SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Anti-Her2 IHC system

Device Trade Name: BOND™ ORACLE™ HER2 IHC System

Applicant's Name and address: Leica Biosystems
Balliol Business Park West
Benton Lane
Newcastle-Upon-Tyne NE128EW
United Kingdom

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P090015

Date of FDA Notice of Approval: April 18, 2012

Expedited: Not applicable.

II. INDICATIONS FOR USE

Bond™ Oracle™ HER2 IHC System is a semi-quantitative immunohistochemical (IHC) assay to determine HER2 (Human Epidermal Growth Factor Receptor 2) oncoprotein status in formalin-fixed, paraffin embedded breast cancer tissue processed for histological evaluation following automated staining on the BOND-MAX™ slide staining instrument. The Bond™ Oracle™ HER2 IHC System is indicated as an aid in the assessment of patients for whom Herceptin® (trastuzumab) treatment is being considered.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Bond™ Oracle™ HER2 IHC System labeling, including the Instructions for use, Interpretation Guide and "Playbook" electronic learning course.

V. DEVICE DESCRIPTION

The Bond™ Oracle™ HER2 IHC System is a standard laboratory IHC assay, developed for use on the Leica Biosystems' Bond™ Fully Automated Advanced Staining System (BondMax™ instrument) with ancillary reagents. The Bond™ Oracle™ HER2 IHC System uses a mouse monoclonal antibody (Clone CB11) for the *in vitro* semi-quantitative detection of c-erb-2 antigen in sections of formalin-fixed, paraffin-embedded normal and neoplastic tissue (1). Visualization of the targeted antigen is achieved by sequential application of the HER2 Primary Antibody, visualization reagents, and chromogen, resulting in a visible reaction at the antigen site. Results are evaluated using a light microscope.

Since the first immunoperoxidase technique, reported by Nakane and Pierce, many developments have occurred within the field of immunohistochemistry. A recent development has been the use of polymeric labeling. This technology has been applied to both primary antibodies and immunohistochemical detection systems (2). The Leica Biosystems (LBS) compact polymer detection utilizes a novel controlled, polymerization technology to prepare polymeric horseradish peroxidase (HRP)-linked antibody conjugates. This detection technology system avoids the use of streptavidin and biotin and therefore eliminates nonspecific staining as a result of endogenous biotin. The LBS compact polymer detection works as follows:

- The specimen is incubated with hydrogen peroxide to block endogenous peroxidase activity;
- Ready-to-use HER2 Primary Antibody or HER2 Negative Control is applied;
- A post primary rabbit anti-mouse linker antibody is applied;
- A poly-HRP goat anti-rabbit immunoglobulin G (IgG) reagent is applied;
- The substrate chromogen, 3, 3'-diaminobenzidine (DAB) is applied. This enables the visualization of a brown precipitate at the antigenic site via an enzyme catalyzed substrate reaction;
- Hematoxylin (blue) counterstaining allows the visualization of cell nuclei. Using the LBS compact polymer detection technology in combination with the Bond™ instrument reduces the possibility of human errors and inherent variability resulting from individual reagent dilution, manual pipetting, and reagent application.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Patient management following initial suspicion of breast cancer, including confirmation of the diagnosis, evaluation of stage of disease, and selection of therapy is vital to ensure the welfare of the patient.

Immunohistochemistry and in situ hybridization (both fluorescence (FISH) and chromogenic (CISH)) are now standard methodologies within clinical practice to provide substantial information with regard to diagnosis, therapeutic prediction and prognosis of breast cancer.

In IHC, there are a number of markers that can be of value in the diagnosis and prognosis of breast cancer. The table below (Table 1) summarizes the markers that are commonly used.

Table 1: Breast Cancer Diagnostic Markers in Immunohistochemistry

DIAGNOSIS	PREDICTION
Myoepithelial markers: •Smooth muscle actin •Cytokeratin 14 •P-cadherin	Estrogen receptor Progesterone receptor
Basement membrane – collagen IV	Epidermal growth factor receptor
Cytokeratin 5/6	HER2 protein (Lonardo et al)
E-cadherin	P53 protein
Epithelial membrane antigen Low molecular weight cytokeratins	Ki-67

Following diagnosis, breast cancer is commonly treated by various combinations of surgery, radiation therapy, chemotherapy and hormone therapy. Prognosis and selection of therapy may be influenced by,

- The age and menopausal status of the patient;
- The stage of the disease;
- The histologic and nuclear grade of the primary tumor;
- The estrogen receptor and progesterone receptor status of the tumor;
- The measures of proliferative capacity of the tumor;
- HER2/neu gene amplification.

Therapy options that are available include using tyrosine kinase inhibitors that block signals needed for tumour growth, in combination with other anticancer drugs or alternative hormone therapy based anti-cancer drugs, such as the aromatase inhibitors (Anastrozole, Letrozole) as alternatives to Tamoxifen.

Clinical studies have shown that a subset of breast cancers may respond to monoclonal antibody therapy, e.g. trastuzumab (Herceptin®) (3), and that reliable assessment of HER2 status is vital to ensure that those patients who may benefit from Herceptin® are correctly identified (4,5,6).

There are several alternatives for the detection of Her2 gene amplification: Other devices utilizing fluorescent *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) methodologies for gene amplification determination in human breast cancer tissue specimens are commercially available. Immunohistochemistry (IHC) is an alternative procedure for detection of gene product over-expression in human breast (7). The first test developed for this purpose was the DAKO IHC assay HercepTest™ (P980018), approved in 1998. It

is a semi-quantitative assay that is suited for both manual and automated staining procedures. Since the introduction of the DAKO HercepTest™, 3 additional IHC assays have been approved. The PATHWAY™ HER2 (CB11) (P990081) and PATHWAY™ HER2 (4B5) (P990081 S002) assays manufactured by Ventana Medical Systems' were approved in 2000 and 2005, respectively; and the BioGenex InSite™ HER2/neu (CB11) assay (P040030) manufactured by BioGenex Laboratories, Inc., was approved in 2004.

HER2/neu receptor gene amplification in breast tumor tissue may also be assessed by the use of in situ hybridization (ISH). This method correlates the gene copy number with protein expression and relies on synthetic oligonucleotide hybridization probes. Three ISH assays have been approved, one fluorescent-based assay, PathVysion™ (P980024), manufactured by Abbott Vysis, received approval in 2002 , one single-color chromogenic-based assay, SPOTLight™ (P050040), manufactured by Invitrogen Inc. received approval in 2008 and the two-color INFORM™ Her2 Dual ISH, manufactured by Ventana Medical Systems, Inc. (P100027) approved in 2011.

Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The Bond™ Oracle™ HER2 IHC System has not been marketed in the US. The Bond™ Oracle™ HER2 IHC System has been marketed in the European Union Countries and in Asian Pacific region since August 2007. At the same time it was also made available (subject to training) for sale to countries in the Asia Pacific region. The table below (Table 2) summarizes the countries in which the product has been available for sale.

The Bond™ Oracle™ HER2 IHC System was the subject of a recall in June 2011. The company subsequently altered its manufacturing processes. These processes have been reviewed by FDA and found acceptable. The current product has not been withdrawn from any market for reasons of safety and effectiveness.

Table 2: Marketing History of the Oracle HER2 Bond IHC System

Country	Distribution Channel	Date of Customer Training
Australia	Leica Microsystems	Mar-08
Austria	A. Menarini Diagnostics	Dec-07
Benelux	A. Menarini Diagnostics	Dec-07
Denmark	Leica Microsystems	Mar-08
Finland	Immunodiagnostic	Nov-07

	Oy via DSA	
France	A. Menarini Diagnostics	Dec-07
Germany	A. Menarini Diagnostics	Dec-07
Greece	A. Menarini Diagnostics	Dec-07
Italy	A. Menarini Diagnostics	Dec-07
New Zealand	Leica Microsystems	Mar-08
Portugal	A. Menarini Diagnostics	Dec-07
Republic of Ireland	Leica Microsystems	Oct-07
Saudi Arabia	Medical Business Centre	Nov-07
South Africa	Southern Cross Biotechnology (PTU/Ltd)	Nov-07
Spain	A. Menarini Diagnostics	Dec-07
Sweden	Leica Microsystems	Mar-08
Switzerland	Medite Medizintechnik Ak	Nov-07
Turkey	Sitogen	Nov-07

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

A potential risk associated with misuse of the assay or a false positive test result is to 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 more aggressive therapy from a treatment regimen, potentially resulting in a poor clinical outcome.

IX. SUMMARY OF NON-CLINICAL STUDIES

Several studies were conducted in order to determine the robustness of the assay and test the ranges of the selectable options in the automated staining procedure. All non-clinical studies were performed with the Bond™ Instrument platform with updated software. The non-clinical studies conducted in support of the Bond™ Oracle™ HER2 IHC System included reproducibility, stability, on-board and normal tissue panel testing, DAB on-board testing, polymer and post primary testing, optimization, cell line characterization, and staining concordance. These studies were conducted using tissue microarrays that represented a reasonable

distribution of expected IHC scores (5 samples scored 3+, 4 samples scored 2+, 4 samples scored 1+, samples scored 0).

A. Laboratory Studies

The analytical sensitivity and specificity of the mouse monoclonal antibody designated Clone CB11 is documented in the literature (1). The Clone CB11 has been previously reviewed by FDA in P990081 (Ventana Medical Systems, Inc., PATHWAY™ HER2) which was approved on November 28, 2000. Leica Biosystems sought to support the preclinical element for the Bond™ Oracle™ HER2 IHC System by reference to published literature and the Ventana PATHWAY™ HER2 PMA. The referenced PMA included studies of reactivity of the antibody with normal and neoplastic tissues as well as with characterization of the reactive epitope of the SKB-3 cell line.

1. Analytical Sensitivity

Testing of positive control tissues with the Bond™ Oracle™ HER2 IHC System was able to accurately reproduce 0, 1+, 2+, and 3+ staining patterns.

Included in the Bond™ Oracle™ HER2 IHC System are 4 cell line controls for procedural validation of IHC with Bond™ Oracle™ HER2 IHC System. Staining of these cell lines is intended as a control for the staining procedure and to indicate the validity of the reagent performance. Such controls are part of established guidelines for proper performance of laboratory techniques (12) and specifically for IHC (13). These cell lines have been characterized for IHC Score, receptor load, gene copy number and ratio, and fluorescent analysis. The HER2 Control Cell Line Slide Profile is shown in the table below (Table 3):

Table 31: Analytical Sensitivity of the Bond™ Oracle™ HER2 IHC System on Control Cell Lines

Cell Line	Bond™ Oracle™ HER2 IHC System Profile	HER2 Receptor Load per Cell*	HER2 Gene Amplification Status+	
			Copy Number	Gene Ratio
SK-BR-3	3+	$4.3x10^5$	13.35	3.55
MDA-MB-453	2+	$1.4x10^5$	5.73	2.05
MDA-MB-175	1+	$6.3x10^4$	3.33	1.20
MDA-MB-231	0	$9.3x10^3$	3.15	1.13

*HER2 receptor load analysis as assessed by flow cytometry.
+HER2 gene amplification status as assessed by dual color FISH.

Based upon the data presented it appears that the antibody can effectively distinguish between a 2+ cell line (borderline for amplification) and 1+ cell line (negative for gene amplification) when Her2 Receptors/cell are used as a comparative method.

2. **Analytical Specificity**

a. *Normal Tissue Panel testing*

Analytical specificity of the Bond™ Oracle™ HER2 IHC System was determined by testing the antibody on 29 normal tissues (Table 4).

Table 4: (Immunoreactivity – Normal Panel)

Normal Tissue Type	Staining Pattern	
	HER2 Primary Antibody	HER2 Negative Control
Adrenal	Negative	Negative
Brain, Cerebellum	Negative	Negative
Brain, Cerebrum	Negative	Negative
Breast	Negative	Negative
Bone Marrow	Negative	Negative
Colon	Negative	Negative
Esophagus	Negative	Negative
Eye	Negative	Negative
Hypophysis	Moderate cytoplasmic staining observed in hypophyseal cells (1/3)	Negative
Kidney	Negative	Negative
Larynx	Negative	Negative
Liver	Negative	Negative
Lung	Negative	Negative
Ovary	Negative	Negative
Pancreas	Negative	Negative
Parathyroid	Negative	Negative
Peripheral Nerve	Negative	Negative
Prostate	Negative	Negative
Salivary Gland	Negative	Negative
Skin	Negative	Negative
Small Intestine	Negative	Negative
Spleen	Negative	Negative
Stomach	Weak cytoplasmic staining observed in gastric glands (2/3)	Negative
Striated Muscle	Negative	Negative
Testis	Negative	Negative
Thymus	Negative	Negative
Thyroid	Negative	Negative
Tonsil	Negative	Negative
Uterine Cervix	Negative	Negative

b. *Expression of HER2 in Tumors*

The HER2 oncoprotein is expressed at levels detectable by CB11 immunohistochemistry in up to 20% of adenocarcinomas from various tumor types. Between 10% and 20% of invasive ductal carcinomas of the breast are positive for HER2 oncoprotein (9). 90% of cases of ductal carcinoma *in situ* (DCIS) of comedo type are positive (10), together with almost all cases of Paget's disease of the breast (11). In P990081, apparent positive cytoplasmic staining was observed in human neoplastic tissues of lung, prostate, colon, cervix and ovary. However, with exception of breast tissue, the continuous membranous staining critical for positive scoring of HER2 (14) was not observed.

3. Optimization Studies

a. *System Optimization*

i. DAB Testing: DAB on-board testing was performed to confirm that the Bond™ System assay performance was consistent when the DAB chromogen was mixed at minimum and maximum dispense volumes.

Two HER2 Control Cell Line Sections were used together with 10 sections from a HER2 multi-block containing tissue exhibiting HER2 expression levels of 3+, 2+, 1+ and 0. All slides were run on the Bond and stained with the Oracle HER2 Bond IHC System. All stained slides were scored by a single trained observer experienced in IHC.

The results of this testing demonstrated no variation in scoring that could have lead to an incorrect result when utilizing the Oracle HER2 Bond IHC System as specified in the IFU at the minimum or maximum values.

ii. On-board Testing: On-board testing evaluated 40 HER2 Control Slides (for Bond™ Oracle™ HER2 IHC System), 10 tissue sections for ISH (Hodgkin's Lymphoma for Bond™ EBER probe) and 10 sections for IHC (Tonsil for Bond™ Ready to Use [RTU] Ki67). Normal tissue panel testing evaluated normal tissue types as recommended by Guidance for Submission of Immunohistochemistry Applications to the FDA. Each normal tissue was represented with tissues from 3 different cases on a standard tissue microarray (TMA).

On-board and normal tissue panel testing was performed to validate that the Bond™ Oracle™ HER2 IHC System meet specifications for dilution of reagents, dispense volumes, ability to use reagents directly from storage at 4°C, and the ability to run HER2 staining protocols with other approved reagents without interference or cross over.

All of the above specifications were met.

iii. Post-primary and Polymer testing: Testing was performed to confirm that the manufacturing processes and procedures in place for the post primary and polymer were suitable to produce consistent results. Specifically, testing was performed on the post primary and polymer detection reagents in order to determine that artificially altered concentrations, beyond manufacturing tolerance of these component parts of the Oracle HER2 Bond IHC System would not alter the staining performance.

The results of this testing demonstrated no variation in scoring that could have lead to an incorrect result when utilizing the Oracle HER2 Bond IHC System as specified in the IFU at the minimum or maximum values.

b. *HER2 Assay Optimization*

Immunohistochemical staining of tissue is dependent on the handling, fixation and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation, embedding methods, or to inherent irregularities within the tissue (15). Excessive or incomplete counterstaining may also compromise correct interpretation of the results.

The impact of pre-analytical conditions, (i.e. tissue fixation type and time) on Bond™ Oracle™ HER2 IHC System performance was evaluated using xenograft tumors generated from the MCF7 human carcinoma cell line.

i. HER2 Clone Suitability: Immunohistochemical optimization experiments were performed to determine clone suitability for inclusion in the Bond™ Oracle™ HER2 IHC System.

ii. HER2 Antibody Concentration: Immunohistochemical optimization experiments were performed to determine final concentration of the ready to use HER2 Primary Antibody.

iii. HER2 Antibody Format: Immunohistochemical optimization experiments were performed to determine the optimal manufacturing format of the HER2 Primary Antibody.

iv. Detection System Format: Immunohistochemical optimization experiments were performed to determine the choice of detection

system reagents to be incorporated into the Bond™ Oracle™ HER2 IHC System.

v. HER2 Epitope Retrieval Protocol: Immunohistochemical optimization experiments were performed to determine the choice of epitope retrieval protocol to be incorporated into the Bond™ Oracle™ HER2 IHC System.

vi. HER2 Immunohistochemical Staining Protocol: Immunohistochemical optimization experiments were performed to determine the optimal Bond™ System protocol profile.

The recommended instructions for use for the Bond™ Oracle™ HER2 IHC System are based upon the parameters established in these studies. These are not selectable variables for the HER2 test on the Bond™ System. All testing of the device is based upon these locked parameters.

4. **Stability**

a. *Control Cell Line Slide Stability*
Stability was confirmed if Control slides were scored as expected (0, 1+, 2+, 3+) following staining according to the pre-set protocol on the BOND instrument.

i. Real Time: Stability studies were performed to determine the stability of the HER2 Control Cell Lines that are utilized in the Bond™ Oracle™ HER2 IHC System (HER2 Control Cell Line Slides). Thus far, data generated have shown the HER2 Control Cell Line Slides to be stable for 68 weeks at 4°C storage.

ii. Transport: Simulated transport studies have shown that the sections are unaffected by elevated temperatures that may be experienced during transportation. These studies were performed by cycling cut sections at recommended storage conditions at 4°C for 24 hours, followed by 2 days at 30°C, followed by a return to 4°C for 24 hours, followed by 2 days at 40°C, and then finally a return to recommended storage conditions for 24 hours. Results showed that the control slides are still viable after 69 weeks at 4°C.

b. *Cut-Slide Stability*
Based on a previous PMA (P990081), it is noted that the CB11 epitope in paraffin embedded human tumor tissue sections is stable for 6 months prior to staining.

c. *Kit Stability*
Stability testing for the Bond™ Oracle™ HER2 IHC System to determine shelf-life was performed at Leica Biosystems Newcastle. Stability testing of all reagents was performed using a Bond™ instrument to assess IHC staining performance.

i. Real Time: All components underwent real-time stability testing to examine the long-term robustness and stability of reagents when exposed to conditions that are anticipated during transport, storage, and use; a control batch of reagents, that was stored at -80°C, was used at each time point. Three batches of reagents were used to determine the long-term stability (Real-Time Shelf-Life Test) out to 42 months. Testing at time 0 for batch 1 was halted due to absence of tumor in the test tissue. Based on the stability data, the kit is stable up to 19 months at 4°C. The Sponsor has limited the claim to 12 months in order to account for storage of up to 6 months prior to use.

ii. Transport: One batch of reagents was used to determine the stability after transport (Transport Test). Testing was conducted after thermal cycling that was designed to simulate conditions that reagents might conceivably experience during transport. Testing was done up to 42 months after reagents were sequentially stored at 4°C for at least 24 hours, 30°C for 2 days, 4°C for at least 2 days, 30°C for 2 days, and 2°C to 8°C (recommended storage conditions) for at least 24 hours.

iii. On-board: One batch of reagents was used to determine the stability of On-board reagents (In-Use Test), done after thermal cycling designed to simulate the routine conditions of use that reagents might conceivably experience on a Bond™ instrument. Testing was done up to 42 months after reagents were stored at 37°C for 7 hours followed by storage at 4°C for 14 hours for 15 cycles at each time point.

The results of the reagent Stability Testing are summarized in Table 5.

Table 5: Bond™ Oracle™ HER2 IHC System Stability Testing

Time Point (wks)	Batch Tested	Results	Overall Claim Generated (months)				
			4°C	25°C	37°C	In-Use	Transport
10	1	PASS	2.5	9	18	N/A	2.5
13	1	PASS	N/A	11	24	N/A	N/A
17	1	PASS	4	N/A	N/A	N/A	4
20	1	FAIL**	N/A	FAIL	FAIL		N/A
0	2, 3	PASS	0	N/A	N/A	N/A	N/A
5	2, 3	PASS	N/A	N/A	9	N/A	N/A
27	1	PASS	6	N/A	N/A	6	N/A
53	1	PASS	12	N/A	N/A	N/A	12
79	1	PASS	19	N/A	N/A	19	19
10	2, 3	PASS	2.5	N/A	N/A	N/A	N/A

17	2, 3	PASS	4	N/A	N/A	N/A	N/A
27	2, 3	PASS	6	N/A	N/A	N/A	N/A
53	2, 3	PASS	12	N/A	N/A	N/A	N/A
79	2, 3	PASS	19	N/A	N/A	N/A	N/A

Note: **Accelerated Testing failed

Storage of assay reagents at elevated temperatures is not recommended. However, stability data from two master lots supports product stability through 19 Months for the Bond™ Oracle™ HER2 IHC System components when stored as recommended at 4°C. Subsequent testing with three lots of material was performed and supported a claim of 12 month shelf following up to 6 months of storage.

5. Reproducibility

a. *Within-Run Precision*

Within-run precision testing was performed at Leica Biosystems Newcastle (LBN) using a formalin-fixed, paraffin-embedded composite TMA comprised of 20 invasive breast carcinoma tissue cores, 4 mm in diameter. The 20 cases were selected based on previously assigned HER2 scores. On this basis, 5 cases of HER2 3+, 4 cases of HER2 2+, 4 cases of HER2 1+, and 4 cases of HER2 0, were included.

Within-run precision testing of the Bond™ Oracle™ HER2 IHC System was evaluated on a total of 40 consecutive sections from a TMA comprising of 20 invasive breast tumors and 40 HER2 Control Cell Line Slides. All slides were stained with the Bond™ Oracle™ HER2 IHC System on the Bond™ instrument. Sections were stained during 1 continuous period using a Bond™ Oracle™ HER2 IHC System from the same manufacturing batch. Stained sections were blinded and assessed in a randomized fashion by a single experienced observer to determine within-run precision. An evaluation of the slides from the within-run investigation indicated that 733/800 positive data points could be interpreted. Forty data points were excluded due to the presence of DCIS. A further 27 data points could not be interpreted due to a loss of an invasive tumor (specific to 3 cores). Variation in staining occurred in 61 (8.32%) out of possible 733 staining events. On 37 occasions, variation from 3+ to 2+ (n=20) and from 1+ to 0 (n=17) were observed and would therefore not represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. The remaining 24 (3.27%) occasions represented a change from clinically negative (0 or 1+) to clinically positive (2+ or 3+). The value for the within-run precision testing was 96.7% (95% confidence intervals (CI) = 95.15% to 97.81%).

b. *Between-Run Precision*

Between-run precision testing was performed at Leica Biosystems Newcastle using a formalin-fixed, paraffin-embedded composite TMA comprised of 20 invasive breast carcinoma tissue cores, 4 mm in diameter. The 20 cases were selected based on previously assigned HER2 scores. On this basis, 5 cases of HER2 3+, 4 cases of HER2 2+, 4 cases of HER2 1+, and 4 cases of HER2 0, were included.

Between-run precision testing of the Bond™ Oracle™ HER2 IHC System was evaluated on a total of 24 consecutive sections taken from a TMA comprising of 20 invasive breast tumors and 24 HER2 Control Cell Line Slides. All slides were stained with the Bond™ Oracle™ HER2 IHC System on the Bond™ instrument. The slides were evaluated in 8 independent runs, performed within the same laboratory, on 3 separate days using a Bond™ Oracle™ HER2 IHC System from the same manufacturing batch. Stained slides were blinded and assessed in a randomized fashion by a single experienced observer to determine the between-run precision. An evaluation of the slides from the between-run investigation indicated that 456/480 positive data points could be interpreted; 24 data points could not be interpreted due to the loss of an invasive tumor (specific to 5 cores). Variation in staining occurred for 42 (9.21%) out of possible 456 data points. On 30 occasions, variation from 3+ to 2+ (n=10) and from 1+ to 0 (n=20) were observed and would therefore not represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. The remaining 12 (2.63%) represented a change from clinically negative (0 or 1+) to clinically positive (2+ or 3+). The value for the between-run precision testing was 97.5% (95% CI = 97.5% to 98.61%).

c. *Lot-to-Lot Reproducibility*

To determine lot-to-lot reproducibility, 3 lots of Bond™ Oracle™ HER2 IHC System were manufactured under Good Manufacturing Practices (GMP) on 3 separate occasions and evaluated on 24 breast tumor sections taken from 4 different formalin-fixed, paraffin-embedded tissue blocks (representing 0, 1+, 2+, and 3+ HER2 staining intensities) and 3 HER2 Control Cell Line Slides. Three independent runs were performed within the same laboratory on 3 separate occasions, each using a separate manufacturing lot of the Bond™ Oracle™ HER2 IHC System. All slides were stained with the Bond™ Oracle™ HER2 IHC System. Stained slides were masked and assessed in a randomized fashion by a single trained observer to determine lot-to-lot reproducibility. An evaluation of the slides stained in the lot-to-lot study indicated that all slides were stained with similar staining intensities. No cases varied from positive to negative and

based upon the performance of the HER2 Control Cell Line Slides, the runs were considered valid. Thus, lot-to-lot staining results are consistent using the Bond™ Oracle™ HER2 IHC System.

d. *Between-Laboratory Reproducibility*
The between-laboratory reproducibility testing of the Bond™ Oracle™ HER2 IHC System was evaluated at 3 sites, LBN, and 2 independent laboratories, on a total of 192 sections from a TMA comprising of 20 invasive breast tumors and 24 HER2 Control Cell Line Slides. Of the 192 TMA sections stained, 96 were stained with the HER2 Primary Antibody and 96 with the HER2 Negative Control reagent. All slides were stained with the Bond™ Oracle™ HER2 IHC System on the Bond™ instrument. The slides were evaluated in 8 independent runs performed within each of the 3 different study sites using a Bond™ Oracle™ HER2 IHC System from the same manufacturing lot. Stained slides were blinded and assessed in a randomized fashion by a single experienced observer at LBN to determine the between-laboratory variability.

An evaluation of the slides from the within-run investigation indicated that 1477/1920 positive data points could be interpreted. Of these, 443 positive data points could not be interpreted due to: Inadequate performance of the HER2 Control Cell Slide (160 data points), deviation from the test plan (240 data points), loss of invasive tumor in the TMA block (23 data points), uninterpretable staining due to inadequate washing by the Bond™ instrument (20 data points).

An evaluation of the slides in the between-laboratory precision investigation indicated that variation in staining occurred in 79 (5.28%) out of a possible 1477 staining events. Of these, 14/1477 (0.95%) occasions represented variations from 0 to 1+ or 2+ to 3+ and as such, did not represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. The results value for this analysis was 99.05% (95% CI = 98.42% to 99.46%).

Of the 14 clinically insignificant changes observed in this study, 5/1477 (0.34%) staining events occurred at LBN (Site A), 8/1477 (0.54%) staining events occurred at Site B, and 1/1477 (0.07%) staining events occurred at Site C. The remaining 65/1477 (4.40%) occasions were variations from 2+ to a 1+ or 0 and therefore would represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. The Pass value for this analysis was 95.6% (95% CI = 94.42% to 96.54%).

Of the 65 clinically significant changes, 11/65 (16.92%) staining events occurred at LBN (Site A), 24/65 (36.92%) staining events

occurred at Site B, and 30/65 (46.15%) staining events occurred at Site C. Of the clinically significant changes, on no occasion did a 3+ change to a negative (0 or 1+).

e. *Inter-Observer Reproducibility*
The inter-observer reproducibility was assessed using 40 invasive breast cancer cases (resection specimens) that were sectioned and provided to the 3 sites for staining and interpretation. The sections were blinded and randomized at each site prior to scoring. Inter-observer agreement between the 2 independent study sites, was 87.5% (95% CI = 73.3% to 95.8%). The agreement between the independent study sites and LBN was 92.5% (95% CI = 79.6 to 98.4%) and 85% (95% CI = 70.1% to 94.3%), at site 1 and site 2, respectively.

B. Animal Studies
None.

C. Additional Studies
None.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The applicant performed a clinical study to establish a reasonable assurance of safety and effectiveness of Bond™ Oracle™ HER2 IHC System. The clinical study consisted of comparisons between Bond™ Oracle™ HER2 IHC System and Dako HercepTest™ and comparisons between Bond™ Oracle™ HER2 IHC System and Vysis PathVysion™ FISH test.

A. Study Design

The clinical concordance of Bond™ Oracle™ HER2 IHC System to Dako HercepTest™ and Vysis PathVysion™ FISH test was examined to determine the suitability of the Bond™ Oracle™ HER2 IHC System for use as an aid in determination of treatment for Herceptin® therapy.

The samples for this study were randomly selected from clinical archives at US-based institutions and were collected between 2003 and 2008. The database for this PMA reflected data collected through December 31, 2009 and included 452 patient samples. There were two investigational sites. The study was conducted as an 18-month, US-based, masked evaluation. Each study site was supplied with formalin-fixed, paraffin-embedded breast cancer samples of known HER2 status. Cohorts of 160 and 292 specimens were tested at Site 1 and Site 2, respectively. Cohorts tested at each site had an equal representation of weakly positive/positive (2+, 3+) and negative (0, 1+), based on retrospective HER2 IHC scores. Of these, 12 samples were considered unsuitable due to the lack of sufficient invasive tumor and 9 additional samples could not be scored as a result of tissue lifting from the slide surface;

these samples were removed from the study, resulting in a final study population of 431 samples.

Bond™ Oracle™ HER2 IHC, Dako HercepTest™ and Abbott Molecular PathVysion™ HER2 FISH test were conducted on serial sections of FFPE tissue specimens. As a control, all cases were stained with the HercepTest™ according to the manufacturer's instructions as specified in the package insert. HercepTest™ is a legally marketed alternative IHC device with similar indications for use as that proposed for the Bond™ Oracle™ HER2 IHC System. Sequential sections from each case were then stained with the Bond™ Oracle™ HER2 IHC System and PathVysion™ HER2 FISH test. All cases were de-linked from unique patient identifying information and were accompanied by clinical data relating to tumor size, tumor stage, tumor grade, and ER status.

All stained slides were masked and scored in a randomized fashion by trained observers at each of the 2 sites. Stained slides were evaluated for HER2 and chromosome 17 copy number in at least 20 nuclei to determine HER2 gene amplification status for each case according to the device labeling. Percent positive, percent negative and percent overall agreements between Bond™ Oracle™ HER2 IHC System and HercepTest™ results or PathVysion™ HER2 FISH results were determined.

The study results were analyzed by a statistician independent of both the clinical investigators and the Sponsor.

1. **Clinical Inclusion and Exclusion Criteria**
   a. *Inclusion Criteria:* Enrollment of patient samples in the method comparison study requires that archived formalin-fixed, paraffin-embedded invasive breast carcinoma tissue blocks of known HER2 protein status contain sufficient material for inclusion in the study and sufficient tumor in each section for evaluation of staining patterns.
   b. *Exclusion Criteria:* Samples that were excluded from analysis were unsuitable due to: the absence of any invasive breast tumor in the sample and poor fixation and/or processing of the original clinical sample.

2. **Follow-Up Schedule**
   No patient follow-up was necessary as all samples are left over archived samples.

3. **Clinical endpoints**
   a. *Method Comparison Study-IHC*
   The primary clinical endpoints of the study were concordance between Bond™ Oracle™ HER2 IHC System and the HercepTest™. The

acceptance criterion was defined as greater than 75% overall concordance between the 2 tests with a 95% lower bound of 70%.

i. For the 2x2 concordance analysis, scores were interpreted as negative if the staining intensity was 0 or 1+, and positive for scores of 2+ or 3+.
ii. For the 3x3 concordance analysis, scores were interpreted as negative if the staining was 0 or 1+, equivocal for scores of 2+ and positive for scores of 3+. Data was then analyzed for positive staining agreement and negative staining agreement between the control and experimental devices.

b. Method Comparison Study-FISH

Leica Biosystems conducted FISH analysis on the same original clinical samples to compare the concordance between the Bond™ Oracle™ HER2 IHC System assay or DAKO HercepTest™ data and a clinical reference method (Abbott Molecular PathVysion™ HER2 FISH Test).

This study was a blinded central laboratory study utilizing archived tissue specimens that had been de-identified and unlinked from patient information to compare HER2 protein levels determined by the Bond™ Oracle™ HER2 IHC System assay to amplification status determined by the PathVysion™ HER2 FISH assay. All assays were conducted on serial sections of FFPE tissue specimens. Stained slides were evaluated for HER2 and chromosome 17 copy number in at least 20 nuclei to determine HER2 gene amplification status for each case according to the device labeling. Percent positive, percent negative and percent overall agreements between Bond™ Oracle™ HER2 IHC System or HercepTest™ results and PathVysion™ HER2 FISH results were determined. Of the original 431 samples in the IHC comparison, one sample did not hybridize properly in the FISH test. 1 sample was counted as equivocal by both Herceptest and ORACLE BOND system without having an additional 20 nuclei counted. A second case was similarly counted as equivocal by the by just the ORACLE BOND system. This produced 429 data points in the Herceptest to FISH comparison and 428 samples in the Oracle Bond System to FISH comparison.

The primary objective of this study was to determine the positive and negative percent agreement rates and the corresponding score 95% confidence intervals (CI) for the Bond™ Oracle™ HER2 IHC System assay results in comparison with PathVysion™ HER2 FISH assay results. The ORACLE BOND results were considered acceptable if the lower bound of the 95% CI was 75% or greater for positive, negative, and overall concordance.

The secondary objectives of this study were to determine overall percent agreement and its score 95% confidence interval of the Control IHC assay results in comparison with PathVysion™ HER2 FISH assay results and to determine if any significant differences could be detected between the two IHC assays that may raise concerns about the safety and effectiveness of the Bond™ Oracle™ HER2 IHC System.

B. Specimen Accountability-All Clinical Studies

A total of 452 samples were tested. The accounting of patient samples is shown in Table 6 below. Of these, 21 samples were considered unsuitable and therefore removed from the study. Of these 21 samples, 12 were eliminated due to the absence of any invasive breast tumor in the sample and 9 were eliminated due to poor fixation and/or processing of the original clinical sample; which resulted in tissue lifting/folding during the staining process making interpretation impossible.

Table 6: Accounting of Clinical Study Specimens

Status	Site 1	Site 2	All sites
Cases considered for inclusion in the study	160	292	452
Cases with successful initial staining using both IHC methods	159	250	409
Cases with successful initial Bond™ Oracle™ HER2 IHC System Staining	159	270	429
Cases with successful initial Bond™ Oracle™ HER2 IHC System Staining	159	265	424
Number of repeat stain cases Oracle™	1	42	43
Number of successful repeat staining cases	0	22	22
Number of stained cases available for IHC comparison	159	272	431
Number of cases stained with PathVysion™ HER2 FISH staining	158	272	430
Number of evaluable cases stained with PathVysion™ HER2 FISH staining and HercepTest tests	158	271	429
Number of evaluable cases stained with PathVysion™ HER2 FISH staining and both IHC tests	158	270	428

Table 7 provides a chart showing the reasons why various samples were removed from the study.

Data from both sites were pooled by LBN and provided to BioStat International, Inc. for analysis.

Table 7: Non-Evaluable Cases All Reasons

Number of cases	Reason for non-evaluability
21	21 total IHC samples of 452 samples failed
12	12 cases failed H&E examination due to lack of tumor tissue present
9	9 Cases failed the IHC preparation step due to poor tissue fixation and processing that caused folding of the tissue on the slide.
1	1 additional case failed PathVysion™ HER2 FISH due to lack of hybridization
2	2 FISH cases were counted as equivocal (only 20 nuclei counted) with Oracle Bond system (1 case with HercepTest)

C. Study Population Demographics and Baseline Parameters

All cases associated with this study were collected at U.S. sites from women who have undergone surgical biopsy for breast cancer. Race and ethnicity associated with the cases in this study was not available. Demographic data (age) was available for 159 cases at Site 1, 272 cases at Site 2, and 431 cases overall. Available demographic data are summarized in Table 8.

Table 8 Summary of Demographics

Demographic Category	Characteristic /Statistic	All Samples	Site 1	Site 2	P-value
Sample size		**n = 431**	**n = 159**	**n = 272**	
Age (years)	**n**	**431**	**159**	**272**	
	Mean	62.3	58.7	64.3	
	Standard Deviation	14.2	13.9	14.0	**<0.0001**
	Median	61.0	57.0	65.0	
	Range	27.0-94.0	27.0-94.0	31.0-94.0	
Tumor size (CM)	**n**	**428**	**159**	**269**	**0.0300**
	Mean	2.1	2.3	2.0	
	Standard Deviation	1.6	1.7	1.6	
	Median	1.7	1.9	1.5	

Demographic Category	Characteristic /Statistic	All Samples	Site 1	Site 2	P-value
	Range	0.2-10.0	0.2-10.0	0.4-10.0	
Tumor Stage	**n**	**430**	**159**	**267**	**0.0338**
	0,1	221	73	148	
	2, 2a, 2b	150	62	88	
	3, 3a, 3b, 3c	52	24	28	
	4	7	0	7	
Tumor Grade	**n**	**390**	**159**	**231**	**0.0220**
Poorly Differentiated		186	88	98	
Moderately Differentiated		138	51	87	
Moderately Well Differentiated		6	0	6	
Well Differentiated		60	20	40	
Diagnosis	**n**	**431**	**159**	**272**	**0.0945**
Invasive Ductal Carcinoma		370	141	229	
Invasive Lobular Carcinoma		44	17	27	
Invasive Mucinous Carcinoma		8	0	8	
Mixed Invasive Ductal and Lobular Carcinoma		4	0	4	
Other		5	1	4	

D. Safety and Effectiveness Results

1. IHC-vs-IHC Comparison Results

a. *2 x 2 IHC Concordance*

In this primary analysis the test results from the two tests (Bond™ Oracle™ HER2 IHC System and DAKO HercepTest™) are categorized as negative (0, 1+) or positive (2+, 3+). The frequencies of four possible combinations are displayed in a 2x2 table format (see Table 9). Then, the overall concordance rate based on this 2x2 table was calculated accompanied by a 95% exact confidence interval (based on the binomial distribution).

The observed agreement for 431 samples between the two tests in a 2x2 analysis show a concordance of 92.34% (398/431) with a 95% CI of 89.42% – 94.67%.

The percentage positive agreement or the ability of Bond™ Oracle™ HER2 IHC System to correctly identify HercepTest™-positive cases (the percentage of specimens scored positive by both Bond™ Oracle™ HER2 IHC System and HercepTest™ out of all the

HercepTest™-positive cases) was 84.87% (129/152) with a 95% CI of 78.17% – 90.16%. The percentage negative agreement or the ability of the test to correctly identify HercepTest™-negative cases (the percentage of specimens scored negative by both Bond™ Oracle™ HER2 IHC System and HercepTest™ out of all the HercepTest™-negative cases) was 96.42% (269/279) with a 95% CI of 93.51 % – 98.27%.

Table 9: Concordance between Bond™ Oracle™ HER2 IHC System with HercepTest™ (2x2)

		Dako HercepTest™ IHC scores		
		Negative	**Positive**	**Totals**
Bond™ Oracle™ HER2 IHC System	**Negative**	269	23	292
	Positive	10	129	139
	Totals	279	152	431
2x2 Concordance (95% CI) = 92.34% (89.42 – 94.67%); p<0.0001				
Percent Positive Agreement = 84.87% (78.17% – 90.16%)				
Percent Negative Agreement = 96.42% (93.51 % – 98.27%)				

b. *3 x 3 IHC Concordance*

Data was grouped as negative (0 or 1+), equivocal (2+) or positive (3+) for 3x3 analysis and showed a concordance of 86.54% (373/431) with a 95% CI of 82.95% – 89.62 %. Therefore, the null hypothesis (H0) that agreement was no greater than 75% was rejected with a p-value<0.0001.

The percentage positive agreement for 3+ (the percentage of specimens scored 3+ positive by both Bond™ Oracle™ HER2 IHC System and HercepTest™ out of all the 3+ HercepTest™ positive cases) in this study was 73.33% (66/90) with a 95% CI of 62.97% – 82.11%. The percentage negative agreement was 96.42% (269/279) with a 95% CI of 93.51 % – 98.27 (See Table 10).

Table 10: Concordance between Bond™ Oracle™ HER2 IHC System with HercepTest™ (3x3)

		Dako HercepTest™ IHC scores			
		Negative (0/1+)	**2+**	**3+**	**Totals**
Bond™ Oracle™ HER2 IHC System	**0/1+**	269	23	0	292
	2+	10	38	24	72
	3+	0	1	66	67
	Totals	279	62	90	431
3x3 Concordance (95% CI) = 87.6% (84.0 to 99.0%); p<0.0001					
Percent Positive Agreement = 73.33% (62.97% – 82.11%)					
Percent Negative Agreement = 96.42% (93.51 % – 98.27%)					

2. IHC-vs-FISH Comparison Results

An additional method comparison between IHC (Bond™ Oracle™ HER2 IHC System or HercepTest™) and FISH (Vysis PathVysion™)was performed to resolve the unexpected discrepancies in the positive agreement between the two IHC tests.

a. Bond™ Oracle™ HER2 IHC System vs. FISH:

Using Vysis PathVysion™ FISH as the reference method, the positive percent agreement for Bond™ Oracle™ HER2 IHC System was 93.8% and the negative percent agreement was 85.8%, with an overall 2x2 concordance of 87.6% (where IHC scores of 2 and 3 are considered positive)(Table 11).

Table 11. Concordance between Leica Bond™ Oracle™ HER2 IHC System and HER2/ CEP 17 FISH

		PathVysion™ HER2/ CEP 17 FISH scores		
		Positive	Negative	Total
Leica Bond™ Oracle™ HER2 IHC System Scores	0/1+	6	284	290
	2+	30	41	71
	3+	61	6	67
	Total	97	331	428
2x2 Concordance (95% CI) = 61+30+284/428 = 87.6% (84.1 – 90.4%)				
Percent Positive Agreement = 61+30/97 = 93.8% (86.9 – 97.4 %)				
Percent Negative Agreement = 284/331 = 85.8% (84.1 – 90.4%)				

b. HercepTest™ vs. FISH: Using Vysis PathVysion™ FISH as the reference method, the positive percent agreement for HercepTest™ was 92.7% and the negative percent agreement was 81.6%, with an overall 2x2 concordance of 84.1% (where IHC scores of 2 and 3 are considered positive)(Table 12).

Table 12. Concordance between HercepTest™ and HER2/ CEP 17 FISH

		PathVysion™ HER2/ CEP 17 FISH scores		
		Positive	Negative	Total
DAKO HercepTest™ IHC scores	0/1+	6	271	277
	2+	10	51	62
	3+	80	10	90
	Total	97	332	429
2x2 Concordance (95% CI) = 80+10+271/429 = 84.1% (80.3 – 87.3%)				
Percent Positive Agreement = 80+10/97 = 92.7% (85.6 – 96.7%)				
Percent Negative Agreement = 271/332 = 81.6% (77.1 – 87.3%)				

Based on the data in tables 11 and 12, the discrepancies in positive agreement between the two IHC tests (Bond™ Oracle™ HER2 IHC and HercepTest™) are limited to the samples with 2+ scoring. As these are the equivocal samples in IHC testing, the positive nature of the scoring would be confirmed by the required reflex testing to ISH. Further, in both data sets, the same 6 samples were negative by IHC but considered positive by FISH (6.1% false negative rate). This is typical of IHC to FISH comparisons where approximately 5% of samples that show HER2 amplification do not show overexpression of HER2 protein. These results confirm that the failure of the Bond™ Oracle™ HER2 IHC to meet acceptance criteria in the 3 x 3 IHC concordance test does not affect the safety and effectiveness of the test.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Exploratory Analyses

IHC 4x4 Concordance

An additional exploratory endpoint was concordance between the two tests in 4x4 analysis. The acceptance criterion was defined as greater than 75% overall concordance between the 2 tests with a 95% lower bound of 70%.

a. *Results*

For the exploratory 4x4 concordance study, data was categorized as 0, 1+, 2+, or 3+. The 4x4 analysis showed a concordance of 69.14% (298/461) with a 95% CI of 64.54% – 73.47%. Results presented in the 4x4 format (Table 13) were for descriptive purposes only and do not represent the basis for meeting the success criteria of this Clinical Validation Study.

Table 13: Concordance between Bond™ Oracle™ HER2 IHC System with HercepTest™ (4x4)

		Dako HercepTest™ IHC scores				
		0	**1+**	**2+**	**3+**	**Totals**
Bond™ Oracle™ HER2 IHC System	**0**	160	29	0	0	189
	1+	0	122	24	0	146
	2+	0	0	145	0	145
	3+	0	0	22	271	293
	Totals	160	151	191	271	773
Overall concordance (95% CI) = 90.30% (88.00% – 92.20%)						
0 concordance (95% CI) = 100.00% (98.00% – 100%)						
1+ concordance (95% CI) = 80.79% (73.73% – 86.33)						
2+ concordance (95% CI) = 75.92% (69.36% – 81.45%)						
3+ concordance (95% CI) = 100.00% (98.81% – 100%)						

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

The Bond™ Oracle™ HER2 IHC System is considered safe when applied for its intended use of *in vitro* diagnostic testing. Non-agreement of results between this device and the comparator device do not represent an increased risk to patients.

A potential risk associated with misuse of the assay or a false positive test result is to 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 more aggressive therapy from a treatment regimen, potentially resulting in a poor clinical outcome.

The adverse effects of the device are based on data collected in a clinical study conducted to support PMA approval as described above. A study using patient samples with unknown Her2 gene amplification status was not conducted. Instead, test results from this device were compared favorably to that from the DAKO HercepTest™, which was approved for the same indication for use.

Further, the device was shown to have reasonable reproducibility that is essential to prolonged safety of the device. However, potential institutional differences should be recognized and individual proficiency should be assessed prior to implementation of this test (15, 16).

The HER2 primary and negative antibodies contain ProClin™ 950, a preservative containing the active ingredient 2-methyl-4-isothiazolin-3-one. Symptoms of overexposure to ProClin™ 950, the preservative used in the Bond™ Oracle™ reagents, may include irritation to skin, eye, mucous membranes and the upper respiratory tract. The concentration of ProClin™

950 in the reagents is 0.09%. The solutions do not meet the Occupational Safety and Health Administration criteria for a hazardous substance.

These safety precautions are sufficient to protect the end user when using the Bond™ Oracle™ HER2 IHC System.

B. Effectiveness Conclusions

The information demonstrating the potential of the device to be used effectively is based on data collected in a clinical study conducted to support PMA approval as described above. The use of unknown patient samples was not conducted. Instead, test results from this device were compared favorably to that from DAKO HercepTest™, which was approved for the same indication for use.

Reproducible results were achievable with archived formalin-fixed, paraffin embedded patients tumors tissue collected stored up to 6 months at room temperature prior to testing using controls that are stable up to 17 months when stored at room temperature and reagents that are stable up to 12 months when stored at 4°C.

Results of the testing of the Bond™ Oracle™ HER2 IHC System indicate that the System is effective in identifying patients with breast cancer for whom Herceptin® therapy is being considered. The data provided demonstrate that the Bond™ Oracle™ HER2 IHC System is stable, may be processed and interpreted reproducibly, and appropriately selects patients for Herceptin® therapy.

C. Overall Conclusions

The results from the non-clinical and clinical studies presented in this original PMA application submission establish reasonable assurance that the Bond™ Oracle™ HER2 IHC System is safe and effective for its intended use when used in accordance with product labeling.

Benefit/Risk:

Evaluation of the Bond™ Oracle™ HER2 IHC System indicates that the System performs consistently and provides clinically relevant results in assessing patients with breast cancer being considered for Herceptin® therapy.

Patients falsely identified as being 2+ or 3+ positive for the presence of HER2 protein might receive unnecessary treatment with Herceptin® therapy alone or in combination with other therapies. Such unnecessary therapeutic intervention might subsequently result in adverse side effects associated with Herceptin® therapy. The known risks of Herceptin® treatment include

infusion toxicity (chills, fever, pain, pain at the tumor site, asthenia, nausea, vomiting and headache), cardiotoxicity, and in rare cases, death. Other unknown adverse events may also occur for patients exposed to these therapies.

A false negative result (0 or 1+) could result in patients not being selected for receiving the potential benefits of Herceptin® therapy alone or Herceptin® in combination with other therapies.

There is a benefit in knowing HER2 protein status in invasive breast cancer patients so that clinicians can make more informed decisions to improve the overall management of their breast cancer patients.

Overall, the results support the conclusion that the benefits outweigh the risks associated with the Bond™ Oracle™ HER2 IHC System.

XIV. CDRH DECISION

CDRH issued an approval order on April 18, 2012. The final conditions of approval are cited in the approval order.

The applicant's manufacturing facilities were inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. 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.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

1. Corbett IP, Henry JA, Angus B et al. NCL-CB11, A new monoclonal antibody recognizing the internal domain of the c-erbB-2 oncogene protein effective for use on formalin-fixed, paraffin-embedded tissue. Journal of Pathology. 1990; 161:15-25.
2. Lonardo F, Di Marco E, King CR, Pierce JH, Segatto O, Aaronson SA, et al. The normal ErbB-2 product is an atypical receptor-like tyrosine kinase with constitutive activity in the absence of ligand. New Biologist. 1990; 2: 992-1003.

3. Carter P, Presta L, Gorman CM, Ridgway JBB, Henner D, Wong WLT, et al. Humanization of an anti-pl85HER2 antibody for human cancer therapy. Proceedings of the National Academy of Science USA. 1992; 89: 4285-4289.
4. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. p185HER2 monoclonal antibody has anti-proliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. Molecular & Cell Biology. 1989; 9: 1165-1172.
5. Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, et al. Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. Cancer Immunology and Immunotherapy. 1993; 37: 255-263.
6. Baselga J, Norton L, Albanell J, Kim Y-M, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin®) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/ neu overexpressing human breast cancer xenografts. Cancer Research 1998; 58: 2825-2831.
7. Nakane PK and Pierce GB. Enzyme labeled antibodies: Preparations and applications for the localization of antigens. Journal of Histochemistry and Cytochemistry. 1967; 14: 929-931.
8. Tsutsumi Y, Serizawa A and Kawai K. Enhanced polymer one-step staining (EPOS) for proliferating cell nuclear antigen and Ki-67 antigen-applications to intraoperative frozen diagnosis. Pathology International. 1995; 45(2): 108-115.
9. Walker FIA, Bartlett JMS Dowsett M, Ellis IO, Hanby AN, Jasani B, Miller K and Pinder SE. HER2 Testing in the UK- Further Update To Recommendations. Journal of Clinical Pathology. 2008.
10. Dickson, RB and Lippman, ME. Genes, Oncogenes, and Hormones Boston, Kluwer Academic Publishers, 1992.
11. Keatings, L. et al. c-erbB-2 oncoprotein expression in mammary and extramammary Paget's disease: an immunohistochemical study. Histopathology. 1990; 17: 234-247.
12. The National Committee for Clinical Laboratory Standards (NCCLS). Quality assurance for immunocytochemistry; Approved guideline. NCCLS document MM4-A (1-56238-396-5) NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 1999; 19087-1898: USA
13. Elias JM, Gown AM, Nakamura RM, Wilbur DC, Herman GE, Jaffe ES, et al. Special Report: Quality control in immunohistochemistry. American Journal of Clinical Pathology 1989; 92: 836-843.
14. Press MF, Cordon-Cardo C, Slamon DJ. Expression of the HER-2/neu proto-oncogene in normal human adult and fetal tissues. Oncogene 1990; 5: 953-962.
15. Nadji, M. and Morales, A. R. Immunoperoxidase, part I: the techniques and its pitfalls. Laboratory Medicine 1983; 14:767.
16. Jackson P. 2007. Quality Assurance in Immunohistochemistry. In: Immunohistochemistry, 2007 (ed. Renshaw S). PP 205-237. Scion Publishing Ltd.