



PATHOGENESYS LLC

**Method Validation Study:  
Immunohistochemistry for EGFr**

**Protocol No. PATH-0016**

**Version 01  
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**PROTOCOL**

**PATH-0016**

**Study Title**

**Method Validation Study:  
Immunohistochemistry for EGFR**

**Goal**

To characterize the performance specifications of an immunohistochemical assay for EGFR on paraffin sections.

**Study Director**

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**Sponsor**

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**Testing Facility**

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**Study Type**

non-GLP study  
Quality Auditor –N/A

**Approvals**

IRB approvals- PATH-0001

## I. Introduction

The analysis to detect the presence of HER1 (c-erb-b1, epidermal growth factor receptor, EGFr) antigen in paraffin sections of human tissues described herein is intended for in vitro diagnostic use and whose performance characteristics are confirmed in compliance with 21 CFR Part 803. This test utilizes the FDA-approved diagnostic kit from DakoCytomation. The intended use of this kit includes the use as a semi-quantitative, Class II immunohistochemical assay to determine the over-expression of EGFr. In addition, to whatever extent this test may be useful for the determination of whether the presence of EGFr may be predictive of responses to other forms of therapy, this assay is qualified for use as a Class I semi-quantitative measure for EGFr expression in various human tissues. This latter use is intended to limit potential harm to patients while qualifying this test as a “building block” toward establishment of performance characteristics of this test as a biomarker of other therapies.

In as such, the performance characteristics of this test are studied in compliance with the Guidance for Submission of Immunohistochemistry Applications to the FDA.

As with any immunohistochemical based test, the limitations may include some degree of false positive and false negative reactions, often as a result of the untoward effects of pre-analytical processing. Therefore, it should be emphasized that the use of this test should only be employed by well-trained and Board-certified Pathologists during the histologic examination phase. Histologic interpretation by adequately trained individuals can be useful towards the recognition and ultimate disposition of problematic cases.

## II. Objective and Plan:

The objective of this study is to verify the performance specifications of this kit’s ability to measure EGFr in formalin-fixed, paraffin-embedded human tissue. Successful verification of this test will pave the way for use of this test in the conduct of clinical trials designed to ascertain the test’s full potential, for the identification of EGFr positive tissues, and as a tool for consideration of cancer treatment.

This validation study involves the characterization of the performance specification including the analysis of test precision, sensitivity, and specificity.

## III. Detailed Methodology Description

### A. Analyte Measurement:

- a. This assay is performed in strict accordance with the instructional guide provided with the kit and Pathogenesis SOPs.
  - i. Anti-EGFr clone, 2-18C9, antibody titer, and antigen-retrieval have been pre-established by DakoCytomation as part of the DAKO EGFR pharmDx kit.

### B. Validation Controls:

- a. Paraffin-embedded Human normal tissues and Human breast carcinoma.
  - i. In some cases, 1 mm fragments of normal or diseased tissues have been embedded as tissue microarrays, containing no more than 12 tissue fragments per block/slide.

### C. Specificity Analysis:

- a. The specificity of this test is confirmed by the examination of sections from formalin-fixed, paraffin-embedded blocks representing over 20 different normal tissues, of which the pattern of reactivity is generally known. This includes the analysis of specific tissues from three different individual Human Subjects.
- b. It is expected that this assay demonstrates a weak to high levels of immunoreactivity on most epithelial tissue, especially squamous epithelium, liver, kidney, and prostate. Perineureum of peripheral nerves have high levels of immunoreactivity. Signal is expected to be absent in brain, muscle, lymphoid tissues, and bone marrow. Weak reactivity can be expected in most tissues that contain reactive fibroblasts.

**D. Sensitivity Analysis:**

- a. The sensitivity of this test is confirmed by the examination of sections from formalin-fixed, paraffin-embedded blocks of over 20 different breast carcinoma tissues.
- b. It is expected that this assay demonstrates some degree of EGFR expression in over 70% of all breast cancer cases. Depending upon the number and nature of the selected specimens for this analysis, a rate slightly higher or lower than expected may result from statistical under-representation of one or more sub-groups.

**E. Reproducibility:**

- a. The reproducibility of this test is confirmed by the examination of sections containing several tissue fragments, stained on several different days. This analysis should include up to 20 different data points to show statistical significance.
- b. It is expected that this assay demonstrates a good interassay precision at the level of the determination of positive versus negative (less than 20% CV) to qualify this test as semi-quantitative.

**IV. Specificity Analysis:**

- A. The table below contains the result of interpretation of slides stained as described above by a Board-certified Pathologist and representing results from 20 normal tissues stained by Immunohistochemistry for EGFR expression analysis.

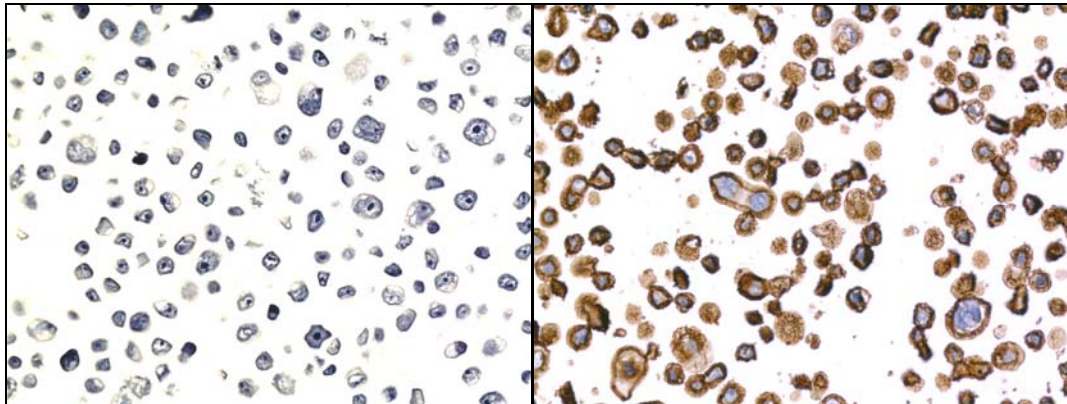
Specimen Number	Specimen Type	Morphology	Normal Present?	Tumor Present?	NTMH	NTCH	NTNH
S3918-90	NORMAL BREAST	Adequate	YES	NO	40	20	0
S5053-90	NORMAL BREAST	Adequate	YES	NO	0	0	0
D4736	NORMAL BREAST	Adequate	YES	NO	120	60	0
D4732	NORMAL BREAST	Adequate	YES	NO	30	60	0
D4716	NORMAL BREAST	Adequate	YES	NO	60	20	0
S4443-90	NORMAL COLON	Adequate	YES	NO	10	0	0
D5037	NORMAL COLON	Adequate	YES	NO	20	20	0
	NORMAL COLON	Adequate	YES	NO	200	100	0
	NORMAL COLON	Adequate	YES	NO	90	50	0
D6304	NORMAL ENDOMETRIUM	Adequate	YES	NO	50	0	0
D4700	NORMAL KIDNEY	Adequate	YES	NO	80	20	0
D4713	NORMAL KIDNEY	Adequate	YES	NO	70	110	0
D4709	NORMAL KIDNEY	Adequate	YES	NO	150	30	0
D4708	NORMAL LIVER	Adequate	YES	NO	160	30	0
D4701	NORMAL LIVER	Adequate	YES	NO	180	150	0
D4739	NORMAL LIVER	Adequate	YES	NO	180	180	0
D4706	NORMAL LUNG	Adequate	YES	NO	150	20	0
D4710	NORMAL LUNG	Adequate	YES	NO	300	30	0
S6795-89	NORMAL LYMPH NODE	Adequate	YES	NO	0	0	0
D6627	NORMAL LYMPH NODE	Adequate	YES	NO	0	0	0
D4725	NORMAL LYMPH NODE	Adequate	YES	NO	0	0	0
D4729	NORMAL PANCREAS	Adequate	YES	NO	40	20	0
D7496	NORMAL PROSTATE	Adequate	YES	YES	60	60	0
D4727	NORMAL SKIN	Adequate	YES	NO	200	70	0
D4734	NORMAL SKIN	Adequate	YES	NO	150	60	0
D4732	NORMAL SKIN	Adequate	YES	NO	280	120	0
D4722	NORMAL SMALL BOWEL	Adequate	YES	NO	50	0	0
D4726	NORMAL SPLEEN	Adequate	YES	NO	0	0	0
D4702	NORMAL SPLEEN	Adequate	YES	NO	0	0	0
D4728	NORMAL SPLEEN	Adequate	YES	NO	0	0	0
D4723	NORMAL STOMACH	Adequate	YES	NO	10	30	0
D4712	NORMAL TESTIS	Adequate	YES	NO	20	20	0
D4733	NORMAL UTERUS	Adequate	YES	NO	20	20	0
D4743	SKELETAL MUSCLE	Adequate	YES	NO	0	0	0

This data was summarized from portions of the raw data (presented in tabular form as Attachment A). The raw data was used to calculate separate H-Scores for membrane staining (Normal Test Article Membrane H-Score or NTMH), for cytoplasm staining (Normal Test Article Cytoplasm H-Score or NTCH), and for nuclear staining (Normal Test Article Nuclear H-Score or NTNH), using the following formula;  $H\text{-Score} = (\% \text{ at } 0) * 0 + (\% \text{ at } 1+) * 1 + (\% \text{ at } 2+) * 2 + (\% \text{ at } 3+) * 3$ .

- B. These results demonstrate a good correlation between the expected expression of EGFR in most epithelial tissues and the results of staining for EGFr. Tissues with the highest levels of and most consistent expression include skin, liver, lung, and colon. Tissues that consistently lack specific staining include lymph node, spleen, skeletal muscle. Skin is a good tissue positive control; since there is strong membrane staining that gradually lightens toward the surface epithelial layers. These results are further summarized below.

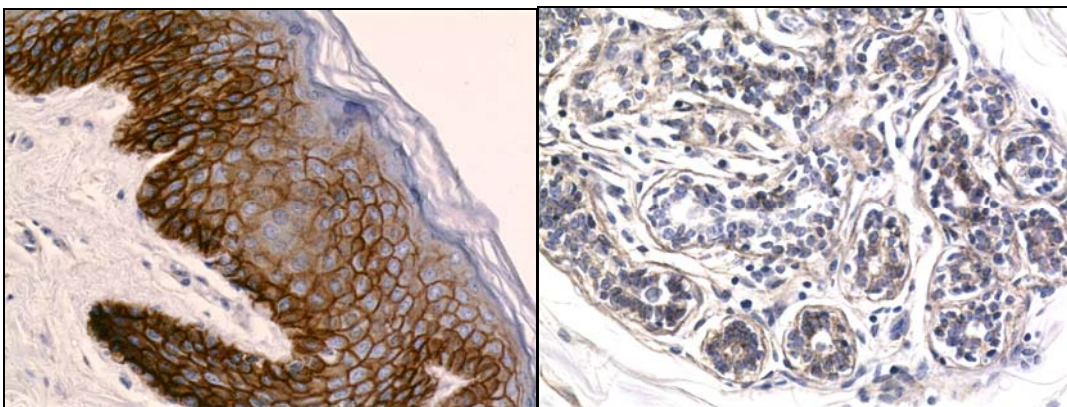
Specimen Type	Percent Positive	Interpretation
NORMAL BREAST	80% (4/5)	Compatible with Literature
NORMAL COLON	100% (4/4)	Confined to Surface
NORMAL ENDOMETRIUM	100% (1/1)	Compatible with Literature
NORMAL KIDNEY	100% (3/3)	Compatible with Literature
NORMAL LIVER	100% (3/3)	Compatible with Literature
NORMAL LUNG	100% (2/2)	Compatible with Literature
NORMAL LYMPH NODE	0% (0/3)	Compatible with Literature
NORMAL PANCREAS	100% (1/1)	Compatible with Literature
NORMAL PROSTATE	100% (1/1)	Compatible with Literature
NORMAL SKIN	100% (3/3)	Compatible with Literature
NORMAL SMALL BOWEL	100% (1/1)	Weak Staining Only
NORMAL SPLEEN	0% (0/3)	Compatible with Literature
NORMAL STOMACH	100% (1/1)	Weak Staining Only
NORMAL TESTIS	100% (1/1)	Leydig Cells Only
NORMAL UTERUS	100% (1/1)	Compatible with Literature
SKELETAL MUSCLE	0% (1/1)	Compatible with Literature

Selected photomicrographs of tissues stained for EGFr are shown below.



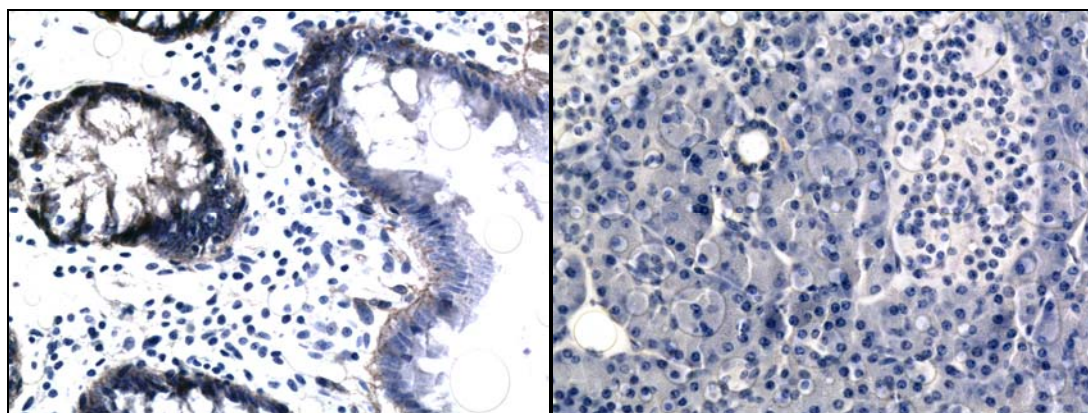
Negative Control Cell Line

Positive Control Cell Line



Normal Human Skin

Normal Human Breast



Normal Human Colon

Normal Human Pancreas

## V. Sensitivity Analysis:

- A. The table below contains the result of interpretation of slides stained as described above by a Board-certified Pathologist and representing results from 20 breast carcinoma cases stained by Immunohistochemistry for EGFR over-expression analysis.

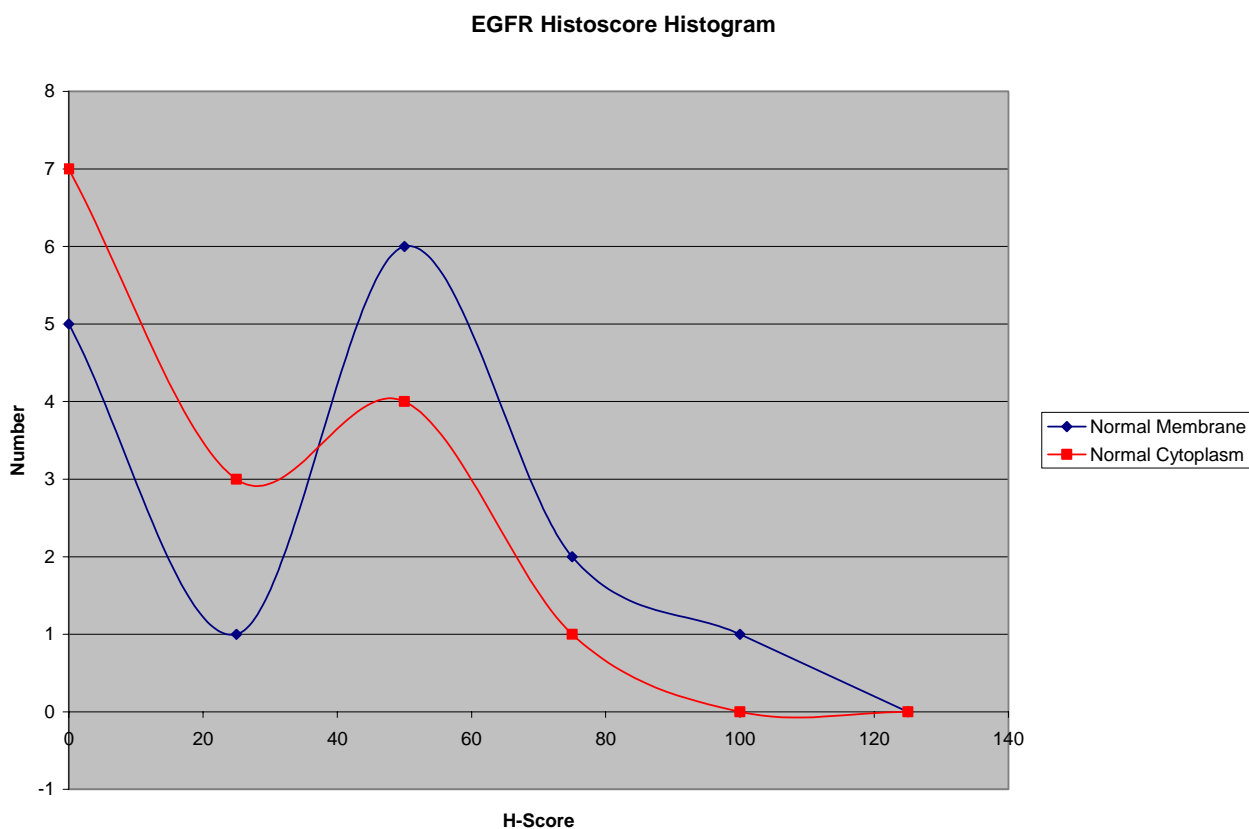
Specimen Number	Specimen Type	Morphology	Normal Present?	Tumor Present?	H-Scores												
					NBMH	NBCH	NBNH	TBMH	TBCH	TBNH	NTMH	NTCH	NTNH	TTMH	TTCH	TTNH	
S4442-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	0	10	0	0	0	0	
S2887-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	10	0	0	10	0		
S7490-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	0	0	0	0	0		
S7559-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	30	10	0	0	10		
S6797-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	0	0	0	0	0		
S6857-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	50	50	0	10	10		
D3859	CERVICAL CA	Adequate	YES	YES	0	0	0	0	0	0	0	0	0	0	0		
D7496	PROSTATE CA	Adequate	YES	YES	0	0	0	0	0	0	60	60	0	10	0		
S6197-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	90	0	0	0	0		
S5907-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	50	50	0	0	10		
S2780-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	30	0	0	10	0		
S4951-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	60	30	0	70	0		
S2298-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	30	0	0	40	0		
S7559-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	50	50	0	0	0		
S5150-90	BREAST CA	Adequate	YES	YES	0	0	0	0	0	0	10	0	0	10	0		
AVERAGE					0	0	0	0	0	0	30.67	18	0	10	2.667		
% POSITIVE					0	0	0	0	0	0	66	53	0	33	26		

This data was summarized from portions of the raw data (presented in tabular form as Attachment B). The raw data was used to calculate separate H-Scores for background (Isotype control) of membrane staining (Normal Background Membrane H-Score or NBMH and Tumor Background Membrane H-Score or TBMH), of cytoplasm staining (Normal Background Cytoplasm H-Score or NBCH and Tumor Background Cytoplasm H-Score or TBCH), and of nuclear staining (Normal Background Nuclear H-Score or NTNH and Tumor Background Nuclear H-Score or TTNH); and for test article staining of membrane (Normal Test Article Membrane H-Score or NTMH and Tumor Test Article Membrane H-Score or TTMH), of cytoplasm staining (Normal Test Article Cytoplasm H-Score or NTCH and Tumor Test Article Cytoplasm H-Score or TTCH), and for nuclear staining (Normal Test Article Nuclear H-Score or NTNH and Tumor Test Article Nuclear H-Score or TTNH), using the following formula;  $H\text{-Score} = (\% \text{ at } 0) * 0 + (\% \text{ at } 1+) * 1 + (\% \text{ at } 2+) * 2 + (\% \text{ at } 3+) * 3$ .

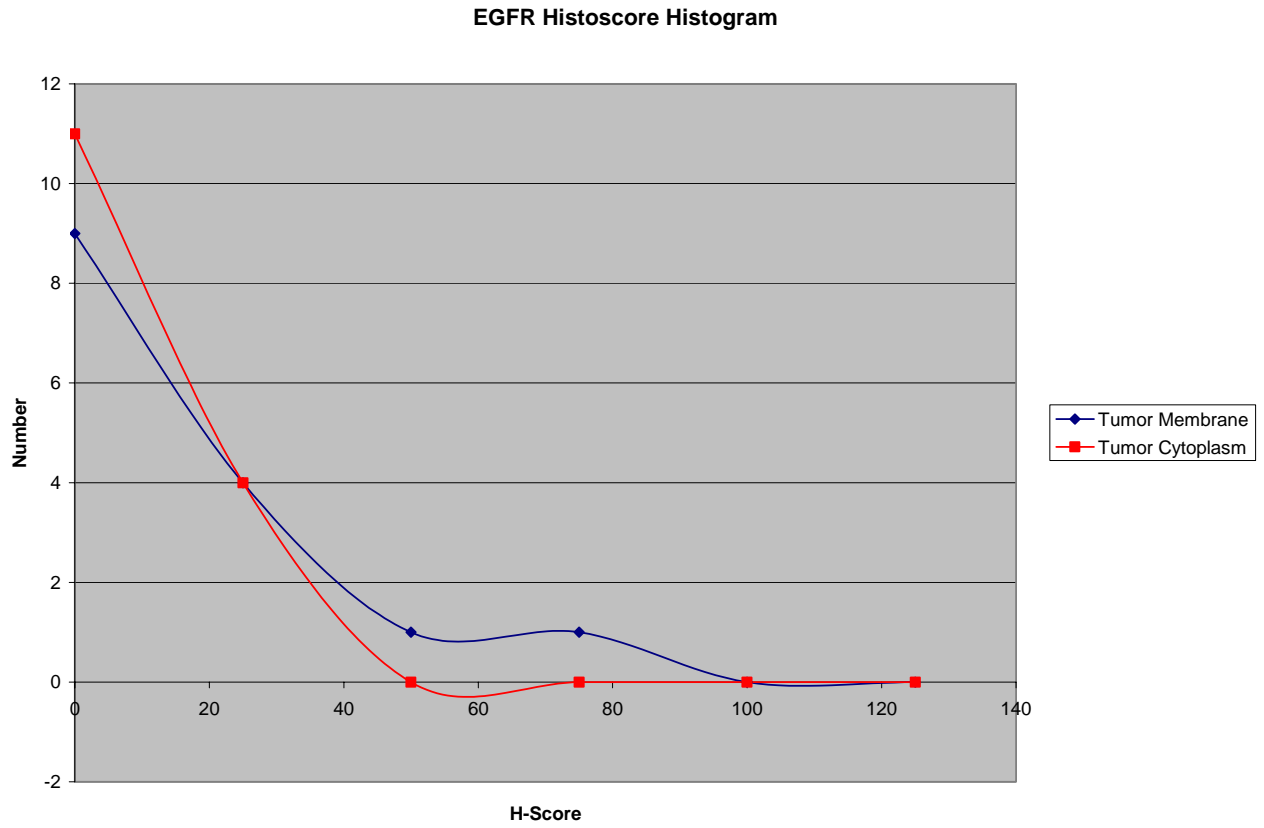
- B. These results demonstrate that the expected rate of expression of EGFR in breast carcinoma as determined by membrane staining was slightly below the expected rate. This is likely due to the unusual amount of specimen over-digestion. In some cases, the tumor cells were so badly over-digested that the slides could not be read (data not included). Too much protease digestion causes

membranes to fall off the slide, making assessment of membranes impossible. Cytoplasmic staining is not expected, but can frequently be seen in association with membrane staining. A few cases, that were negative for membrane staining, were positive for cytoplasmic staining. Inclusion of the cytoplasmic cases with the membrane raises the positivity rate to acceptable levels. Great care should be exercised when scoring cases that have excessive over-digestion.

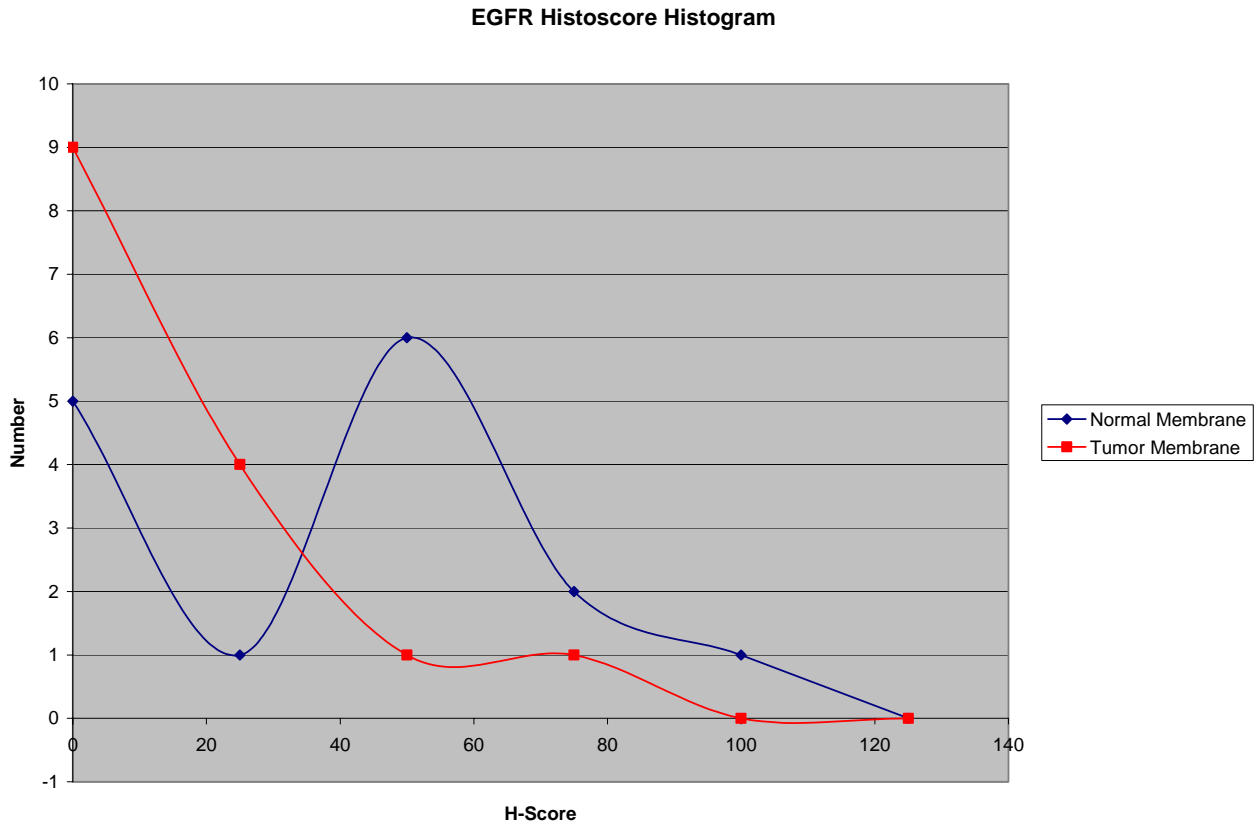
- C. No nuclear staining was observed.
- D. The EGFR positive rate for normal adjacent breast tissue was high, consistent with literature. There was a good trend toward correlation between cytoplasmic staining and membrane staining, although not statistically significant.
- E. Frequency histograms representing the data for staining of carcinoma case is presented in the graphs below.



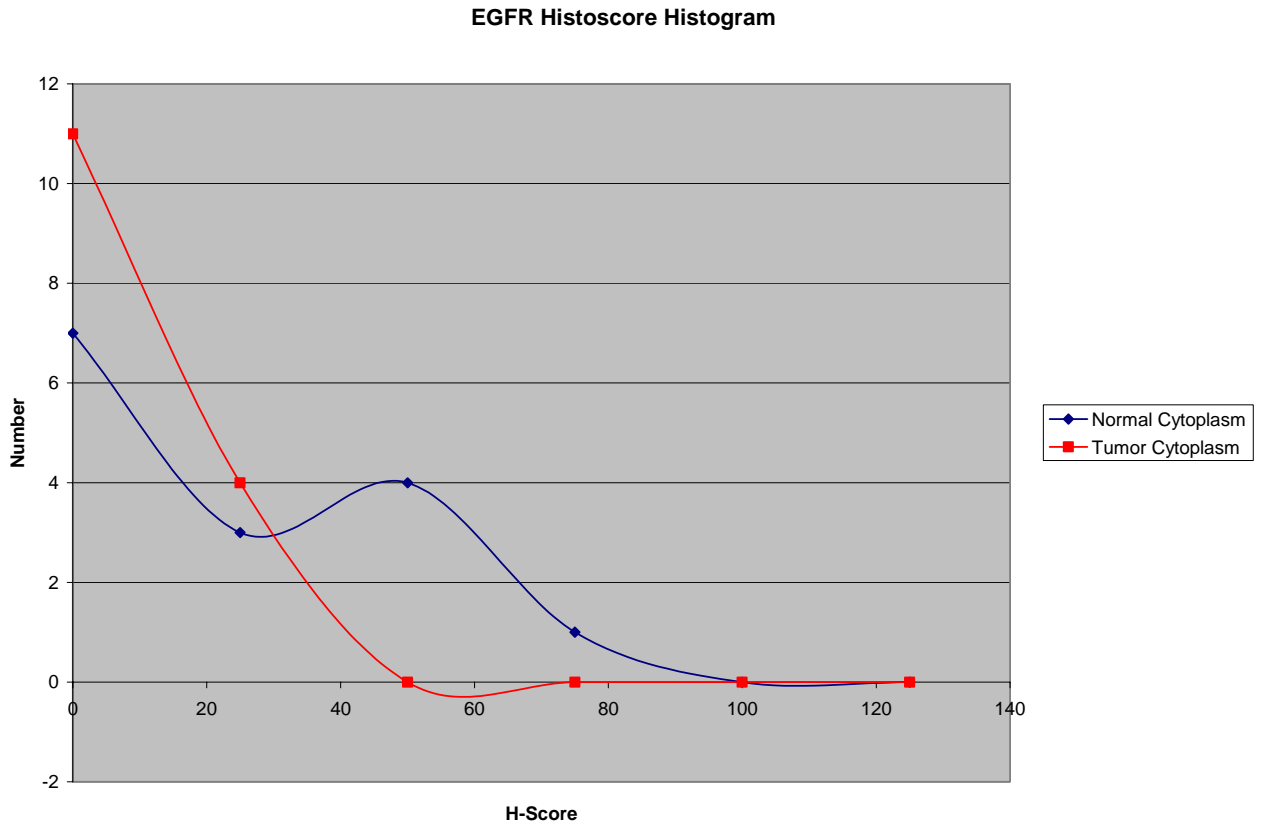
The histogram above of the frequency of membrane and cytoplasmic staining in normal tissues generally confirms the trend of bi-compartment staining mentioned earlier. There appear to be two populations as judge by the degree of staining of normal tissues, one with moderate membrane and/or cytoplasmic EGFR expression and one with weak to no expression (or possible false negatives).



The histogram above of the frequency of membrane and cytoplasmic staining in breast carcinoma cells and show that few cases show moderate staining, with most weak to no staining.

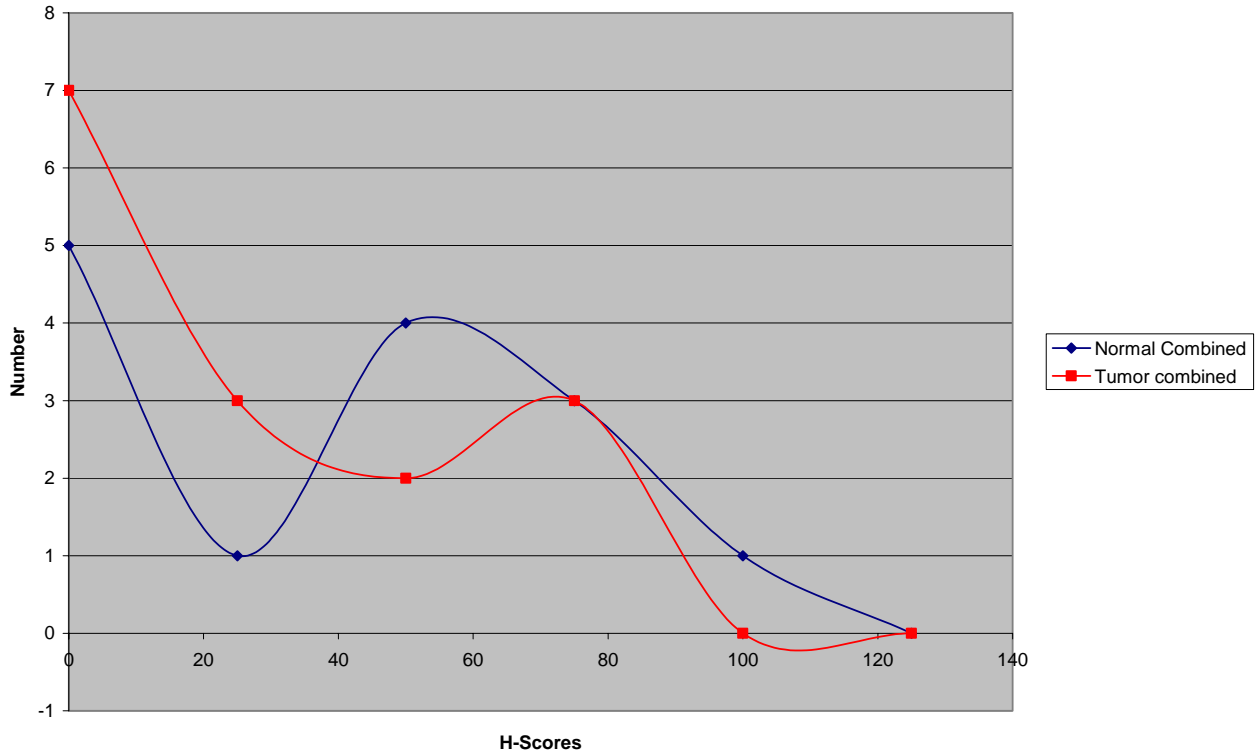


The histogram above of the frequency of membrane staining in normal tissues and carcinoma shows the trend of carcinoma to either stain slightly more intensely than normal glands, or more often, weaker.



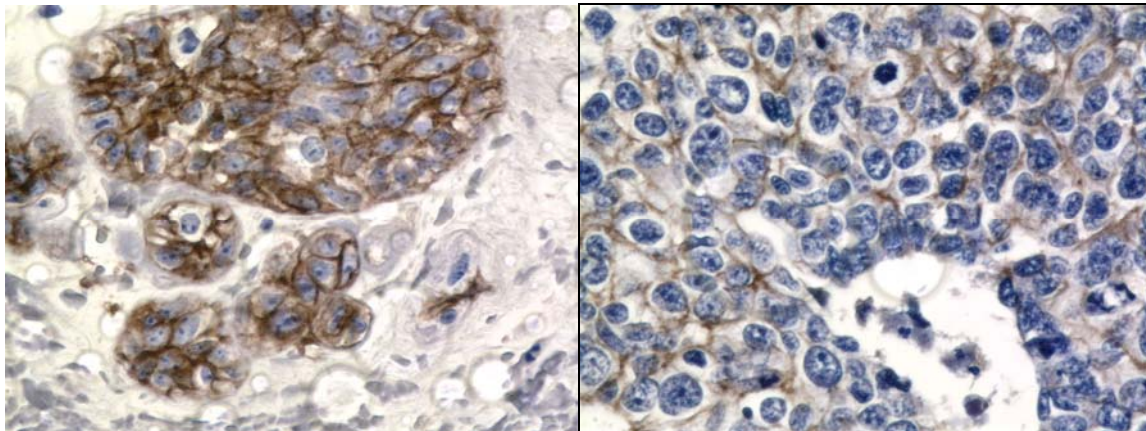
The histogram above of the frequency of cytoplasmic staining in normal tissues and carcinoma demonstrate roughly the same trend as the membrane staining comparison.

**EGFR Histoscore Histogram**



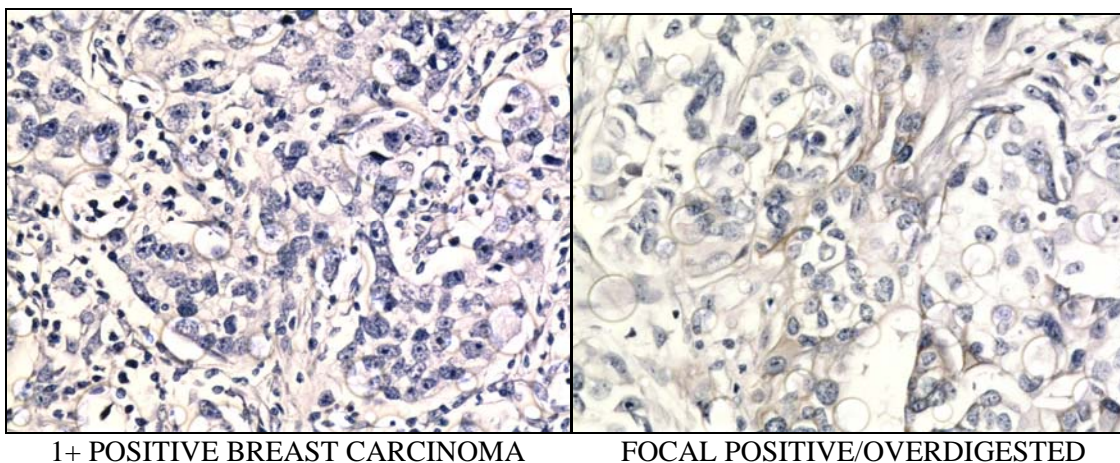
The histogram above of the frequency of the combined scores for membrane and cytoplasmic staining in normal tissues and carcinoma again show the same trend toward weaker or more intense staining in carcinoma as compared to normal gland staining.

The above analyses suggest that normal gland staining for EGFr expression might make a reasonable quality control measure. When present, the staining of carcinoma cells could be contrasted with normal gland staining. When normal glands present are negative for EGFr, tumor cells should be read with a more critical eye. In the cohort of case above, only two cases showed negative normal gland staining and positive tumor cells and both of these cases were weakly stained. Statistical analysis was not meaningful due to the low number of cases.



3+ EGFR POSITIVE BREAST CARCINOMA

2+ EGFR POSITIVE BREAST CARCINOMA



## VI. Reproducibility:

- A. The tables below contains the result of interpretation of slides stained as described above by a Board-certified Pathologist and representing results of the analysis of 5 tissue sections by Immunohistochemistry for EGFR expression analysis over four days. Only the simple score of intensity is shown in the table below.

Specimen Number	Tumor Membrane Staining				Average Score
	3/30/2004	3/31/2004	4/1/2004	4/2/2004	
S5150-90	10% 1+	10% 2+	10% 2+	10% 1+	1.5
S4951-90	20% 3+	20% 3+	20% 3+	30% 3+	3
S2780-90	10% 1+	10% 1+	10% 1+	10% 1+	1
S2298-90	10% 2+	10% 2+	10% 2+	10% 3+	2.25
Skin	50% 3+	50% 3+	50% 3+	70% 3+	3

These results suggest reasonable correlation between sample stained on separate days, especially at the very high level staining and very low. Slightly more variability was found at the 1+ to 2+ boundary.

Specimen Number	Tumor Membrane Staining				Average		
	3/30/2004	3/31/2004	4/1/2004	4/2/2004	Score	STDEV	CV
S5150-90	10	30	40	10	22.5	15	66.6667
S4951-90	100	100	90	160	112.5	32.0156	28.4583
S2780-90	10	10	10	10	10	0	0
S2298-90	40	40	40	30	37.5	5	13.3333
Skin	220	220	220	260	230	20	8.69565
					Average> 23.4308		

Consistent with the semi-quantitative nature of immunohistochemistry, the average H-score variability was low (CV just over 20). One outlier was seen, but in a case only demonstrating weak staining.

Specimen	Tumor Membrane Staining				Average
	3/30/2004	3/31/2004	4/1/2004	4/2/2004	
S5150-90	Positive	Positive	Positive	Positive	Positive
S4951-90	Positive	Positive	Positive	Positive	Positive
S2780-90	Positive	Positive	Positive	Positive	Positive
S2298-90	Positive	Positive	Positive	Positive	Positive
Skin	Positive	Positive	Positive	Positive	Positive

With “positive” defined as any significant staining identified, excellent correlation was found in the five cases stain of 4 separate days.

### VIII. Acceptable Specifications:

- A. This assay meets the acceptance criteria for a Semi-Quantitative Immunohistochemical assay

Dynamic Range: 0 to 3+ (negative to strong staining) for EGFR  
 Specificity: adequate (expected pattern of expression in normal tissues)  
 Sensitivity: adequate (see limitations below)  
 Precision: adequate

Notwithstanding the intended use as a tool for guiding certain forms of anti-EGFr therapy, the use of this test for **other** purposes must be accompanied by the phrase, “This test was developed and its performance characteristics determined by Pathogenesis. It has not been cleared or approved by the U.S Food and Drug Administration for use other than as an assessment tool for patients considered for Anti-EGFr Therapy.”

### IX. Limitations:

- A. This validation study is specific for use for staining of formalin-fixed, paraffin-embedded tissues only for the intended use of qualifying patients with metastatic breast cancer as candidates for Anti-EGFr therapy and as an indicator of EGFR expression in tissue sections.
- B. Accordingly, this study does not qualify the assay for use in tissues fixed with other fixatives, in frozen tissue sections or in cytological preparations.
- C. Caution should be applied to the histologic examination of stained slides demonstrating over-digestion by protease. Only those cells with intact cellular membranes should be included in the analysis. Repeat staining after post-fixation of the de-paraffinized slide in 10% neutral buffered formalin prior to staining. Ensure or encourage adequate fixation of specimens prior to embedding.

Study Director: \_\_\_\_\_

igned by James Thor  
 Y authenticity with App  
 Monday, 17 Mar. 2004

James Thompson M.D. Ph.D.  
 Medical Director

Date: \_\_\_\_\_

## ATTACHMENT A

Specimen Number	S3918-90	S5053-90	D4736	D4732	D4716	S4443-90	D5037			D4700	D4713
Specimen Type	NORMAL BREAST	NORMAL BREAST	NORMAL BREAST	NORMAL BREAST	NORMAL BREAST	NORMAL COLON	NORMAL COLON	NORMAL COLON	NORMAL COLON	NORMAL KIDNEY	NORMAL KIDNEY
Morphology	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate
Normal Present?	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Tumor Present?	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
NBM0	100	100	100	100	100	100	100	100	100	100	100
NBM1	0	0	0	0	0	0	0	0	0	0	0
NBM2	0	0	0	0	0	0	0	0	0	0	0
NBM3	0	0	0	0	0	0	0	0	0	0	0
NBC0	100	100	100	100	100	100	100	100	100	100	100
NBC1	0	0	0	0	0	0	0	0	0	0	0
NBC2	0	0	0	0	0	0	0	0	0	0	0
NBC3	0	0	0	0	0	0	0	0	0	0	0
NBN0	100	100	100	100	100	100	100	100	100	100	100
NBN1	0	0	0	0	0	0	0	0	0	0	0
NBN2	0	0	0	0	0	0	0	0	0	0	0
NBN3	0	0	0	0	0	0	0	0	0	0	0
TBM0	N	N	N	N	N	N	N	N	N	N	N
TBM1	N	N	N	N	N	N	N	N	N	N	N
TBM2	N	N	N	N	N	N	N	N	N	N	N
TBM3	N	N	N	N	N	N	N	N	N	N	N
TBC0	N	N	N	N	N	N	N	N	N	N	N
TBC1	N	N	N	N	N	N	N	N	N	N	N
TBC2	NN	NN	N	N	N	N	N	N	N	N	N
TBC3	N	N	N	N	N	N	N	N	N	N	N
TBN0	N	N	N	N	N	N	N	N	N	N	N
TBN1	N	N	N	N	N	N	N	N	N	N	N
TBN2	N	N	N	N	N	N	N	N	N	N	N
TBN3	N	N	N	N	N	N	N	N	N	N	N
NTM0	60	100	40	70	60	90	80	10	40	50	50
NTM1	40	0	20	30	20	10	20	20	40	30	30
NTM2	0	0	20	0	20	0	0	30	10	10	20
NTM3	0	0	20	0	0	0	0	40	10	10	0
NTC0	80	100	60	80	80	100	80	40	80	90	30
NTC1	20	0	20	20	20	0	20	30	0	0	30
NTC2	0	0	20	20	0	0	0	20	10	10	40
NTC3	0	0	0	0	0	0	0	10	10	0	0
NTN0	100	100	100	100	100	100	100	100	100	100	100
NTN1	0	0	0	0	0	0	0	0	0	0	0
NTN2	0	0	0	0	0	0	0	0	0	0	0
NTN3	0	0	0	0	0	0	0	0	0	0	0
TTM0	100	100	N	N	N	N	N	N	N	N	N
TTM1	0	0	N	N	N	N	N	N	N	N	N
TTM2	0	0	N	N	N	N	N	N	N	N	N
TTM3	0	0	N	N	N	N	N	N	N	N	N
TTC0	100	100	N	N	N	N	N	N	N	N	N
TTC1	0	0	N	N	N	N	N	N	N	N	N
TTC2	0	0	N	N	N	N	N	N	N	N	N
TTC3	0	0	N	N	N	N	N	N	N	N	N
TTN0	100	100	N	N	N	N	N	N	N	N	N
TTN1	0	0	N	N	N	N	N	N	N	N	N
TTN2	0	0	N	N	N	N	N	N	N	N	N
TTN3	0	0	N	N	N	N	N	N	N	N	N
Comments											
Read by	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson
NBMC	100	100	100	100	100	100	100	100	100	100	100
NBCC	100	100	100	100	100	100	100	100	100	100	100
NBNC	100	100	100	100	100	100	100	100	100	100	100
TBMC	0	0	0	0	0	0	0	0	0	0	0
TBCC	0	0	0	0	0	0	0	0	0	0	0
TBNC	0	0	0	0	0	0	0	0	0	0	0
NTMC	100	100	100	100	100	100	100	100	100	100	100
NTCC	100	100	100	120	100	100	100	100	100	100	100
NTNC	100	100	100	100	100	100	100	100	100	100	100
TTMC	100	100	0	0	0	0	0	0	0	0	0
TTCC	100	100	0	0	0	0	0	0	0	0	0
TTNC	100	100	0	0	0	0	0	0	0	0	0
NBMH	0	0	0	0	0	0	0	0	0	0	0
NBCH	0	0	0	0	0	0	0	0	0	0	0
NBNH	0	0	0	0	0	0	0	0	0	0	0
TBMH	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
TBCH	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
TBNH	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
NTMH	40	0	120	30	60	10	20	200	90	80	70
NTCH	20	0	60	60	20	0	20	100	50	20	110
NTNH	0	0	0	0	0	0	0	0	0	0	0
TTMH	0	0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
TTCH	0	0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
TTNH	0	0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!



ATTACHMENT B

Specimen Number	S4442-90	S2887-90	S7490-90	S7559-90	S6797-90	S6857-90	S1587-90	D3859	D7496	S6197-90	S5907-90	S2780-90	S4951-90	S2298-90	S7559-90	S5150-90
Specimen Type	BREAST CA	BREAST CA	BREAST CA	BREAST CA	BREAST CA	BREAST CA	BREAST CA	CERVICAL CA	NORMAL PROSTATE	BREAST CA	BREAST CA	BREAST CA	BREAST CA	BREAST CA	BREAST CA	BREAST CA
Morphology	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate
Normal	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Present? Tumor	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Present? NBM0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NBM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBM2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBC0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NBC1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBC2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBC3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBN0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NBN1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBN2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBN3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TBM0	100	100	100	100	100	100	N	100	100	100	100	100	100	100	100	100
TBM1	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBM2	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBM3	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBC0	100	100	100	100	100	100	N	100	100	100	100	100	100	100	100	100
TBC1	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBC2	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBC3	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBN0	100	100	100	100	100	100	N	100	100	100	100	100	100	100	100	100
TBN1	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBN2	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TBN3	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
NTM0	100	100	100	90	100	80	80	100	80	60	80	80	70	80	70	90
NTM1	0	0	0	0	0	0	0	0	0	10	0	10	10	10	10	10
NTM2	0	0	0	0	0	10	10	0	0	10	10	10	10	10	20	0
NTM3	0	0	0	10	0	10	10	0	20	10	0	10	0	0	0	0
NTC0	90	90	100	90	100	80	100	100	80	100	80	100	80	100	70	100
NTC1	10	10	0	10	0	0	0	0	0	0	0	10	0	10	0	0
NTC2	0	0	0	0	0	10	0	0	0	10	0	10	0	20	0	0
NTC3	0	0	0	0	0	10	0	0	20	0	10	0	0	0	0	0
NTN0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NTN1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NTN2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NTN3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TTM0	100	100	100	100	100	90	N	100	90	100	100	90	50	70	100	90
TTM1	0	0	0	0	0	10	N	0	10	0	0	10	20	20	0	10
TTM2	0	0	0	0	0	0	N	0	0	0	0	0	10	10	0	0
TTM3	0	0	0	0	0	0	N	0	0	0	0	0	10	0	0	0
TTC0	100	90	100	90	100	90	N	100	100	100	90	100	100	100	100	100
TTC1	0	10	0	10	0	10	N	0	0	0	10	0	0	0	0	0
TTC2	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TTC3	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TTN0	100	100	100	100	100	100	N	100	100	100	100	100	100	100	100	100
TTN1	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TTN2	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
TTN3	0	0	0	0	0	0	N	0	0	0	0	0	0	0	0	0
Comments	OVERDIGE STED															
Read by	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson	James Thompson
NBMC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NBCC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NBNC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
TBMC	100	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100
TBCC	100	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100
TBNC	100	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100
NTMC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NTCC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NTNC	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
TTMC	100	100	100	100	100	100	0	100	100	100	100	100	90	100	100	100
TTCC	100	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100
TTNC	100	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100
NBMH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBCH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NBNH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TBMH	0	0	0	0	0	0	#VALUE!	0	0	0	0	0	0	0	0	0
TBCH	0	0	0	0	0	0	#VALUE!	0	0	0	0	0	0	0	0	0
TBNH	0	0	0	0	0	0	#VALUE!	0	0	0	0	0	0	0	0	0
NTMH	0	0	0	30	0	50	50	0	60	90	50	30	60	30	50	10
NTCH	10	10	0	10	0	50	0	0	60	0	50	0	30	0	50	0
NTNH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TTMH	0	0	0	0	0	10	#VALUE!	0	10	0	0	10	70	40	0	10
TTCH	0	10	0	10	0	10	#VALUE!	0	0	0	10	0	0	0	0	0
TTNH	0	0	0	0	0	0	#VALUE!	0	0	0	0	0	0	0	0	0