to FISH reproducibility, the CISH reproducibility of 99.03% is similar to FISH reproducibility of 99.07% at the same study sites.

D. Benefit/Risk

The safety and effectiveness of the SPOT-Light® HER2 CISH™ Kit was demonstrated by the Invitrogen sponsored clinical study. The Invitrogen study provided comparative data between the SPOT-Light® HER2 CISH™ Kit method and PathVysion™ FISH method, along with data from the HercepTest™ method.

Potential risks of the SPOT-Light® HER2 CISH™ Kit are associated with incorrect result interpretations. A false positive test result would likely assign patients to receive a more aggressive adjuvant therapy regimen than needed, possibly exposing the patient to serious side effects and, in rare cases, death.

Alternatively, a false negative test result may exclude a patient who might benefit from therapy, potentially resulting in a poor outcome.

The SPOT-Light® HER2 CISH™ Kit is safe and effective when used according to the manufacturer's instructions and for its approved intended use (together with other clinical and pathological information) for HER2 gene amplification in formalin-fixed, paraffin-embedded (FFPE) tissue sections using Chromogenic *In Situ* Hybridization (CISH).

XI. Conclusions Drawn From the Studies

A. Safety conclusions

The SPOT-Light® HER2 CISH™ Kit is an *in vitro* diagnostic test and does not transfer hazardous material into the patient. As a diagnostic test, the SPOT-Light® HER2 CISH™ Kit involves testing on formalin-fixed, paraffin-embedded human breast cancer tissue sections. Instructions for the safe use of the product are included in the package insert, when used according to the stated instructions and in conjunction with clinical and other laboratory information, therefore the assay presents no additional safety hazard to the patient being tested.

B. Effectiveness conclusions

The efficacy of the CISH kit was assessed by the total agreement rate between CISH and FISH as well as the total agreement rate between CISH and IHC.
The Pre-clinical and Clinical results provide valid scientific evidence of the clinical utility and support the SPOT-Light® HER2 CISH™ Kit testing as an alternative to the PathVysion™ FISH and HercepTest™ IHC testing.

C. Overall Conclusions

The results of the clinical study to assess the performance of the SPOT-Light® HER2 CISH™ Kit test establish reasonable assurance that this test is safe and effective for its intended use when utilized in accordance with product labeling.

XII. Panel Recommendation

In accordance with the provisions of Section 515 (c (2) of the Act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Devices Panel, an FDA advisory committee, for review and recommendation.

XIII. CDRH Decision

CDRH issued an approval order on July 1, 2008. The applicant's manufacturing facility was inspected on January 9-25, 2008 and was found to be in compliance with the device Quality System Regulation (21 CFR 820).

SUMMARY OF SAFETY and EFFECTIVENESS (SSED)

XIV. Approval Specifications

Directions for Use: see device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Positive-approval Requirements and Restrictions: see Approval Order