

**SUMMARY OF SAFETY AND
EFFECTIVENESS DATA (SSED)**

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I. GENERAL INFORMATION

| | |
|--|--|
| Device Generic Name: | Anti- Her-2/neu (c-erbB-2) Mouse Monoclonal Antibody Immunohistochemical Detection System |
| Device Trade Name: | BioGenex InSite™ Her-2/neu |
| Applicant Name and Address: | BioGenex Laboratories, Inc. 4600 Norris Canyon Road San Ramon, CA 94583 |
| Premarket Approval Application Number: | P040030 |
| Date of Panel Recommendation: | None |
| Date of Notice of Approval to the Applicant: | December 22, 2004 |

II. INDICATIONS FOR USE

InSite™ Her-2/neu Mouse Monoclonal Antibody (Clone CB11) kit is intended for *In Vitro* Diagnostic use in Immunohistochemistry (IHC) assays to semi-quantitatively localize by light microscopy the over-expression of Her-2/neu (i.e., c-erbB-2) in formalin-fixed, paraffin-embedded normal and neoplastic tissue sections. InSite™ Her-2/neu is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered. Clinical interpretation of InSite™ Her-2/neu immunostaining results (absence or presence; semi-quantitative intensity score) should be complemented by appropriate controls and morphological tissue analysis and be evaluated by a qualified pathologist within the context of patient clinical history and other diagnostic results.

III. CONTRAINDICATIONS None

IV. WARNINGS AND PRECAUTIONS

Warnings and Precautions for use of the device are stated in the product labeling.

V. DEVICE DESCRIPTION

Reagents Provided

InSite™ Her-2/neu Primary Antibody (Clone CB11) consists of two choices of dispenser: one dispenser contains 6 ml (10 tests) of prediluted reagent for manual use. A second choice of dispenser contains 20 ml (200 tests) of prediluted reagent for

automated use. The antibody is diluted in Phosphate buffered saline containing bovine serum albumin carrier protein and 0.09% sodium azide as a preservative.

In addition, the following materials and reagents are provided as a part of the test kit

| InSite™ Her-2/neu (Cat. No. RD134-60K) | | For 60 Tests | |
|--|---|--------------|--------------|
| Item Number | Description | Volume | No. of Vials |
| HK083-5K | Power™ Block | 6 ml | 1 |
| HK111-5K | Peroxide Block | 6 ml | 1 |
| HK330-5K | HRP Label | 6 ml | 1 |
| HK340-5K | MultiLink® | 6 ml | 1 |
| HK128-5K | DAB Substrate Buffer | 2.5 ml | 6 |
| HK124-5K | Liquid DAB Chromogen | 2 ml | 1 |
| HK126-5K | DAB Substrate | 2 ml | 1 |
| HK119-5M | Negative Control | 3 ml | 1 |
| HK080-5K | Citra Plus (10X Concentrated) | 100 ml | 1 |
| HK583-YAK | Super Sensitive™ Wash Buffer (20X Concentrated) | 100 ml | 1 |
| CL134-MT | Positive Control Slides* | 5 slides | 1 pack |
| HK100-5K | Hematoxylin | 6 ml | 1 |

| InSite™ Her-2/neu (Cat. No. RD134-YCX) | | For 200 Tests | |
|--|-------------------------------|---------------|--------------|
| Item Number | Description | Volume | No. of Vials |
| HK083-20K | Power™ Block | 20 ml | 1 |
| HK111-20K | Peroxide Block | 20 ml | 1 |
| HK330-20K | HRP Label | 20 ml | 1 |
| HK340-20K | MultiLink® | 20 ml | 1 |
| HK128-20X | DAB Substrate Buffer | 20 ml | 5 |
| HK124-7K | Liquid DAB Chromogen | 4 ml | 1 |
| HK126-7K | DAB Substrate | 3 ml | 1 |
| HK119-20X | Negative Control | 20 ml | 1 |
| HK080-5K | Citra Plus (10X Concentrated) | 100 ml | 2 |
| CL134-MT | Positive Control Slides* | 5 slides | 1 pack |
| HK100-20K | Hematoxylin | 20 ml | 1 |

* Positive Control Slides: Each slide will contain sections of formalin-fixed, paraffin-embedded breast carcinoma cell lines representing different levels of Her-2 expression.

In addition, the following materials and reagents are necessary to perform the assay.

A. Additional Reagents Not Provided

- EZ-DeWax™ (BioGenex; Cat. No. HK585-5K)
- Xylene
- Ethanol (Absolute and 95%)
- Distilled or deionized water
- Ammonia water (1% ammonium hydroxide)
 - Permanent: For use with DAB substrate (Fisher Cat. No. SP15-100)
- 10% Buffered Formalin (for specimen preparation)
- Super Sensitive™ Wash Buffer (20X) (BioGenex; Cat. No. HK583-YAK) (N.B. For use with InSite™ Her-2/neu, Cat. No. RD134-YCX only.)

B. Laboratory Equipment Not Provided

- Oven or incubator (capable of maintaining 56-60°C)
- BioGenex Automated Staining System
- Humidity Chamber
- Microwave oven
- Staining Jars or baths
- Timer (capable of 3-20 minute intervals)
- Wash Bottles
- Absorbent Wipes
- Microscopes slides (pre-treated with poly-L-Lysine) (BioGenex; Cat. No. XT002-SL)
- Coverslips (VWR; Cat. No. 48366-089)
- Lens paper
- Light microscope with magnification of 200X

Principle of Device Methodology

Immunohistochemical applications on formalin-fixed, paraffin-embedded tissue sections rely on the sequential detection of defined molecular entities. In such applications, an antigen of interest is first detected in the tissue by a primary antibody with a high level of specificity for the antigen. This primary antibody is in turn detected by an appropriate species-specific biotinylated secondary antibody. An enzyme conjugated to streptavidin is then applied which binds the biotin-labeled secondary antibody. Finally, the location of the primary antibody is visualized by the application of a colorimetric chromogen that precipitates in the presence of the streptavidin conjugated-enzyme. Results are interpreted using a light microscope.

InSite™ Her-2/neu (Clone: CB11) may be used to detect Her-2 receptor antigen in formalin-fixed, paraffin-embedded tissue sections after pretreatment with Citra Plus Antigen Retrieval. This pretreatment is essential for optimal reactivity of InSite™ Her-2/neu with Her-2 receptor antigen in routinely processed surgical pathology

specimens. In general, Immunohistochemical staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (link antibody), an enzyme complex and a chromogenic substrate interspersed with washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. Finally, the specimen may be counterstained and mounted/coverslipped. Results are interpreted using light microscopy and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

InSite™ Her-2/neu may be used in manual Immunohistochemical applications or in automated applications, such as on the BioGenex i6000™ Automated Staining System or the OptiMax® Plus Consolidated Staining System. The latter Automated Staining Systems will perform all the staining steps as described in their respective Operator's Manual. After completion of the staining procedure, the stained slides are removed from the instrument and mounted manually with an appropriate mounting medium.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Multiple methods exist for the identification of Her-2/neu receptor expression in breast cancer biopsy tissue. Alternative assays reliant on immunohistochemistry incorporate the use of alternative primary antibodies and detection reagents. Analyte Specific Reagents for Her-2/neu immunohistochemistry and assays for research are currently marketed by several companies.

Her-2/neu receptor gene amplification in breast tumor tissue may also be assessed by use of Fluorescent *In Situ* Hybridization (FISH). This method correlates gene copy number with protein expression and relies on synthetic oligonucleotide hybridization probes.

VII. MARKETING HISTORY

The BioGenex InSite™ Her-2/neu IHC Test System (kit) has not been marketed in the United States or any other country

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

BioGenex InSite™ Her-2/neu is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered. Patients falsely assigned as positive following assessment would be considered eligible for treatment. Because the design of the clinical studies did not include treatment of patients with negative assay results, the risks or benefits of treatment in this patient population are unknown. The risks of Herceptin® treatment to Her-2/neu -positive patients included infusional toxicity and cardiotoxicity.

Patients falsely assigned as negative will not receive the potential benefits of therapy with Herceptin®.

The labeling recommends that the testing laboratory employ positive and negative tissue culture controls as well as actual positive and negative tissue controls to reduce the potential for an erroneous test result.

IX. SUMMARY OF PRECLINICAL STUDIES

Preclinical testing of the BioGenex InSite™ Her-2/neu included analytical specificity, stability/stress studies and a number of different reproducibility studies. Also included were studies performed to characterize control cell lines and to demonstrate the antigen stability of these cell lines, when stored as freshly cut, paraffin-embedded sections on glass slides. Except where noted, all of the studies were performed with the component reagents of the final kit.

Antibody Specificity Studies

a) Analytical Specificity/Crossreactivity Study

Specificity was determined by Western Blot analysis of two cell lines, SKBR-3 and AU565, known to over-express the Her-2 receptor (Akiyami *et al.*, 1986; Siegall *et al.*, 1995). SKBR-3 also expresses moderate levels of Her-3 receptor.

Results: In both cell lines, the Anti-Her-2/neu Monoclonal Antibody (Clone CB11) detected one protein band at ~195kDA in protein cell extracts immobilized on nitrocellulose membrane. This is the approximate molecular weight reported for the Her-2/neu receptor protein (Akiyama *et al.* (1986). No cross-reactivity was seen with the 160kDA Her-3 receptor protein moderately expressed by cell line SKBR-3 (Siegall *et al.*, 1995). Mild reactivity was seen with proteins ranging from 83-118kDA in both cell lines. Most likely, these protein bands represent cytoplasmic Her-2 receptor or its protein degradation products. These results correlate well with previous Western Blot and immunoprecipitation analysis of Clone CB11 and the Her-2 receptor (Akiyama *et al.*, 1986; Corbette *et al.*, 1990).

Conclusion: These studies demonstrate that BioGenex InSite™ Her-2/neu (Clone CB11) reacts with the Her2-neu receptor and does not cross-react with the Her-3 receptor present in the SKBR-3 cell line. These studies are sufficient because clone CB11 has been FDA-approved as the Ventana Medical Systems' PATHWAY Her2 primary antibody and it was also used in the original clinical trial assay to establish the clinical outcome of the drug, Herceptin®. Thus the specificity of this clone is well accepted to be specific for Her-2/neu.

b) Clinical Specificity/Crossreactivity/Tour of Normal Tissues Throughout the Body

Eighty-six normal tissue sections were stained with the components of the BioGenex InSite™ Her-2/neu test using the BioGenex i6000™ Automated Staining System. The following normal tissues were evaluated: Adrenal (3); Bone Marrow (3); Brain/Cerebellum (3); Brain/Cerebrum (3); Breast (3); Cervix (3); Colon (3); Esophagus (3); Heart (3); Kidney (3); Liver (3); Lung (3); Mesothelial Cells (3); Ovary (3); Pancreas (3); Parathyroid (1); Peripheral Nerve (3); Pituitary (1); Prostate (3); Salivary Gland; (3); Skeletal Muscle (3); Skin (3); Small Intestine (3); Spleen (3); Stomach (3); Testis (3); Thymus (3); Thyroid (3); Tonsil (3); and Uterus (3).

Results:

No specific staining was seen in any of the tissues. Non-specific cytoplasmic staining was seen in some of the tissues. Cytoplasmic staining is not to be interpreted as a positive result.

Conclusions: BioGenex InSite™ Her-2/neu test performed acceptably on these normal tissue samples by showing no cross-reactivity. The results for the BioGenex InSite™ Her-2/neu test are published in its package insert so that users will know what to expect with regard to staining with this product in normal tissues that may be found in the background of metastasized tumor specimens.

2) Accelerated Stability/Stress Testing of the Complete Kit

One lot of the whole kit consisting of two kits with the exception of the control slides was manufactured. One of the two kits manufactured was subjected to a round trip shipping process during the month of September to a Southern state in the United States under our standard product shipping conditions. The kit and one package of 5 Control Slides were then stored at 37° C for two weeks. Cell line control slides with multiple cell lines, which have reactivity ranging in semi-quantitative intensity scores of 0-3+, were used for testing. After being stored at the elevated temperature for the allotted time, the kit and the pre-treated control slides were tested with two fresh control slides and one additional fresh control slide as the negative control. One fresh kit from the same lot was tested alongside as a positive control.

Results: The staining results from the incubated kit and the fresh kit gave comparable scoring. All negative slides gave negative results. Each cell line from duplicates within the same lot and from different lots scored within one point variation.

Conclusions:

Results met the pre-determined acceptance criteria. The accelerated stability data supports a 6-month expiration date. Dating for this product will be set at 6 months from manufacture when stored at 2-8° C. Real-time stability studies and stress testing are on-going. A protocol for on-going real-time stability studies and acceptance criteria were suggested to the sponsor that included a more rigorous testing of real breast tumor specimens of variable Her-2/neu reactivity.

3) Reproducibility Studies**a) Intra-Run Reproducibility**

Each specimen was run in a masked randomized fashion with one slide tested with the negative reagent control to assess background staining. The 5 different formalin-fixed, paraffin-embedded breast cancer tissues chosen for evaluation of BioGenex InSite™ Her-2/neu intra-run reproducibility ranged in semi-quantitative immunostaining intensity score from 0 ~ 3+. All tissues were stained on the BioGenex i6000™ Automated Staining System with the same lot of BioGenex InSite™ Her-2/neu antibody and the same lot of detection reagents.

Results: Uniform reproducibility in semi-quantitative immunostaining intensity score was seen across all slides of each specimen evaluated. Results from the Intra-run reproducibility study are presented in Table 1 below.

Table 1. Summary of Staining Intensities of BioGenex InSite™ Her-2/neu on Breast Tissue Sections obtained in a Single Run:

| | First Slide | | Second Slide | | Third Slide | |
|------------------------------|-------------|------|--------------|------|-------------|------|
| | Her-2/neu* | NC** | Her-2/neu* | NC** | Her-2/neu* | NC** |
| Quality Control Sides | 3+ | 0 | N/A | N/A | N/A | N/A |
| S98-388 | 0 | 0 | 0 | 0 | 0 | 0 |
| S97-3352A | 1+ | 0 | 1+ | 0 | 1+ | 0 |
| S97-229 | 1+ ~ 2+ | 0 | 1+ ~ 2+ | 0 | 1+ ~ 2+ | 0 |
| S97-1324A | 2+ | 0 | 2+ | 0 | 2+ | 0 |
| S97-2357B | 3+ | 0 | 3+ | 0 | 3+ | 0 |

* BioGenex InSite™ Her-2/neu

** NC, Negative Control

Conclusion: This study demonstrates adequate intra-run reproducibility for BioGenex InSite™ Her-2/neu.

b) Inter-Run Reproducibility

Each specimen was run in a masked randomized fashion with one slide tested with the negative reagent control to assess background staining. The 5 different formalin-fixed, paraffin-embedded breast cancer tissues chosen for evaluation of BioGenex InSite™ Her-2/neu intra-run reproducibility ranged in semi-quantitative immunostaining intensity score from 0 ~ 3+. All tissues were stained on the BioGenex i6000™ Automated Staining System with the same lot of BioGenex InSite™ Her-2/neu antibody and the same lot of detection reagents on three separate days.

Results: Results from the Inter-run reproducibility study are demonstrated in Table 2 below.

Table 2. Summary of Staining Intensities of BioGenex InSite™ Her-2/neu on Breast Tissue Sections Obtained in Three Separate Runs.

| | First Run (Day 1) | | Second Run (Day 2) | | Third Run (Day 3) | |
|------------------------------|-------------------|------|--------------------|------|-------------------|------|
| | Her-2/neu* | NC** | Her-2/neu* | NC** | Her-2/neu* | NC** |
| Quality Control Sides | 3+ | 0 | 3+ | 0 | 3+ | 0 |
| S98-388 | 0 | 0 | 0 | 0 | 0 | 0 |
| S97-3352A | 0 ~ 1+ | 0 | 0 | 0 | 0 ~ 1+ | 0 |
| S97-229 | 1+ ~ 2+ | 0 | 1+ ~ 2+ | 0 | 1+ | 0 |
| S97-1324A | 2+ ~ 3+ | 0 | 2+ | 0 | 2+ | 0 |
| S97-2357B | 3+ | 0 | 3+ | 0 | 3+ | 0 |

* BioGenex InSite™ Her-2/neu

** NC, Negative Control

Minor variability of inter-run reproducibility was inferred from the reproducibility of semi-quantitative immunostaining intensity scores. One specimen returned mildly discrepant semi-quantitative immunostaining intensity scores of 1+ ~ 2+, 1+ ~ 2 and 1+ across three days.

Conclusion: Except for specimen S97-229, the diagnostic scores of negative (0 ~ 1+) or positive (2+ ~ 3+) were not compromised between runs. The variability seen in this study is well documented. Low reproducibility in semi-quantitative immunostaining score is typical within the scoring range of 1+ ~ 2+. Therefore, it is commonly accepted that semi-quantitative scores of 2+ be reevaluated by additional observers or FISH analysis before final diagnostic decision (Thomson *et al.*, 2001; Tsuda *et al.*, 2001a, 2001b & 2002). This study demonstrates adequate inter-run reproducibility for BioGenex InSite™ Her-2/neu.

c) Manual vs. Automated Methodology Reproducibility

A single section from each of the same five breast tumor formalin-fixed, paraffin-embedded tissue blocks was used and subjected to

immunohistochemical staining using all of the ingredients of the InSite™ Her-2/neu kit as separate reagents either a manual or automated protocol using the BioGenex i6000™ Automated Staining System. The results of this study are presented in Table 3 below.

Table 3. Summary of Staining Intensities of InSite™ Her-2/neu on Breast Tissue Sections Obtained in a Manual vs. Automated Assay System.

| | Manual | | Automated* | |
|-------------------------------|-------------|-------|-------------|-------|
| | Her-2/neu** | NC*** | Her-2/neu** | NC*** |
| Quality Control Slides | 3+ | 0 | 3+ | 0 |
| S98-388 | 0 | 0 | 0 | 0 |
| S97-3352A | 0 | 0 | 0 | 0 |
| S97-229 | 1+ | 0 | 1+ ~ 2+ | 0 |
| S97-1324A | 2+ | 0 | 2+ | 0 |
| S97-2357B | 3+ | 0 | 3+ | 0 |

* Performed on a BioGenex i6000™ Automated Staining System

** BioGenex InSite™ Her-2/neu

*** NC, Negative Control

Results: Mild variability between manual vs. automated methodology reproducibility was seen. One specimen returned discrepant semi-quantitative immunostaining intensity scores of 1+ and 1+ ~ 2+ between the manual and automated protocol, respectively.

Conclusion: Except for specimen S97-229, the diagnostic scores of negative (0 ~ 1+) or positive (2+ ~ 3+) were not compromised. Such variability is well documented. Low reproducibility in semi-quantitative immunostaining score is typical within the scoring range of 1+ ~ 2+. Therefore, it is commonly accepted that semi-quantitative scores of 2+ be reevaluated by additional observers or FISH analysis before final diagnostic decision (Thomson *et al.*, 2001; Tsuda *et al.*, 2001a, 2001b & 2002). This study demonstrates adequate reproducibility between manual and automated methodology for BioGenex InSite™ Her-2/neu.

d) Detection Systems Reproducibility

The reproducibility of InSite™ Her-2/neu primary antibody staining using three different BioGenex Super Sensitive™ Detection Systems was tested. Single sections from each of the five different breast tumor formalin-fixed, paraffin-embedded tissue specimens were stained using three different chromogen-based Super Sensitive™ Detection systems: DAB (used in final Her-2/neu test kit), AEC, and Fast Red. The first two staining systems are detected using horseradish peroxidase while Fast red is detected using alkaline phosphatase. All tissues were stained with the same lot of InSite™ Her-2/neu

Monoclonal Antibody and either BioGenex Super Sensitive™ MultiLink® Detection kit for DAB, AEC or Fast Red.

Results: The results of this study are presented in Table 4 below.

Table 4 Summary of Staining Intensities of InSite™ Her-2/neu Monoclonal Antibody on Breast Tissue Sections Visualized by Three Different Chromogens.

| | DAB | | AEC | | Fast Red | |
|-------------------------------|------------|------|------------|------|------------|------|
| | Her-2/neu* | NC** | Her-2/neu* | NC** | Her-2/neu* | NC** |
| Quality Control Slides | 3+ | 0 | N/A | N/A | N/A | N/A |
| S98-388 | 0 | 0 | 0 ~ 1+ | 0 | 0 | 0 |
| S97-3352A | 0 | 0 | 0 ~ 1+ | 0 | 0 ~ 1+ | 0 |
| S97-229 | 1+ | 0 | 1 | 0 | 0 ~ 1+ | 0 |
| S97-1324A | 2+ | 0 | 2 | 0 | 1+ ~ 2+ | 0 |
| S97-2357B | 3+ | 0 | 3 | 0 | 3 | 0 |

* BioGenex InSite™ Her-2/neu monoclonal antibody

** NC, Negative Control

There was only minor variability in reproducibility of the BioGenex Super Sensitive™ Detection Systems when used with the BioGenex InSite™ Her-2/neu Monoclonal Antibody. One specimen returned discrepant semi-quantitative immunostaining intensity scores of 2+, 2 and 1+ ~ 2+ across Super Sensitive™ Detection Systems using DAB, AEC and Fast Red, respectively.

Conclusion: Except for specimen S97-1324A, the diagnostic scores of negative (0 ~ 1+) or positive (2+ ~ 3+) were not compromised. Such variability is well documented. Low reproducibility in semi-quantitative immunostaining score is typical within the scoring range of 1+ ~ 2+. Therefore, it is commonly accepted that semi-quantitative scores of 2+ be reevaluated by additional observers or FISH analysis before final diagnostic decision (Thomson *et al.*, 2001; Tsuda *et al.*, 2001a, 2001b & 2002). This study demonstrates adequate reproducibility between three different BioGenex Super Sensitive™ detection systems for BioGenex InSite™ Her-2/neu primary antibody. However, the BioGenex InSite™ Her-2/neu test kit is currently available only with the AB chromogen.

e) Lot-to-lot reproducibility of the Complete Kit

Three independent kit lots were made according to manufacturing procedures. Cell line control slides were used for testing the three lots. They contain formalin-fixed paraffin-embedded tissue culture cells with reactivities ranging in semi-quantitative intensity scores from 0 – 3+. For each lot, three slides were used. One slide was stained using the negative control reagent as negative control.

Results: The staining results from all three lots gave comparable scoring. All negative slides gave negative results. Each cell line from duplicate slides within the same lot and from different lots scored within one point variation.

Conclusion: Overall acceptance criteria were met. This study demonstrated adequate lot-to-lot reproducibility for the BioGenex InSite™ Her-2/neu test kit. For future lot-to-lot studies, a protocol for test kit acceptance criteria was suggested to the sponsor that included a more rigorous testing of a real 2+ breast tumor specimen along with the Positive and Negative Control Slide. They agreed to incorporate this change into their quality control protocols (lot release, lot-to-lot reproducibility, accelerated stability, and real-time stability testing).

f) Inter-Laboratory Reproducibility

All three investigators followed a common manual Immunohistochemistry staining procedure, as described in the package insert for the BioGenex InSite™ Her-2/neu test kit. The laboratories were also provided with the same lot of InSite™ Her-2/neu antibody and all necessary ancillary DAB staining reagents. In brief, three different, geographically distinct laboratories were provided with unstained breast tumor tissue sections from 30 different cases of formalin-fixed, paraffin-embedded tissue blocks. These cases consisted of 10 each of 3+ and 2+ Her-2/neu staining scores, and 5 each of 1+ and 0 Her-2/neu staining scores.

Results: The results of this study are presented in Table 5 below.

Table 5. Summary of Inter-Laboratory Concordance for InSite™ Her-2/neu.

| | | LABS | | | |
|---------|---|------|---|-------|--------|
| | | A | B | C | Counts |
| RESULTS | - | - | - | - | 5 |
| | - | - | - | + | 1 |
| | - | - | + | - | 2 |
| | - | - | + | + | 1 |
| | + | + | + | - | 2 |
| | + | + | + | + | 19 |
| | | | | TOTAL | 30 |

Most of the discrepant results were between 1+ and 2+ staining scores. In general however, very good reproducibility of positive vs. negative scores across the three laboratories was observed for all breast tumor tissue specimens used. This is based on the concordance of staining results between labs which ranged from 83% - 90%, with Labs A and B at 90%; Labs A and C at 86%; and Labs B and C at 83%.

Conclusion: Such variability is well documented. Low reproducibility in semi-quantitative immunostaining score is typical within the scoring range of 1+ ~ 2+. Therefore, it is commonly accepted that semi-quantitative scores of 2+ be reevaluated by additional observers or FISH analysis before final diagnostic decision (Thomson *et al.*, 2001; Tsuda *et al.*, 2001a, 2001b & 2002). This study demonstrated adequate between laboratory reproducibility for the BioGenex InSite™ Her-2/neu test kit.

4) Characterization and antigen stability on control cell lines

a) Characterization of Control Cell Lines

The three cell lines chosen were grown in tissue culture, formalin-fixed, paraffin-embedded and sectioned for staining with BioGenex InSite™ Her-2/neu antibody and ancillary Super Sensitive Fast Red staining reagents. This staining system had been previously demonstrated to give results equivalent to the DAB staining system found in the BioGenex InSite™ Her-2/neu test kit.

Results: The results of this study can be found in Table 6 below.

Table 6: Comparison of BioGenex InSite™ Her-2/neu Staining Intensity with Previously Reported Her-2/neu Protein Expression in Control Cell Lines MDA-231, MDA-175 and SKBR-3

| Cell Line | Receptors / Cell (Data from Literature) | IHC Staining Intensity Score |
|-----------|--|---------------------------------|
| MDA-231 | 21,600 ± 6,700 | 0 |
| MDA-175 | 92,400 ± 12,000 | 1+ |
| SK-BR3 | 2,390,000 ± 130,000 | 3+ |

Control breast cancer cell lines MDA-231, MDA-175 and SKBR-3 were previously characterized regarding their expression of Her-2/neu receptor protein (Brown, 2002; Dako A/S, 1998; Rodriguez *et al.*, 1993). As shown above, Her-2/neu expression of ~20,000 receptors/cell produces a semi-quantitative score of 0 using BioGenex InSite™ Her-2/neu. Membrane expression at five times greater (i.e. ~100,000) than a baseline score of 0 is reflected by a score 1+, and an expression at 100 fold greater than baseline will produce a score of 3+.

Conclusions: Cell lines with increasingly greater amounts of Her-2 receptor protein will produce increasingly greater semi-quantitative immunohistochemistry scores when stained using BioGenex InSite™ Her-2/neu primary antibody and ancillary staining reagents. Semi-quantitative scores of 3+ reflect receptor protein expression levels of 2×10^6 or more, while semi-quantitative scores of 0 to 1+ reflect receptor expression levels of Her-2 receptor protein that are as much as two orders of magnitude less.

Hence, cell lines MDA-MB-231, MDA-MB-175, and SKBR-3 are semi-quantitatively characterized by BioGenex InSite™ Her-2-neu as 0, 1+ and 3+ respectively.

b) Her-2/neu Antigen Stability in Control Cell Line Tissue Sections

Formalin-fixed paraffin embedded tissue sections of control cell lines MDA-231, MDA-175 and SKBR-3 were tested using BioGenex InSite™ Her-2/neu primary antibody and ancillary staining reagents. Slide sections were stored for either 1, 15 or 45 days at ambient temperature before immunostaining. Ambient temperature corresponded to 20° - 26° C.

Results: The results of this study can be found in Table 7 below.

Table 7: Comparison of BioGenex InSite™ Her-2/neu Staining Intensity Achieved over a 45- Day Period in Control Cell Lines MDA-231, MDA-175 and SKBR-3

| Cell line | Receptors / cell (Data from Literature) | IHC Score | | |
|------------|--|-----------|--------|--------|
| | | Day 1 | Day 15 | Day 45 |
| A. MDA-231 | 21,600 ± 6,700 | 0 | 0 | 0 |
| B. MDA-175 | 92,400 ± 12,000 | 1+ | 1+ | 1+ |
| SKBR-3 | 2,390,000 ± 130,000 | 3+ | 3+ | 3+ |

Conclusions: As Table 2 shows, Her-2/neu antigen is sufficiently stable to allow accurate semi-quantitative measurement of antigen expression by InSite™ Her-2/neu in freshly cut paraffin embedded sections of tissue culture cells when stored up to a period of 45 days at room temperature (20° - 26° C).

5) Overall Conclusions for Preclinical Studies: The preclinical testing performed on BioGenex InSite™ Her-2/neu demonstrated that this product is specific for the Her-2/neu receptor protein, is reproducible when studied with a number of variables, and possesses preclinical stability and performance sufficient to aid in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered.

X. SUMMARY OF CLINICAL STUDIES

One study was conducted with clinical samples to demonstrate the agreement between InSite Her-2/neu antibody plus ancillary reagents vs. the reference assay, the DakoCytomation HercepTest®. The study was performed with the component reagents of the final kit, rather than with a lot of assembled kit. This study was performed in a single blinded manner. The clinical study was adequate to illustrate the performance of the InSite Her-2/neu kit.

THE OBJECTIVE OF THE CLINICAL STUDIES

The objective of this study using clinical samples was to determine the acceptability of BioGenex InSite™ Her2/neu as an alternative to the FDA approved DakoCytomation HercepTest® (PMA No. P980018) for use as an aid in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered. This study was performed in a single blinded manner. Both a 2x2 and 3x3 concordance analysis was conducted. The acceptance criterion was defined as > 75% concordance between the two systems within a 95% confidence interval.

All testing for the clinical study was done in-house at BioGenex Laboratories.

STUDY POPULATIONS

A total of 352 identical pairs of formalin-fixed, paraffin-embedded slides of anonymized breast tumor specimen tissue sections were evaluated in this study. Positive and negative scoring specimens were equally represented. The breast tissue sections that were used in this study had been provided by Oncotech, under a research collaborative arrangement. These specimens were sectioned from tissue blocks that were classified as tissue discards from their archival tissue bank. All patient ID information had been de-linked from the ID numbers assigned to each tissue section. Based on the documented Her-2/neu status of these tissue blocks, sections were prepared from tissue blocks that were randomly selected from a pool of positive Her-2/neu cases. Then, an equal number of tissue blocks were randomly chosen from archival cases that showed negative results for Her-2/neu.

STUDY SUMMARIES

A total of 352 identical slide pairs of formalin-fixed, paraffin-embedded breast tumor tissue sections were evaluated in this study. Positive and negative scoring specimens were equally represented. Agreement was determined by staining one slide each of identical breast tumor specimen pairs with either BioGenex InSite™ Her-2/neu and ancillary DAB staining reagents or DakoCytomation HercepTest®. Two lots of HercepTest® kit and two lots of InSite™ Her-2/neu antibody and reagents were used in the study and staining was carried out by two histotechnologists. The breast tumor tissue slides stained using HercepTest® were prepared and processed manually following the manufacturer's instructions as specified in the package insert. Breast tumor tissue slides stained with InSite™ Her-2/neu were pre-treated manually using BioGenex EZ-DeWax™ and Citra Plus Antigen Retrieval. These slides were then processed using InSite™ Her-2/neu and counterstained with hematoxylin. The InSite™ testing was carried out on the BioGenex i6000™ Automated Staining System following the protocol recommended by the manufacturer. The slides were blinded and read independently by two pathologists using manual microscopy and the interpretation criteria approved by the FDA in the DakoCytomation HercepTest® package insert.

RESULTS OF THE CLINICAL STUDIES

Even though the staining was carried out by two different histotechnologists and the results were interpreted by two independent, blinded pathologists, no statistically significant difference could be attributed to the results obtained for these different groups, thus all of the data were pooled

(1) Results Presented as 2x2 Concordance Analysis

Table 8 below summarizes the immunostaining results obtained from 352 pairs of identical breast tumor specimens when stained with both InSite™ Her-2/neu and HercepTest® and scored as either 'positive' (2+ or 3+) or 'negative' (0 or 1+).

Table 8: 2x2 Concordance Results.

| | | HercepTest® | | Total |
|-------------------|---|-------------|-----|-------|
| | | - | + | |
| InSite™ Her-2/neu | - | 128 | 7 | 135 |
| | + | 36 | 181 | 217 |
| Total | | 164 | 188 | 352 |

Concordance =87.8% (83.9%, 91.0% 95% Confidence Interval)

Conclusion of the 2 x 2 Results

The overall 2x2 concordance of the InSite™ Her-2/neu with HercepTest® is 87.8% (309/352), with a two-side 95% confidence interval of 83.9% to 91.0%. The null hypothesis, H_0 : concordance \leq 75%, was rejected with p-value < 0.00001.

Percent Positive Agreement (the percentage of specimens scored as positive by HercepTest® that were also scored positive by InSite™ Her-2/neu) was 96.3% (181/188), with a 95% confidence interval of 92.5% to 98.5%. Percent Negative Agreement (the percentage of specimens scored negative by HercepTest® that were also scored negative by InSite™ Her-2/neu) was 78.0% (128/164), with a 95% confidence interval of 70.9% to 84.1%.

The Kappa measure of agreement was 0.752, with a 95% confidence interval of 0.683 to 0.820. The sample value of Kappa measure has a large-sample normal distribution. Its estimated asymptotic standard error is 0.0350. The null hypothesis, H_0 : agreement is no better than chance, was rejected with p-value < 0.00001.

(2) Results Presented as 3x3 Concordance Analysis

Table 9 below summarizes the immunostaining results obtained from 352 pairs of identical breast tumor specimens when stained with both InSite™ Her-2/neu and HercepTest® and scored as either 3+, 2+ or 'negative' (0 or 1+).

Table 9: 3x3 Concordance Results.

| HercepTest® | | | | |
|--------------------------|----------|-----------|-----------|--------------|
| InSite™ Her-2/neu | - | 2+ | 3+ | Total |
| - | 128 | 5 | 2 | 135 |
| 2+ | 25 | 80 | 9 | 114 |
| 3+ | 11 | 14 | 78 | 103 |
| Total | 164 | 99 | 89 | 352 |

Concordance = 81.3% (76.8%, 85.2% 95% Confidence Interval)

Conclusion of the 3 x 3 Results

The overall 3x3 concordance of the InSite™ Her-2/neu with HercepTest® is 81.3% (286/352), with a two-side 95% confidence interval of 76.8% to 85.2%. The null hypothesis, H_0 : concordance \leq 75%, was rejected with p-value = 0.0022.

Percent Positive Agreement (the percentage of specimens scored as 2+ by HercepTest® that were also scored 2+ by InSite™ Her-2/neu) for scores of 2+ was 80.8% (80/99), with a 95% confidence interval of 71.7% to 88.0%. The Percent Positive Agreement of InSite™ Her-2/neu for scores of 3+ was 87.6% (78/89), with a 95% confidence interval of 79.0% to 93.7%. Percent Negative Agreement (the percentage of specimens scored negative by HercepTest® that were also scored negative by InSite™ Her-2/neu) was 78.0% (128/164), with a 95% confidence interval of 70.9% to 84.1%.

The Kappa measure of agreement was 0.714, with a 95% confidence interval of 0.653 to 0.776. The sample value of Kappa measure has a large-sample normal distribution. Its estimated asymptotic standard error is 0.0313. The null hypothesis, H_0 : agreement is no better than chance, was rejected with p-value < 0.00001.

Conclusion of the Clinical Studies

As can be seen in Table 9, there are numerous discrepant results. Many of these lie in the 2+ range. Previous work by many independent investigators has demonstrated that specimens with a Her-2/neu score of 2+ achieved a low percentage of inter-observer agreement. Other factors, such as run to run variability in the immunostaining process over a period of time, may contribute to the difficulty of defining the 2+ staining intensity range. Therefore, it is generally accepted that specimens giving a Her-2/neu score of 2+ should be reevaluated by multiple observers and/or subjected to FISH analysis.

All of the analyses met the study criterion of \geq 75% concordance and thus demonstrated acceptable agreement with the DakoCytomation HercepTest® (PMA No. P980018). This data thus demonstrates that the BioGenex InSite™ Her2/neu kit may be used as an acceptable alternative to aid in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered.

IX. OVERALL CONCLUSIONS DRAWN FROM ALL OF THE STUDIES

The results of the preclinical and clinical testing performed on BioGenex InSite™ Her2/neu demonstrate that this product is reproducible and is specific to Her-2/neu expression on cell membranes with analytical and clinical performance characteristics appropriate for use as an aid in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered.

Risk Benefit Analysis

The testing performed using BioGenex InSite™ Her2/neu indicates that the assay performs consistently and that the assay results are clinically relevant for use as an aid in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered.

Patients falsely assigned as Her-2/neu-positive following testing might receive unnecessary treatment with Herceptin® or Herceptin® in combination with other therapies and might experience unnecessary adverse side effects associated with Herceptin® therapy. These may include infusion toxicity and/or cardiotoxicity.

A false negative immunostaining score result (0 or 1+) would result in patients who may not be selected for therapy with Herceptin® or Herceptin® in combination with other therapies. Because the design of the clinical studies did not include treatment of patients with negative assay results, the risks or benefits of treatment in this patient population are unknown.

Based on the information in the studies provided, the FDA has concluded that the benefits of using the BioGenex InSite™ Her2/neu kit for its intended use outweigh the risks associated with using it.

Safety

The BioGenex InSite™ Her2/neu kit is an *in vitro* diagnostic test and does not contact the patient. Instructions for the safe use of the product are included in the package insert.

Effectiveness

The results of testing performed with the BioGenex InSite™ Her2/neu indicated that the assay is effective in the assessment of breast cancer patients for whom Herceptin® (Trastuzumab) therapy is being considered. CDRH has, therefore, concluded that the device is safe and effective for the stated indication.

X. PANEL RECOMMENDATIONS

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 Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XI. CDRH DECISION

CDRH issued an approval order for the applicant's PATHWAY Anti-c-KIT (9.7) Primary Antibody on December 22, 2004.

The applicant's manufacturing facility was inspected in November 17-19, 2004 and found to be in compliance with the Quality System Regulation (21 CFR 820).

XII. APPROVAL SPECIFICATIONS

Directions for use: See labeling

Hazards to Health from Use of the Device: See Indications, Warnings and precautions in the labeling.

Postapproval Requirements and Restrictions: See approval order.