Summary of Safety and Effectiveness Data

I. General Information

Device Generic Name:	Mouse monoclonal antibody for detection of c-erbB-2 antigen in histological tissue sections
Device Trade Name:	Ventana Medical Systems' PATHWAY™ Her 2
Applicant's Name and Address:	Ventana Medical Systems, Inc. 3865 North Business Center Drive Tucson, AZ 85705
PMA Number:	P990081
Date of Panel Recommendation:	None
Product Code/Panel:	MVC - 88 Immunohistochemistry Test for Her 2/neu.
Date of Notice of Approval of the Application:	NOV 28 2000

II. Indications for Use

Ventana Medical Systems' PATHWAY™ Her 2 (clone CB11) is a mouse monoclonal antibody intended for laboratory use for the semi-quantitative detection of c-erbB-2 antigen in sections of formalin fixed, paraffin embedded normal and neoplastic tissue on a Ventana automated immunohistochemistry slide staining device. It is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® treatment is being considered.

III. Contraindications, Warnings and Precautions

Contraindications: None known.

Warnings and Precautions: See product labeling.

IV. Background

Breast cancer is the most common newly diagnosed malignancy in American women and is the second leading cause of cancer-related death[1]. Increased awareness of the importance for screening for early signs of breast cancer has resulted in the removal of smaller primary tumors, which are now often less than 1 cm in diameter. These small tissue samples can be studied by routine immunohistochemistry (IHC), making this technique an important tool for the pathologist. IHC tests such as those for estrogen receptor and progesterone receptor have been used to assess prognosis and assist in the planning of therapy for breast cancer patients. Another important marker for breast cancer evaluation is the c-erbB-2 oncoprotein (also called HER 2 or $p185^{neu}$ protein).

The c-erbB-2 gene is localized to chromosome 17q and encodes for an intracellular membrane protein detected in the cellular membrane[2]. The c-erbB-2 oncoprotein is closely related to the epidermal growth factor receptor (EGFR) and, like EGFR has tyrosine kinase activity. C-erbB-2 gene amplification and the corresponding overexpression of c-erbB-2 protein have been identified in a variety of human tumors, including approximately 25% of breast carcinomas.[3] In node positive breast cancer, amplification of the c-erbB-2 gene and overexpression of c-erbB-2 protein have been correlated with adverse disease outcome.[3,4,5] C-erbB-2 status has also been shown to be of use for determination of therapy for breast cancer patients.[6]

The FDA approved therapeutic Herceptin® has been shown to benefit breast carcinoma patients by arresting, and in some cases reversing the growth of their cancer. The drug is a humanized monoclonal antibody (Trastuzumab) that binds to Her-2/*neu* protein on cancer cells.[6] *In vitro* diagnostics for the determination of Her-2/*neu* positivity in breast carcinomas aid the clinician in determining the appropriateness of therapy with Herceptin.®

Interpretation of the results of any detection system for c-erbB-2 must take into consideration the fact that c-erbB-2 protein is expressed in both breast cancer tumors and healthy tissue, albeit at differing levels and cellular locations. Immunohistochemical preparations have the advantage of ensuring intact tissue morphology to aid in the interpretation of the localization within the cell, staining intensity and distribution of the c-erbB-2 signal in the tissue. Histological tests such as IHC-based breast cancer prognostic tests should only be interpreted by a specialist in breast pathology, and the results should be used carefully in conjunction with other known clinical and laboratory data available for each patient.

V. Device Description

The demonstration of antigens in tissue and cells by immunostaining is a process involving first the binding of an antibody to an antigen of interest and second,

visualization of the bound primary antibody by an indirect biotin-avidin system coupled to an enzyme. PATHWAY™ Her 2 primary antibody specifically binds to c-erbB-2 antigen located in the plasma membrane and cytoplasmic regions of a variety of normal and neoplastic tissues. The primary antibody is located by a biotin conjugated secondary antibody formulation that recognizes rabbit or mouse immunoglobulins. This step is followed by the addition of a streptavidin-enzyme conjugate that binds to the biotin on the secondary antibody. The primary antibody-secondary antibody-avidin enzyme complex is visualized by using a precipitating enzyme generated product.

PATHWAY™ Her 2 was developed for use on Ventana's automated immunohistochemistry slide staining devices.
PATHWAY™ Her 2 is an immunohistochemical antibody indicated for aid in the assessment of patients being considered for Herceptin® treatment. The test is interpreted and reported as negative for c-erbB-2 overexpression (0 and 1+ staining intensity) weakly reactive (2+ staining intensity) and strongly reactive (3+ staining intensity). In this manner PATHWAY™ Her 2 was shown to provide 82.9 to 94.7% concordant results to the DAKO HercepTest™ approved for use with Herceptin®.

VI. Alternative Practices and Procedures

There are several alternative laboratory practices that can be used to measure Her-2 overexpression. Immunohistochemical staining in tissue sections may be achieved by other available primary antibodies and detection chemistries. There is one other FDA approved immunohistochemical test for the detection of c-erbB-2 protein utilizing a rabbit polyclonal antibody (P980018, The DAKO HercepTest™).

There are also two approved fluorescence *in situ* hybridization (FISH) tests for the detection of Her-2 gene amplification in tissues, which has been correlated to protein overexpression: Ventana INFORM® HER-2/neu Gene Detection System (P940004) and Vysis PathVysion (P980024).

VII. Marketing History

Ventana c-erbB-2 Primary Antibody (PATHWAY™ Her 2) has been marketed in the United States and abroad as an Analyte Specific Reagent (ASR). Countries in which PATHWAY™ Her 2 have been marketed as an ASR are: Austria, Belgium, Canada, China, Finland, France, Germany, Israel, Italy, Puerto Rico, Republic of South Africa, Spain, Sweden, Switzerland, and the United states. This represents products sold since launch (2/98) through May 3, 2000. Ventana c-erbB-2 Primary Antibody has never been withdrawn from the market, either domestically or abroad.

VIII. Potential Adverse Effects of the Device on Health

A diagnosis of breast carcinoma is established using hematoxylin and eosin (H&E) staining in conjunction with the clinical presentation of the disease. C-erbB-2 detection is

intended for use in a panel of detection immunohistochemistries to further characterize the carcinoma.

Ventana c-erbB-2 primary antibody is indicated as an aid in determining therapy with Herceptin®. A false positive result may lead to patients with undetectable or low levels of c-erbB-2 expression (0 or 1+) to be treated with Herceptin®. The benefit of Herceptin® treatment in this patient population is not known. The risk of Herceptin® treatment include adverse effects such as infusional toxicity (chills, fever, pain, pain at the tumor site, asthenia, nausea, vomiting and headache), and cardiotoxicity.[6] A false negative result would result in patients not being treated with Herceptin®. Clinical studies have shown that between 4% and 53% of immunohistochemically positive patients benefit from Herceptin® therapy either alone or in combination with other therapies.[6] A false negative result would deny patients the possibility of this benefit.

IX. Summary of Studies

1. Preclinical/Nonclinical Studies

Preclinical studies included reagent and antigen stability studies, antibody specificity studies, and reproducibility studies. Also included are studies performed to characterize control cell lines and the stability of these cell lines on cut slides, although these are a separate product.

a. Reagent Stability of Ventana c-erbB-2 Primary Antibody

The primary study for determination of stability dating was storage at 2-8° C for 12 months or longer. Testing intervals are day 0, day 3, week 1, week 2, and month 1 through month 12. In addition, stability was analyzed after a mock shipping stress (2 days at 45° C followed by continuous storage at 2-8° C for 12 months) tested at day 0, day 3, week 1, week 2, and month 1 through month 12. The final test performed was at 45° C continuous test for 1 month, or until failure, with testing intervals of day 0, day 3, week 1, week 2, and month 1. Intended storage is at 2-8° C. The two lots tested for establishing dating were evaluated through month 18 with both ongoing conditions (2-8° C continuous and 2 day 45° C followed by 2-8° C continuous) still passing.

The tissue used for stability studies was paraffin-embedded invasive ductal breast carcinoma. Testing of PATHWAY™ Her 2 includes antigen enhancement, and was visualized using Ventana's DAB detection kit. Three slides were used to test for staining. Cells expected to be negative in the positive ductal breast carcinoma slides are used as a negative tissue control to look for unintended cross-reactivity.

The test parameter measured was staining intensity at time points indicated. Slides were masked and scored in comparison with rated reference slides of intensity levels of 0, 1, 2, 3, and 4+. Passing criteria were set as maintaining intensity when compared to rated reference slides of 3.0+ or better. Should the sample time point fail, the assay shall be

repeated twice. A second failure constitutes a product failure. Dating for this product has been set at 12 months from manufacture when stored at 2-8° C.

b. C-erbB-2 antigen stability in cut slides of breast carcinoma tissue and cell lines

The objective of this study was to determine the stability of the c-erbB-2 antigen in freshly cut tissue sections. Thirty sections of three paraffin embedded control cell lines and eight well characterized breast carcinomas were cut and stored at room temperature in a slide box and tested at day 1, day 3, week 1, week 2, week 3, week 4, week 5, week 6, week 7, week 8, month 2, month 3, through month 6, month 8, 10 and 12. Cases were to be discontinued after failing two time points in a row. The slides were masked and evaluated by a qualified pathologist. A failure was considered a drop of 1 intensity level from baseline for two consecutive time points. No drop in immunoreactivity for c-erbB-2 antigen was detected at month 6. The study was, therefore, extended to 12 months.

Cut slide stability for tissue samples and dating for IHC use of the cell line controls was established at 8 months. The 10-month time point was the first that demonstrated a drop in staining intensity. Some of the cases and the Level 3 cell line control exhibited a drop of one intensity level from baseline. Staining at this time point also showed increased granularity of cell membrane staining. Month 8 was chosen as the expiration date because of the increased incidence of cytoplasmic background staining at month 10 and also a slight lowering of cell membrane intensity on both the positive tissue control and case tissues. This observation was confirmed by the staining observed at the 12-month time point.

c. Characterization of control cell lines

Available as a separate product (Ventana catalog # S9100) are three cell line controls for procedural validation of IHC with PATHWAY™ Her 2 primary antibody. These cell lines were characterized for gene copy number and IHC intensity level at Ventana. Receptor analysis was also performed using fluorescent analysis. Results are in Table 1.

Table 1: Characterization of Cell Line Controls

Cell Line	IHC Score	Receptors/Cell	Gene copy range #/cell
MDA-MB-468	0	8.45×10^4	1-4
T-47D	1+	1.13×10^5	1-9
SKBR-3	3+	2.02×10^5	15-19

Literature references were available that describe other laboratories' values for c-erbB-2 receptor content of these cell lines. Rodriguez et. al., using I^{125} labeled TA-1 primary antibody, calculated SKBR-3 cell line as having 1.2×10^6 receptors per cell.[7] Beerli et. al. report a value for T-47D c-erbB-2 receptors of 3×10^4. This value was obtained by a comparative Western blot analysis of T-47D to SKBR-3 that was given as 1×10^6

receptors per cell.[8] Jannot et. al. report a value for MDA-MB-468 cells of $< 5 \times 10^3$. This was also obtained by Western analysis relative to T-47D cell receptor content.[9]

The differences between the values obtained and the literature may be due to the different assays used and differences in the binding affinity of the primary antibodies directed against the c-erbB-2 receptor.

d. Antibody Specificity Studies

The specificity of the primary antibody was supported by Western blotting and immunoprecipitation studies. Clone CB11 was shown to react with a 190 kD protein from SK-BR-3 cell lysates via Western blotting. SK-BR-3 is a breast carcinoma cell line which has a 128-fold over-expression of c-erbB-2 mRNA. The size of the band corresponds well with that reported by Akiyama et. al. for the size of the purified c-erbB-2 protein.[10] Two additional bands at 150 kD and 130 kD were also detected on heavily loaded gels. It has been suggested that the 150 kD and 130 kD bands visualized correspond to precursor or cytoplasmic versions of c-erbB-2.[2,11] The identity of the protein was further indicated by its ability to autophosphorylate in an immune kinase complex assay. This activity is characteristic of receptor tyrosine kinase-like c-erbB-2.[2] Evidence that CB11 detects the internal domain of c-erbB-2 protein was provided by fluorescence activated cell sorter (FACS) analysis. Strongly positive fluorescence when stained with CB11 was seen only when the SK-BR-3 cells were treated with the detergent Saponin, which permeabilized the cell membranes.[2]

e. Characterization with a Panel of Normal Human Tissues

The specificity of the PATHWAY™ Her 2 for use with metastatic breast tumor tissue obtained from elsewhere in the body was tested in a variety of normal tissues to evaluate unexpected background staining. The staining patterns of 78 normal tissues are described in Table 2. Tissues not tested as recommended in "FDA Guidance for Submission of Immunohistochemistry Applications to the FDA", were parathyroid.

Table 2: PATHWAY™ HER 2 on Normal Human Tissue

Tissue	Results	# positive/total
Adrenal	Negative	0/3
Bone Marrow	Negative	0/3
Breast	Negative	0/2
Cerebrum	Negative	0/2
Cerebellum	Negative	0/1
Cervix	Negative	0/3
Colon	1 C of absorptive cells, 1 autolysed, 1 goblet and absorptive cells not present	1/3
Endometrium	C of glandular epithelium	1/3
Esophagus	Negative	0/3
Heart	Negative	0/3
Kidney	M of renal tubule and collecting duct epithelium	1/2
Liver	Negative	0/3
Lung	Negative	0/3
Mesothelium	Negative	0/3
Ovary	Negative	0/3
Pancreas	1 C of acinar cells, 1 C of acinar cells & C of Islets of Langerhans	2/3
Peripheral nerve	Negative	0/3
Pituitary	1 C of adenohypophysis secretory cells & neuro-hypophysis pituicytes	1/2
Prostate	2 C of glandular epithelium	2/3
Salivary Gland	Negative	0/3
Skeletal muscle	Negative	0/3
Skin	Negative	0/3
Small Intestine	2 C of absorptive cells, 1 autolysed	2/3
Spleen	Negative	0/3
Stomach	2 C of glandular epithelium	2/3
Testis	Negative	0/2
Thyroid	2 C of follicular cells,1 E of colloid	3/3
Tonsil	M (1) & C (3) of squamous epithelium (secondary component)	3/3
Thymus	C of epithelial cells	1/1

Abbreviations used: C = cytoplasmic, M = cell membrane, E = extracellular

Non-specific cytoplasmic staining was observed in the following tissues: colon (1 of 3 cases), endometrium (1 of 3 cases), kidney (1 of 2 cases), pancreas (2 of 3 cases), pituitary (1 of 2 cases), prostate (2 of 3 cases), small intestine (2 of 3 cases), stomach (2 of 3 cases), thyroid (3 of 3 cases), tonsil (3 of 3 cases), and thymus (1 of 1 case). One case of kidney and one case of tonsil showed specific cell membrane staining, and one case of thyroid exhibited non-specific extracellular staining. The cell membrane staining of the tonsil was limited to the squamous epithelium, a secondary component.

f. Characterization with a Panel of Neoplastic Human Tissues

The staining patterns of 18 neoplastic tissues, stained at Ventana and evaluated by a qualified pathologist, are described in Table 3. Staining was cytoplasmic and varied in intensity from sample to sample. Based on the criteria for positive staining in breast carcinomas, all of these cases would be considered negative, as the cell membrane was uninvolved.

Table 3: C-erbB-2 on Neoplastic Human Tissue

Tissue	Results*	# Positive/ Total
Lung	C. staining in 5 - 75% of cancer cells, variable intensity	3 / 3
Prostate	C. staining in 50 - 75% of cancer cells, variable intensity	2 / 2
Colon	C. staining in 25 to 100% of cancer cells	3 / 3
Lymphoma	All cases negative	0 / 3
Stomach	All cases negative	0 / 2
Cervix	C. staining in 5 to 25% of cancer cells	2 / 3
Ovary	C. staining in 5% of cancer cells	2 / 2

*Abbreviations used: C = cytoplasmic

g. Reproducibility Studies

i) Inter-run Reproducibility

Inter-run reproducibility of staining with Ventana's PATHWAY™ Her 2 was determined by staining slides containing sections from the same neutral buffered formalin fixed tissue on 20 different runs. The procedure included pretreatment of the slides using microwave antigen enhancement in an acidic buffer. Slides were then stained using a protocol consisting of 32 minute incubation of the primary antibody, Ventana's Basic DAB detection kit, and counterstaining with hematoxylin and bluing reagent. All slides stained with similar staining intensity. Users should verify between run reproducibility results by staining several sets of serial sections with low, medium, and high antigen density on different days.

ii) Intra-run Reproducibility

Intra-run reproducibility of staining with Ventana's PATHWAY™ Her 2 was determined by staining 20 slides containing sections from the same neutral buffered formalin fixed tissue. The procedure included pretreatment of the slides using microwave antigen enhancement in an acidic buffer. Slides were then stained using a protocol consisting of 32 minute incubation of the primary antibody, Ventana's Basic DAB detection kit, and counterstaining with hematoxylin and bluing reagent (20 of 20 slides stained positively). All slides stained with similar staining intensity. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium, and high antigen density in a single run.

iii) Inter-laboratory Staining Reproducibility

Three laboratories, from separate institutions in the United States, participated in the inter-laboratory reproducibility study. Cut slides of four neutral buffered formalin fixed breast carcinoma cases and the three neutral buffered formalin fixed cell line controls

were shipped to the sites for staining on a Ventana Automated slide staining device. The slides were returned to Ventana and masked, then sent for evaluation by a qualified pathologist.

All three sites performed identical pretreatment procedures and staining protocols. The procedure included pretreatment of the slides using microwave antigen enhancement in an acidic buffer. Slides were then stained using a protocol consisting of 32 minute incubation of the primary antibody, Ventana's Basic DAB detection kit, and counterstaining with hematoxylin and bluing reagent. All slides were stained with similar staining intensity (varied by no more than 1 intensity level). No cases varied from clinically positive to clinically negative between the sites, and no sites experienced invalid runs, based upon the performance of the controls. Controls included the three cell lines, a positive tissue control located on the same slide as the test case, and a second slide of each case stained with negative Ig reagent. Evaluating 3 inter-laboratory comparisons of the 2 cases staining positively, variation between 3+ intensity and 2+ intensity occurred four times out of a total of 22 staining events (18%). Thus, there can be some difficulty distinguishing a 2+ from a 3+ reaction.

iv) Intra-laboratory, intra-technician and inter-technician reproducibility

The same three laboratories that participated in the inter-laboratory reproducibility study participated in an intra-laboratory (technician A vs. technician B) and intra-technician (technician A vs. technician A, 1 week apart) study, utilizing the same four breast carcinoma cases and cell line controls. The slides were returned to Ventana and masked, then sent for evaluation by a qualified pathologist. At all three sites, intra and inter technician staining of all the slides stained with similar staining intensity (varied by no more than 1 intensity level). No cases varied from clinically positive to clinically negative between the sites. Evaluating 3 intra-laboratory comparisons (technician A, Run 1 vs. technician B) of the 2 cases staining positively, variation between 3+ intensity and 2+ intensity occurred one time out of a total of 16 staining events (6%). Evaluating 3 intra-technician comparisons (technician A vs. technician A, 1 week apart) of the 2 cases staining positively, variation between 3+ intensity and 2+ intensity occurred three times out of a total of 14 staining events (21%). Thus, there can be some difficulty distinguishing a 2+ from a 3+ reaction.

Additionally these sites were invited to use their own in-house pretreatment methods with the study cases to evaluate inter-technique reproducibility. Again there were no differences of more than one intensity level between methodologies and no variation occurred that changed the clinical interpretation of the results. Evaluating 3 intra-technique comparisons (technician A Run 2 Ventana method vs. technician A Run 2 in-house method) of the 2 cases staining positively, variation between 3+ intensity and 2+ intensity occurred two times out of a total of 14 staining events (14%). Thus, staining with an in-house method was equivalent to the Ventana staining method.

v) Intra-investigator and Inter-investigator scoring reproducibility

Five investigators participated in a scoring reproducibility study. Each investigator was sent the same set of twelve neutral buffered formalin fixed breast carcinoma cases pre-stained with hematoxylin and eosin (H&E), PATHWAY™ Her 2, and negative IgG reagent. The procedure for preparing the slides used in this study included pretreatment of the slides using microwave antigen enhancement in an acidic buffer. Slides were then stained using a protocol consisting of 32 minute incubation of the primary antibody (PATHWAY™ Her 2 or negative IgG reagent), Ventana's Basic DAB detection kit, and counterstaining with hematoxylin and bluing reagent. Investigators were provided with a scoring guide with photographs of breast carcinoma cases stained with PATHWAY™ Her 2, and the study protocol with directions for scoring. Investigators were also instructed to call Ventana if they had questions. Investigators scored the cases twice with a minimum of 4 weeks' interval between scoring. Scores were evaluated for intra-investigator and inter-investigator scoring reproducibility. One investigator missed (forgot to score) one case for round 1 evaluation, which left 74 staining events evaluable for reproducibility. Intra-investigator reproducibility was clinically consistent (positive versus negative) for 72 of the 74 staining interpretations–97%. Out of 34 intra-investigator evaluations of 2+ and 3+ positively staining cases/cell lines, there were 5 (15%) instances of 2+/3+ intra-investigator scoring variability. Thus an investigator trained with the scoring materials provided was able to consistently interpret PATHWAY™ Her 2 staining of breast carcinoma cases and cell line controls, although there was still some variation in the interpretation of 2+ and 3+ staining. Inter-investigator reproducibility was evaluated for both round 1 and round 2. In round 1, 71 of 74 interpretations were clinically consistent among the five investigators–96%. In round 1, out of 34 inter-investigator evaluations of 2+ and 3+ positively staining cases/cell lines, there were 4 (12%) instances of 2+/3+ inter-investigator scoring variability, In round 2, 70 of 75 interpretations were clinically consistent among the five investigators–93%, and out of 35 inter-investigator evaluations of 2+ and 3+ positively staining cases/cell lines there was one instance (3%), of 2+/3+ inter-investigator scoring variability. Thus investigators at different institutions trained with the scoring materials provided were able to consistently interpret PATHWAY™ Her 2 staining of breast carcinoma cases and cell line controls, although there was still some variation in the interpretation of 2+ and 3+ staining. This variability, however, declined with investigator experience; by round two there was only one instance of 2+/3+ inter-investigator variability.

vi) Lot-to-lot reproducibility

To test the lot-to-lot variation of the PATHWAY™ Her 2, the three PATHWAY™ Her 2 cell line controls and two neutral buffered formalin fixed known positive breast carcinoma cases were run with three different GMP lots. The procedure for preparing the

slides used in this study included pretreatment of the slides using microwave antigen enhancement in an acidic buffer. Slides were then stained using a protocol consisting of 32-minute incubation of the primary antibody (PATHWAY™ Her 2 or negative IgG reagent), Ventana's Basic DAB detection kit, and counterstaining with hematoxylin and bluing reagent. Lot 2 exhibited a lower percentage of cells staining in the 1+ cell line control (T-47D), and a drop from 3+ to 2+ for one breast carcinoma case. All other tissues and cell lines stained uniformly with the three lots tested. No tissues or cell lines tested changed from clinically positive or clinically negative when stained with the three different lots of antibody. Thus, lot-to-lot staining results are very consistent with c-erbB-2 primary antibody.

2. Concordance Studies

The safety of PATHWAY™ Her 2, (whether diagnostic information is likely to cause a mischaracterization of the patient sample), and its effectiveness, (how well it detects analytes in the patient sample) was examined through clinical testing by Ventana Medical Systems, Inc.

Comparison of Performance of Ventana c-erbB-2 Primary Antibody and DAKO HercepTest™: Appropriate detection of antigen in breast carcinoma tissue/ appropriate performance for use as an aid in the assessment of Herceptin® therapy.

Objective: This study examined the suitability of PATHWAY™ Her 2 for use as an aid in determination of treatment for Herceptin® therapy. A comparative protocol was designed to examine the correlation of performance between PATHWAY™ Her 2 and HercepTest™, a previously approved FDA diagnostic for this indication. Seventy-five percent (75%) concordance between the two test results with 95% confidence was set as the criteria for success. Concordance was defined as the total number of specimens that agreed with the DAKO HercepTest™ results divided by the total number of samples tested.

Protocol: Three investigators participated in the study. Each investigator evaluated the archived neutral buffered formalin fixed, paraffin embedded breast carcinoma tissue blocks available at their institutions for Her-2/neu status and stratified their cases into positive and negative sub-populations. Seventy-five (75) cases were randomly selected from each pool for a total of 150 case per site, 450 cases total for the study. The slides stained with HercepTest™ were processed and stained according to the manufacturer's instructions specified in the package insert. The procedure used for preparing the slides stained with PATHWAY™ Her 2 included pretreatment of the slides using microwave antigen enhancement in an acidic buffer. Slides were then stained using a protocol consisting of 32-minute incubation of the primary antibody (PATHWAY™ Her 2 or negative IgG reagent), Ventana's Basic DAB detection kit, and counterstaining with hematoxylin and bluing reagent. Cases were evaluated for percent positivity of invasive tumor cells and intensity of staining on a scale from 0 to 3+. Positivity was defined as greater than 10% of the invasive tumor cells staining at an intensity of 2+ or 3+. The

determination of percent positivity was evaluated in the area identified by the pathologist as well preserved and well stained. The staining pattern was established as fully circumferential staining of the cell membrane for 2+ and 3+ intensity and partially circumferential for 1+ staining. Seventy-five percent (75%) concordance between the two test results with 95% confidence was set as the criteria for success. Concordance was defined as the total number of specimens that agreed with the DAKO HercepTest™ results divided by the total number of samples tested.

Study Population: Three investigators participated in the study. Each investigator evaluated the archived neutral buffered formalin fixed, paraffin embedded breast carcinoma tissue blocks available at their institutions for Her-2/neu status and stratified their cases into positive and negative sub-populations. Seventy-five (75) cases were randomly selected from each pool for a total of 150 case per site, 450 cases total for the study. All 450 cases selected for the study were fixed, paraffin embedded primary breast carcinomas with archived c-erbB-2 results. Actual staining data from both tests yielded a positivity rate of 33.5%. This may be due to discrepancies in scoring criteria for c-erbB-2 staining from the archived analysis and were used in this clinical study.

Results: Data was analyzed for concordance, copositivity, and conegativity. Concordance was evaluated in two formats: binary (positive vs. negative) and 3 X 3 (3+, 2+ >10%, negatives—2+ ≤10%, 1+, 0).

Table 4: Concordance of Ventana c-erbB-2 Primary Antibody and HercepTest™

	HercepTest™ Negative	HercepTest™ Positive	Total
c-erbB-2 Primary Antibody Negative	282	17	299
c-erbB-2 Primary Antibody Positive	17	134	151
Total:	299	151	450

Concordance = 92.4% 95% Confidence Interval = 89.6% - 94.7% $p < 0.0001$

Statistical Analysis of Table 4

The observed agreement between the two tests was 92.4% (416/450). The exact 95% confidence interval was 89.6% to 94.7%. The null hypothesis that agreement was no greater than 75% (one-sided hypothesis) was rejected with $p < 0.0001$. The kappa statistic (chance-corrected measure of agreement) was 0.83; the null hypothesis that agreement is no better than chance was rejected with $p < 0.0001$. McNemar's Test of the hypothesis that the proportion positive by CB11 was equal to the proportion positive by HercepTest™ could not be rejected with $p = 1.00$. Treating HercepTest™ as the standard, the sensitivity of CB11 was 88.7% (134/151) with 95% confidence interval of 82.6% to 93.3%. The specificity was 94.3% (282/299) with 95% confidence interval of 91.1% to 96.7%. Finally, the extrapolated agreement based on the Herceptin® clinical trial assay for prevalence[6] was 92.5%, resulting in a kappa statistic of 0.83 ($p < 0.0001$).

Table 5: 3 X 3 Concordance of Ventana c-erbB-2 Primary Antibody and HercepTest™

	HercepTest™			
c-erbB-2 Primary Antibody ↓	**Negatives**	**2+**	**3+**	**Total:**
Negatives	282	14	3	299
2+	13	24	13	50
3+	4	14	83	101
Total:	299	52	99	450

Concordance = 86.4% 95% Confidence Interval = 82.9% - 89.5% $p < 0.0001$

Statistical Analysis of Table 5

The observed agreement was 86.4% (389/450) with 95% confidence interval of 82.9% to 89.5%. The null hypothesis that agreement was no greater than 75% (one-sided hypothesis) was rejected with $p < 0.0001$. The kappa statistic was 0.73; the null hypothesis that agreement was no greater than chance was rejected with $p < 0.0001$. The test of marginal homogeneity could not be rejected with $p = 1.00$. The sensitivity of CB11 relative to HercepTest™ for the intermediate category was 46.2%, with 95% confidence interval of 32.3% to 60.5%. The sensitivity of CB11 for the positive category was 83.8%, with 95% confidence interval of 75.1% to 90.5%. The specificity was the same as that for the two by two table given above; 94.3% with 95% confidence interval of 91.1% to 96.7%. Finally, the extrapolated agreement was 87.1%, resulting in a kappa statistic of 0.74 ($p < 0.0001$).

X. Conclusions drawn from the studies

The studies demonstrated that PATHWAY™ Her 2 appropriately and reproducibly detects antigen in breast carcinoma tissue and can aid in the assessment of breast cancer for patients being considered for Herceptin® therapy, based on its correlation of staining with the DAKO HercepTest™ for which there is an approved PMA for the same indication for use.

Risk Benefit Analysis: Testing has been completed to characterize the performance of the PATHWAY™ Her 2 relative to the DAKO HercepTest™. There was a sufficient level of concordance between the two assays to expect that the clinical experience with Herceptin could be obtained with tissues considered "positive" with the PATHWAY™ Her 2.

PATHWAY™ Her 2 is indicated as an aid in determining therapy with Herceptin®. A false positive result may lead to patients with undetectable or low levels of c-erbB-2 expression (0 or 1+) being treated with Herceptin®. The benefit of Herceptin® treatment in this patient population is not known. The risk of Herceptin® treatment include adverse effects such as infusional toxicity (chills, fever, pain, pain at the tumor site, asthenia, nausea, vomiting and headache), and cardiotoxicity.[6] A false negative result would result in patients not being treated with Herceptin®. Clinical studies have shown that between

4% and 53% of immunohistochemically positive patients benefit from Herceptin® therapy either alone or in combination with other therapies.[6] A false negative result would deny patients the possibility of this benefit.

Safety: PATHWAY™ Her 2 is considered safe when applied for its intended use of *in vitro* diagnostic testing. C-erbB-2 primary antibody does contain ProClin 300, a preservative containing the active ingredients 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one. Symptoms of overexposure to ProClin 300 may include skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of ProClin 300 in this product is 0.05%. This antibody solution does not meet the OSHA criteria for a hazardous substance. A Material Safety Data Sheet is enclosed with each shipment of product, and the enclosed package insert also alerts the user to this potentially irritating ingredient. Ventana feels that these safety precautions are sufficient protect the customer when using Ventana c-erbB-2 Primary Antibody.

Effectiveness: The data Ventana has provided demonstrate that c-erbB-2 Primary Antibody is stable, may be processed and interpreted reproducibly, and appropriately selects patients for Herceptin® therapy. The studies provided support Ventana's intended use for this product.

X. PANEL RECOMMENDATION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XI. FDA DECISION

CDRH issued an approvable order for the applicant's PATHWAY™ HER 2 subject to an FDA inspection that finds the manufacturing facilities, methods and controls in compliance with the applicable requirements of the Quality System Regulation (21 CFR Part 820) on August 23, 2000.

FDA issued an approval order on NOV 28 2000.

The applicant's manufacturing and control facilities were inspected on July 12-13, 2000, and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs). The shelf life of PATHWAY™ Her 2 has been established at 12 months when stored at 2-8° C.

XII APPROVAL SPECIFICATIONS

Directions for use: See labeling

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order.

Postapproval Requirements and Restrictions: See approval order.

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XIII References

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10. Akiyama, T. et. al. The product of the human c-erbB-2 Gene: A 185-kilodalton glycoprotein with tyrosine kinase activity. Science 232: 1644-1646, 1986.

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