

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k061613

B. Purpose for Submission:

New application on approved system

C. Measurand:

Her2/neu protein on formalin-fixed paraffin-embedded breast cancer specimens

D. Type of Test:

Computer-assisted image analyzer for immunohistochemistry

E. Applicant:

TRIPATH IMAGING, INC.

F. Proprietary and Established Names:

Ventana Image Analysis System (VIAS) – PATHWAY® HER2(4B5) Application

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NQN- Microscope, Automated, Image Analysis, Immunohistochemistry, Operator Intervention, Nuclear Intensity and Percent Positivity	Class II	21CFR§ 864.1860 Immunohistochemistry reagents and kits	Pathology (88)

H. Intended Use:

1. Intended use(s):

This antibody is intended for *in vitro* diagnostic (IVD) use.

Ventana® Medical Systems' (VIAS) PATHWAY® anti-HER-2/neu (4B5) primary antibody (PATHWAY HER2 (4B5)) is a rabbit monoclonal antibody intended for laboratory use for semi-quantitative detection of HER2 antigen in sections of formalin fixed, paraffin embedded normal and neoplastic tissue on a Ventana automated slide immunohistochemistry slide staining device. It is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® treatment is considered.

Note: All of the patients in the Herceptin clinical trials were selected using a clinical trial assay. None of the patients in those trials were selected using PATHWAY® anti-HER-2/neu (4B5). PATHWAY® anti-HER-2/neu (4B5) was compared to Ventana® Medical Systems' (Ventana) PATHWAY® Her2 (clone CB11) Primary Antibody on an independent sample and found to provide acceptably concordant results. The actual correlation of PATHWAY® anti-HER-2/neu (4B5) to clinical outcome has not been established.

2. Indication(s) for use:

The **Ventana Image Analysis System** (VIAS™) is an adjunctive computer-assisted image analysis system functionally connected to an interactive microscope. It is intended for use as an aid to the pathologist in the detection, classification and counting of cells of interest based on

marker intensity, size and shape using appropriate controls to assure the validity of the VIAS scores.

In this application, the VIAS is intended to aid a qualified pathologist for the semi-quantitative detection of c-erbB-2 (HER2) antigen in formalin-fixed, paraffin embedded normal and neoplastic tissue specimens immunohistochemically stained for the presence of HER2 proteins using Ventana Medical Systems, Inc.'s (Ventana) Pathway® anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody as well as Ventana's DAB copper chromogen and nuclear hematoxylin.

The Ventana PATHWAY™ Her2 is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® treatment is considered.

The VIAS is an adjunctive computer-assisted methodology to assist the reproducibility of a qualified pathologist in the acquisition and measurement of images from microscope slides of breast cancer specimens stained for the presence of HER2 receptor protein. The accuracy of the test result depends upon the quality of the immunohistochemical staining. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls as specified in the instructions for the Ventana PATHWAY™ Her2 (4B5) to assure the validity of the VIAS-assisted HER2.

Note: All of the patients in the Herceptin clinical trials were selected using a clinical trial assay. None of the patients in those trials were selected using PATHWAY® anti-HER-2/neu (4B5). PATHWAY® anti-HER-2/neu (4B5) was compared to Ventana® Medical Systems' (Ventana) PATHWAY® Her2 (clone CB11) Primary Antibody on an independent sample and found to provide acceptably concordant results. The actual correlation of PATHWAY® anti-HER-2/neu (4B5) to clinical outcome has not been established.

3. Special conditions for use statement(s):

This device is for prescription use only.

4. Special instrument requirements:

The Ventana Image Analysis System – Pathway® anti-HER-2/neu (4B5) Application requires the Ventana Image Analysis System (VIAS) previously cleared in 510(k) submission k053520.

I. Device Description:

The VIAS is an interactive histology imaging device that performs image processing using a microscope, digital color video camera, computer, and image analysis software to acquire and analyze user-selected images on Pathway® anti-HER-2/neu (4B5) histology slides.

The VIAS consists of a single workstation with two main software applications for administration and slide processing. The workstation components include a microscope, motorized stage, digital color video camera, computer, monitor, keyboard, mouse, and barcode reader. The workstation is a table-top unit designed to be placed in the Pathologist office or lab space.

As an interactive system, the VIAS device requires competent human intervention at all steps in the analytical process. The system is designed to complement the routine workflow of a qualified pathologist screening a histological slide with additional quantitative data to assist the reproducibility of the slide interpretation. The system software makes no independent interpretation of the data.

J. Substantial Equivalence Information:

Predicate	Ventana Image Analysis System (VIAS) – Her2(CB11) k051282
Describe the item being compared	
The Ventana Image Analysis System (VIAS™) is the same system in this submission that has been cleared for the previous indications for HER-2/neu (k051282). HER (CB11) is an antibody that when stained indicates over-expression of a protein that is associated with cellular abnormality. The VIAS, with its previously cleared uses, is indicated an aid in the assessment of breast cancer patients for whom Herceptin® treatment is considered.	

Similarities		
Item	Device	Predicate
Indications for Use	As an accessory to an assay which is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® treatment is considered	Same
Hardware and Software	VIAS System	Same
Specimen	Formalin-fixed paraffin-embedded breast cancer specimens stained by immunohistochemical (IHC) technique	Same
Localization of IHC positive stain	Nuclear	Same
Image analysis system	Histologic observation by a pathologist through a controlled microscope/digital camera combination	Same
Method of cell detection	Colorimetric pattern recognition by microscopic examination of prepared cells by size, shape, hue, and intensity as observed by a computer assisted microscope and by visual observation by a healthcare professional	Same
IHC Antigen Detected	c-erbB-2 (HER2)	Same

Differences		
Item	Device	Predicate
Assay used	Ventana's Pathway® anti-HER-2/neu (4B5) Primary Antibody (Pathway® HER (4B5)	Ventana's Pathway™ Her2 (clone CB11)
Primary Antibody	Rabbit monoclonal antibody (4B5)	Mouse monoclonal (CB11)

K. Standard/Guidance Document Referenced (if applicable):

STANDARDS
Title and Reference Number
Laboratory Instruments and Data Management Systems: Design of Software User Interfaces and End-User Software Systems Validation, Operation, and Monitoring; Approved Guideline - Second Edition

STANDARDS
Title and Reference Number
(GP19-A2)
Standard for Software Verification and Validation (1012:1998)
Medical devices - Risk management - Part 1: Application of risk analysis (14971-1)
Other Standards

GUIDANCE			
Document Title	Office	Division	Web Page
Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices - Guidance for Industry and FDA Staff	ODE		http://www.fda.gov/cdrh/ode/guidance/337.html
Guidance for Off-the-Shelf Software Use in Medical Devices; Final	ODE		http://www.fda.gov/cdrh/ode/guidance/585.html
Indications for Use Statement	ODE		http://www.fda.gov/cdrh/ode/indicuse.html
In Vitro Diagnostic Devices: Guidance for the Preparation of 510(k) Submissions	OCER		http://www.fda.gov/cdrh/manual/ivdmanul.html

L. Test Principle:

During the course of a HER-2/neu (4B5) slide evaluation the Pathologist manually screens the slide using the interactive microscope of the *Ventana Image Analysis System*. At any time during this screening process the Pathologist can acquire color images of fields of interest within tumor areas via the digital color camera mounted on top of the microscope. The selection of the tumor areas is the sole responsibility of the Pathologist. The Pathologist can refine his/her selection by marking specific tumor regions within acquired images with an interactive drawing tool. These color images are quantitatively evaluated by the *Ventana Image Analysis System*.

The evaluation includes as a first step the separation of the two dye components DAB (brown) and hematoxylin (blue). The parameters for the dye characterization are stored in a slide type storage structure containing assay specific parameters to process HER-2/neu (4B5) slides. The slide type for the HER-2/neu (4B5) assay contains the name of the assay (*HER-2/neu Clone 4B5*), Counterstain (Hematoxylin), Marker Stain (DAB), Marker Expression Localization (Membrane) and the magnification of the objective used for quantitative analysis (20x). The HER-2/neu (4B5) slide type is optimized for Ventana's PATHWAY™ HER-2/neu (4B5) assay using Ventana's DAB copper chromogen and nuclear hematoxylin.

Based on the two dye images the total area of membranes with significant marker expression and the total cytoplasmic area of all cells included in the selected tumor regions is accumulated over all the fields of view selected by the pathologist for a slide.

The total cytoplasmic area is calculated as sum of the areas of all membranes with significant marker expression detected in the marked tumor regions of the DAB components of the selected images. The detection and differentiation of membranes with significantly elevated marker expression in relation to the background stain of the surrounding cytoplasm is achieved via a local contrast threshold. This threshold assures that membranes accepted for the score calculation have a higher marker expression than the surrounding cytoplasm. Additionally membranes with a high over-expression of HER-2/neu (4B5) end up with larger (wider and more complete) areas than membranes showing low expression.

Establishing the System Score Formula

The final score value is derived from the normalized total membrane area as ratio of total membrane area divided by the total cytoplasmic area. This normalization takes care of the patient-dependent cell size variation. To match the well established manual scoring scale of 1+, 2+, 3+ for HER-2/neu (4B5) slides the system results' scale was adapted to the manual scoring scale. For this purpose the system results derived were derived from a training set of about 200 HER-2/neu (4B5) slides and matched with the manual consensus call of three pathologists for the same slides. This led to a conversion factor between the system and the manual scale of 18.

The result of the **VIAS** system quantification of the HER-2/neu (4B5) over-expression is a continuous number ranging from 0 to 3.5. The manual score, however, consist of discrete bins. The bins used are 0, 1+, 2+, and 3+. **VIAS** provides the possibility for the pathologist to divide the continuous system scoring scale into the number of bins he/she prefers. The "continuous" number can be considered as a confidence measure provided by the system for its own score values. For example, the confidence in a 1+ score derived from a continuous result of 1.47 to truly be a 1+ score is not as high as in a situation where the 1+ is derived from a continuous score of 0.99. By default **VIAS** comes with settings for the four bins 0, 1+, 2+, 3+. These bins were also used throughout the HER-2/neu (4B5) 510(k) study.

Interactive Region Correction

For correct results it is important to segment out normal cells from the regions of interest (marked tumor areas). **VIAS** provides two tools which are designed to do this. When an image is acquired, **VIAS** by default refines the region of interest by excluding most of the stroma cells (see *Defining regions on the field in Chapter 4: Screening a slide*). This region of interest is presented as a suggestion to the operator who can either accept it or further refine it with the drawing tool (see *Defining regions on the field in chapter 4: Screening a slide*). The drawing tool enables the interactive addition or subtraction of objects or regions to the region of interest within the displayed image. The region of interest is the part of the stored image which will be quantitatively evaluated by **VIAS**.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Instrument Precision

To determine the precision of the *Ventana Image Analysis System* inter-assay reproducibility studies were conducted using a set of eight HER-2/neu slides. The slides consisted of formalin-fixed, paraffin-embedded tissue specimens immunohistochemically stained for HER-2/neu (4B5) protein expression using Ventana's PATHWAY™ HER-2/neu (4B5) assay labeled with Ventana's DAB copper chromogen and Ventana's nuclear hematoxylin. The slides were selected in such a way that the 4 bins 0, 1+, 2+, 3+ of the manual scoring scale were represented by two slides each, and that three pathologists agreed with each other on the manual score for each slide. For each slide, the mean, the standard deviation (StdDev), and the coefficient of variation (CV) of the continuous instrument readings were calculated.

For the Inter-Assay (Between Run) Inter-System Reproducibility, one field of view for each of the eight HER-2/*neu* slides of the study sample was measured five times on three different Ventana Image Analysis Systems. The three systems were calibrated by carefully adjusting the microscopes in an identical fashion. To achieve best image quality on all three systems the acquisition of the Black and White Reference Images is controlled during the image process by each system.

Inter-Assay (Between-run) Inter-Instrument (System) Reproducibility

Results of the Inter-Assay (Between Run) Precision Study- System 1

HER-2/ <i>neu</i> (4B5) (n = 5)							
Slide #	Mean Score	StdDev Score	CV [%]	Slide #	Mean Score	StdDev Score	CV [%]
1	0.09	0.013	N/A	2	0.04	0.008	N/A
3	1.05	0.016	1.56	4	1.16	0.020	1.72
5	1.93	0.029	1.53	6	2.15	0.015	0.71
7	3.40	0.023	0.68	8	3.40	0.045	1.33

Results of the Inter-Assay (Between Run) Precision Study- System 2

HER-2/ <i>neu</i> (4B5) (n = 5)							
Slide #	Mean Score	StdDev Score	CV [%]	Slide #	Mean Score	StdDev Score	CV [%]
1	0.04	0.009	N/A	2	0.01	0.000	N/A
3	1.05	0.015	1.45	4	1.08	0.034	3.14
5	1.89	0.033	1.75	6	2.12	0.062	2.93
7	3.36	0.067	2.01	8	3.35	0.045	1.35

Results of the Inter-Assay (Between Run) Precision Study- System 3

HER-2/ <i>neu</i> (4B5) (n = 5)							
Slide #	Mean Score	StdDev Score	CV [%]	Slide #	Mean Score	StdDev Score	CV [%]
1	0.04	0.007	N/A	2	0.00	0.004	N/A
3	1.02	0.018	1.77	4	1.16	0.026	2.23
5	1.95	0.047	2.41	6	2.16	0.059	2.76
7	3.41	0.016	0.48	8	3.41	0.046	1.36

To evaluate the between-run precision on each system the selected field of view for each of the same eight study slides was measured once before repeating the same sequence another four times on the same system. This resulted in five instrument score values for each field of

view per slide, where between the measurements; the slide was removed and placed back on the microscope stage. After finishing with the first system, the study was repeated on system 2 and 3. All measurements were performed by the same operator.

Reproducibility results may vary depending on the composition of the field of view chosen for analysis.

Summary results of the Inter-System Precision Study-Systems 1, 2 3

HER-2/neu(4B5) (n = 3)							
Slide #	Mean Score	StdDev Score	CV [%]	Slide #	Mean Score	StdDev Score	CV [%]
1	0.06	0.033	N/A	2	0.02	0.022	N/A
3	1.04	0.022	2.14	4	1.13	0.054	4.79
5	1.92	0.048	2.50	6	2.14	0.053	2.50
7	3.39	0.051	1.52	8	3.39	0.055	1.62

b. Linearity/assay reportable range:

Linearity is not applicable.

The assay reportable range is 0% to 100% positive tumor cells.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The analytical traceability of the system depends on the Ventana PATHWAY™ Her2 (clone 4B5) kit. **VIAS** operating manual HER-2/neu application recommends the user to follow the package insert of Ventana PATHWAY™ Her2/neu (4B5) for quality control procedures. Ventana PATHWAY™ Her2/neu (4B5) package insert requires the user to run cell lines controls, positive tissue control, negative tissue control, and nonspecific negative reagent control. According to the Ventana PATHWAY™ Her2/neu (4B5) package insert, patient results are considered to be invalid if quality control procedures do not meet the required specifications.

d. Detection limit:

Not applicable

e. Analytical specificity:

Specificity of Ventana PATHWAY™ HER2/neu (4B5) was determined by a study that showed appropriate specific membrane staining for normal and neoplastic tissue. For neoplastic tissue results are as follows:

Tissue type	Number	Negative	Positive
Hepatocellular	5	5	0
Lung	2	2	0
Ovarian	2	1	1
Renal	5	5	0
Stomach	3	3	0
Pancreatic	3	3	0
Thyroid	3	3	0
Breast	4	3	1
Prostate	3	3	0
Colon	3	2	1
Melanoma	2	2	0
Undifferentiated	1	1	0

Tissue type	Number	Negative	Positive
Sarcoma	2	2	0
Carcinoid	2	2	0
Leiomyoma	2	2	0
Lymphoma	3	3	0

For normal tissue results are as follows:

Tissue type	Number	Negative	Positive
Spleen	3	3	0
Skeletal	3	3	0
Ovary	3	3	0
Liver	3	3	0
Cervix	3	3	0
Colon	3	3	0
Esophagus	3	2	1
Breast	3	3	0
Kidney	3	3	0
Tonsil	3	1	2 (focal staining)
Pancreas	3	3	0
Skin	3	3	0
Thyroid	3	3	0
Small intestine	3	3	0
Adrenal	3	3	0
Uterus	3	3	0
Heart	2	2	0
Cerebrum	3	3	0
Lung	3	3	0
Cerebellum	3	3	0
Testis	3	3	0
Stomach	3	3	0
Prostate	3	2	1
Salivary gland	3	3	0
Peripheral nerve	3	3	0
Thymus	2	2	0
Pituitary	2	2	0
Parathyroid	3	2	1 (focal staining)
Mesothelial	3	3	0

f. Assay cut-off:

Each laboratory can set the threshold for positivity preferred by their pathologist for the HER2/neu (4B5) assay. Typical cutoff values used are 1%, 5%, and 10% positive tumor cells. The pathologist makes the final call based on both qualitative and quantitative information seen in the tissue section.

2. Comparison studies:

a. Method comparison with predicate device:

The substantial equivalence studies were based on comparison to conventional manual microscopy performed using the reagents and in accordance with Ventana PATHWAY™ Her2/neu (4B5) instructions for use.

Concordance was evaluated as the agreement between the manual Her2 scores and VIAS Her2/neu (4B5) scores after they had been reviewed by a pathologist. A set of 213 formalin-

fixed, paraffin-embedded breast tissue specimens were obtained from an outside source for this study. They were immunohistochemically stained using Ventana's PATHWAY™ HER-2/*neu* reagents (2 lots) labeled with Ventana's IViewDAB copper chromogen and nuclear *Hematoxylin II*. The slides were selected in such a way that approximately one-third of them were negative slides (0 and 1+), one-third 2+ and one-third 3+ for HER-2/*neu* over-expression.

As preparation for the comparison study one board-certified pathologist screened each slide of the study sample using the microscope of one *Ventana Image Analysis System* and selected and stored between three and six images (along with their corresponding location coordinates) of diagnostically significant fields. For each slide the pathologist also noted down the manual score value as result of the manual scoring of the selected fields. During this process 7 slides were excluded from the initial sample by the pathologist for various reasons. See table below.

Exclusion reason	Slide numbers	Total number
Not enough tumor	1168	4
	1187	
	1202	
	1317	
In situ negative	1204	1
Fixation problem	1253	1
Lifted tissue	1281	1
Total exclusions		7

The images and the coordinates of their related slide locations were then copied to the databases of two additional *Ventana Image Analysis Systems*. Based on the manual score of the pathologist, 206 slides were grouped into four bins 0, 1+, 2+ and 3+. There were 36, 41, 60 and 69 slides in the respective bins.

During the comparison study three different board-certified pathologists performed a manual read in a blinded manner of each slide of the study sample by having the pre-selected fields of interest automatically relocated underneath the microscope of one *Ventana Image Analysis System*. Each pathologist used the microscope of a different system (e.g. pathologist 1 uses system 1, pathologist 2 uses system 2, pathologist 3 uses system 3). Each system was validated and checked for conformity prior to use in this study. For this portion of the trial, the imaging system software was switched to a mode where it did not display any quantitative results to avoid influencing the pathologists' manual calls.

For each slide the stored fields of view were relocated in a sequential manner, and the pathologists assessed each field through the microscope and stored the image for quantitative evaluation by the system. The pathologists based their manual reads exclusively on the pre-selected fields of view which had been chosen by the independent pathologist prior to the reading of the study sample set. For the purpose of the study the pathologists were not screening the entire slide but were comparing their assessment against the scoring of the *Ventana Image Analysis System*. At the end of each slide assessment the pathologist recorded his/her manual score in a table provided for the study.

Based on the recaptured images the system automatically computed the respective continuous (continuous scale 0 to 3.5) and binned score value (discrete bins 0, 1+, 2+ or 3+) for the slide. The slide score results were later retrieved from the system and used in the subsequent data

analysis. The following table shows the contingency tables of the HER-2/*neu* scoring and the resulting concordances with corresponding 95% confidence intervals of the three different VIAS-Pathologist pairs VIAS1-Pathologist 1, VIAS2-Pathologist 2 and VIAS3-Pathologist 3, which participated in the clinical study.

Contingency table and concordance with 95%CI of VIAS1-Pathologist 1 pair

	Pathologist 1				
VIAS 1	0	1+	2+	3+	Total
0	34	2	0	0	36
1+	0	26	7	0	33
2+	0	8	55	1	64
3+	0	0	3	70	73
Total	34	36	65	71	206
<i>Concordance = 0.898 [95%CI = 0.846 – 0.934]</i>					

Contingency table and concordance with 95%CI of VIAS2-Pathologist 2 pair

	Pathologist 2				
VIAS 2	0	1+	2+	3+	Total
0	36	0	0	0	36
1+	6	32	3	0	35
2+	0	21	43	0	64
3+	0	1	5	65	71
Total	36	54	51	65	206
<i>Concordance = 0.854 [95%CI = 0.797 – 0.889]</i>					

Contingency table and concordance with 95%CI of VIAS3-Pathologist 3 pair

	Pathologist 3				
VIAS 3	0	1+	2+	3+	Total
0	32	4	0	0	36
1+	0	29	3	1	33
2+	2	18	39	4	63
3+	0	0	4	70	74
Total	34	51	46	75	206
<i>Concordance = 0.825 [95%CI = 0.765 – 0.873]</i>					

The following table shows the concordance, Kappa values and 95% confidence intervals for discrete 4 bin score scale 0, 1+, 2+, 3+. Rows 3 to 5 show the concordance, weighted Kappa values and the 95% confidence intervals of the weighted Kappa values between the three

different *Ventana Image Analysis System* – Pathologist pairs. Rows 8 to 10 show the concordance, weighted Kappa values and 95% confidence intervals between the three corresponding system calls. Rows 13 to 15 show concordance, weighted Kappa values and 95% confidence intervals between the three study pathologists.

HER-2/neu (4B5)				
4 discrete bins 0, 1+, 2+, 3+				
Pathologist #	System #	Concordance	Weighted Kappa	95% CI
1	1	0.898	0.914	0.816 – 1.012
2	2	0.854	0.876	0.778– 0.973
3	3	0.825	0.844	0.745 – 0. 943
System #	System #	Concordance	Weighted Kappa	95% CI
1	2	0.961	0.967	0.869 – 1.066
1	3	0.956	0.963	0.864 – 1.062
2	3	0.976	0.980	0.881-1.078
Pathologist #	Pathologist #	Concordance	Weighted Kappa	95% CI
1	2	0.845	0.871	0.774-0.968
1	3	0.850	0.867	0.769-0.966
2	3	0.874	0.893	0.793-0.992

The Concordance values between System and Pathologist (concordance range 0.825-0.898) was comparable to concordance values for the Pathologist to Pathologist read (concordance range 0.845 – 0.874) and the corresponding System to System read (concordance range 0.956 – 0.976).

The following table shows the concordance, weighted Kappa values and the 95% confidence intervals around the weighted Kappa values for the agreement between the manual scores of the pathologists with the system scores, the agreement between the system scores and the agreement of the manual scores of the three (3) study pathologists. The scores are grouped into Negative (0, 1+) and Positive (2+, 3+) according to clinical significance. Rows 3 to 5 show the concordance, weighted kappa values and the 95% confidence intervals of the weighted Kappa values for three (3) different Ventana Image Analysis System-Pathologist pairs. Rows 8 to 10 show concordance, weighted Kappa values and 95% confidence intervals between the three (3) corresponding system calls. Rows 13 to 15 show concordance, weighted Kappa values and 95% confidence intervals between the three study pathologists. The fields of view were selected by an independent fourth pathologist before study. During the study, these fields were relocated and the corresponding images reacquired by each pathologist-system pair. During that time the Ventana Image Analysis Systems did not display the results of the quantification to avoid influencing the pathologists' manual calls. The HER-2/neu (4B5) scores are grouped into Negative (0, 1+) and Positive (2+, 3+) according to clinical significance.

HER-2/neu (4B5)				
Negative (0, 1+ / Positive (2+, 3+)				
Pathologist #	System #	Concordance	Weighted Kappa	95% CI
1	1	0.927	0.887	0.701-0.974
2	2	0.879	0.747	0.613-0.881
3	3	0.884	0.753	0.618-0.887
System #	System #	Concordance	Weighted Kappa	95% CI
1	2	0.971	0.935	0.799-1.072
1	3	0.971	0.935	0.799-1.072
2	3	0.990	0.978	0.842-1.115
Pathologist #	Pathologist #	Concordance	Weighted Kappa	95% CI
1	2	0.884	0.757	0.623-0.891
1	3	0.898	0.784	0.619-0.919
2	3	0.947	0.891	0.754-1.027

The concordance values between System and Pathologist pairs (concordance range 0.879-0.927) were comparable to concordance values for the three system calls (concordance range 0.971-0.990) and the corresponding pathologist to pathologist (concordance range 0.884-0.947).

Another study was done by Ventana to examine the correlation of Pathway anti-HER-2/neu (4B5) to Pathway HER2 (CB11) and PathVysion® Her-2 FISH. Six investigators participated in the study. Two sets of three different investigators evaluated two independent cohorts (Cohort 1: n=178, Cohort 2: n=144) using known breast cancer cases stained with HER-2 CB11 and HER2 4B5. Fish data were obtained from patient history. A consensus score from the three readers for each antibody was created for each case to reduce intra-reader variability known to exist with HER-2 scoring. A total of 322 cases were evaluated. The Slides stained with PATHWAY HER-2 (CB11) were processed and stained according to the manufacturer's instructions specified in the Ventana CB11 package insert. There was an average of approximately one year between staining and reading of the CB11 stained slides. Data from the two cohorts are presented separately as follows:

Cohort 1-Consensus IHC scores of three pathologists:				
	CB11			
4B5	3	2	0 and 1	Total
3	29	24	5	58
2	2	13	17	32
0 and 1	0	0	53	53
Total	31	37	75	143

Cohort 1: Performance characteristics for 3 x 3 Presentation
Overall agreement is 29+13+53/143=66.4% (95% C.I. = 38.6%, 59.7%)

Cohort 1: Performance characteristics for 2 x 2 Presentation (HER-2 antibody positive (2+ and 3+) and negative (0+ and 1+) scores are combined.

Positive percent agreement is $29+2+24+13/31+37=100\%$ (95% C.I. = 97.5% - 100%)

Negative percent agreement is $53/75 = 70.7\%$ (95% C.I. = 58.5% - 80.1%)

Overall agreement is $29+24+2+13+53/143=84.7\%$ (95% C.I. = 78.2% - 90.0)

Cohort 2- Consensus IHC scores of three pathologists:				
	CB11			
4B5	3	2	0 and 1	Total
3	72	1	0	73
2	1	12	5	18
0 and 1	0	7	80	87
Total	73	20	85	178

Cohort 2 Performance characteristics for 3 x 3 Presentation

Overall agreement is $72+12+80/178=92.1\%$ (95% CI: 80.1%, 93.1%)

Cohort 2: Performance characteristics for 2 x 2 Presentation (HER-2 antibody positive (2+ and 3+) and negative (0+ and 1+) scores are combined.

Positive percent agreement is $72+12+1+1/73+20 = 92.5\%$ (95% CI: 85.2% - 96.9%)

Negative percent agreement is $80/85 = 94.1\%$ (95% CI: 86.8% - 98.1%)

Overall agreement is $72+12+1+1+80/178=93.3\%$ (95% CI: 88.5% - 96.4%)

Cohort 1- Consensus CB11 IHC scores of three pathologists compared to FISH			
	FISH		
CB11	Positive	Negative	Total
3	32	0	32
2	32	5	37
0 and 1	22	53	75
Total	86	58	144

Cohort 1 Performance characteristics for CB11 and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive)

Positive percent agreement is $32+32/86= 74.4\%$ (95% CI: 63.8% - 83.2%)

Negative percent agreement is $53/58 = 91.4\%$ (95% CI: 80.9% - 97.1%)

Overall agreement is $32+32+53/144=81.2\%$ (95% CI: 73.9% - 87.2%)

Cohort 1- Consensus 4B5 IHC scores of three pathologists compared to FISH			
	FISH		
4B5	Positive	Negative	Total
3	55	3	58
2	25	8	33
0 and 1	6	47	53
Total	86	58	144

Cohort 1: Performance characteristics for 4B5 and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive)

Positive percent agreement is $55+25/86=93.0\%$ (95% C.I. = 87.9% - 96.3%)

Negative percent agreement is $47/58=81.0\%$ (95% C.I. = 73.4% - 86.0%)

Overall agreement is $55+25+47/144=88.2\%$ (95% C.I. = 82.1% - 92.2%)

Cohort 2- Consensus CB11 IHC scores of three pathologists compared to FISH			
	FISH		
CB11	Positive	Negative	Total
3	72	1	73
2	13	7	20
0 and 1	8	77	85
Total	93	85	178

Cohort 2 Performance characteristics for CB11 and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive)

Positive percent agreement is $72+13/93=91.3\%$ (95% C.I. = 85.0% - 96.7%)

Negative percent agreement is $77/85=90.6\%$ (95% C.I. = 83.9% - 96.3%)

Overall agreement is $72+13+77/178=91.0\%$ (95% C.I. = 86.5% - 94.9%)

Cohort 2- Consensus 4B5 IHC scores of three pathologists: compared to FISH			
	FISH		
4B5	Positive	Negative	Total
3	72	1	73
2	11	7	18
0 and 1	10	77	87
Total	93	85	178

Cohort 2: Performance characteristics for 4B5 and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive)

Positive percent agreement is $72+11/93=89.2\%$ (95% C.I. = 82.5% - 95.1%)

Negative percent agreement is $77/85=90.6\%$ (95% C.I. = 84.0% - 96.4%)

Overall agreement is $72+11+77/178=90.0\%$ (95% C.I. = 85.4% - 93.6%)

Inter-pathologist Reproducibility of Comparison Studies Specimens

Since it is well known that different pathologists may have different interpretations of immunohistochemistry slides, three pathologists were employed for each of the two cohorts (for a total of 6 pathologists) to read all samples. A two-out-of-three rule was used to adjudicate the final results. Below is the summary of the variable results obtained by the three pathologists of the comparison study samples for each cohort.

Cohort 1: HER-2 4B5 Scoring for the three Pathologists			
	Investigator 1	Investigator 2	Investigator 3
HER2 Score	4B5 Score	4B5 Score	4B5 Score
3	72	70	73
2	22	19	18
0 and 1	80	89	87
Total	174	178	178

Note: A total of 1 sample varied by more than one grade level (i.e. 0- 2+) when evaluated by the three Pathologists.

Sample 1: one pathologist scored 2+, two pathologists scored 0+

Sample2: one pathologist scored 0+, two pathologists scored 2+

Sample 3: one pathologist scored 0+, the second scored 1+, and the third scored 2+

Cohort 1: CB11 Scoring for the three Pathologists

	Investigator 1	Investigator 2	Investigator 3
HER2 Score	CB11 Score	CB11 Score	CB11 Score
3	72	75	73
2	22	22	18
0 and 1	80	81	87
Total	174	178	178

Note: A total of 1 sample varied by more than one grade level (i.e. 1-3+) when evaluated by the three Pathologists.

Sample 1: one pathologist scored 1+, the second scored 2+, and the third scored 3+

Cohort 2: HER-2 4B5 Scoring for the three Pathologists

	Investigator 4	Investigator 5	Investigator 6
HER2 Score	4B5 Score	4B5 Score	4B5 Score
3	59	65	50
2	30	28	39
0 and 1	52	51	55
Total	141	144	144

Note: A total of 6 samples varied by more than one grade level (e.g. 0, 3+) when evaluated by the three Pathologists.

Sample 1: One pathologist scored 0+, the second scored 0+ and the third scored 2+

Sample 2: One pathologist scored 1+, the second scored 1+, and the third scored 3+

Sample 3: One pathologist scored 0+, the second scored 2+, and the third pathologist scored 2+

Sample 4 and 5: one pathologist scored 0+, the second scored 2+ and the third scored 2+

Sample 6: one pathologist scored 0+, the second scored 3+, and the third scored 3+.

Cohort 2: CB11 Scoring for the three Pathologists

	Investigator 4	Investigator 5	Investigator 6
CB11 Score	CB11 Score	CB11 Score	CB11 Score
3	31	37	28
2	38	32	47
0 and 1	75	75	69
Total	144	144	144

Note: A total of 8 samples varied by more than one grade level (i.e. 0-2+) when evaluated by the three Pathologists.

Samples 1-6: one pathologist scored 0+, the second scored 1+ and the third scored 2+

Samples 7-8: one pathologist scored 0+, the second scored 2+ and the third scored 2+

Following is a tabulation of the ranges of percent agreements across pairs of pathologists (three pairs for each cohort).

Ranges of 2X2* AGREEMENTS FOR THE THREE PATHOLOGISTS

	Overall Percent Agreement	Positive Percent Agreement	Negative Percent Agreement
4B5 vs. CB11			
Cohort 1	82.6 – 86.9%	97.3 – 100.0%	68.0% - 75.4%
Cohort 2	88.2 – 95.5%	87.6 – 95.6%	86.1 – 95.4%
4B5 vs. FISH			
Cohort 1	86.8 – 88.2%	90.7 – 94.2%	79.3 – 81.0%
Cohort 2	87.4 – 89.9%	88.2 – 90.0%	84.5 – 91.8%
CB11 vs. FISH			
Cohort 1	79.9 – 84.0%	73.3 – 80.2%	89.7 – 89.7%
Cohort 2	84.8% - 93.3%	86.7 – 92.5%	82.7 – 94.1%

* 0 and 1+ = Negative. 2+ and 3+ = Positive

b. Matrix comparison:

Not applicable. The only matrix is formalin-fixed paraffin-embedded tissue section stained slide.

3. Clinical studies:

a. Clinical Sensitivity:

No clinical studies were performed. The clinical sensitivity of the test result is dependent on the analytical performance of the Ventana's Pathway® anti-HER-2/neu (4B5) Primary Antibody kit.

b. Clinical specificity:

No clinical studies were performed. The clinical specificity of the test result is dependent on the analytical performance of the Ventana's Pathway® anti-HER-2/neu (4B5) Primary Antibody kit.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

The Ventana's Pathway® anti-HER-2/neu (4B5) Primary Antibody kit is sold by Ventana as a Class I immunohistochemistry (IHC) reagent. No clinical claims are appropriate for a Class I IHC reagent.

N. Instrument Name:

Ventana Image Analysis System (VIAS)

O. System Descriptions:

1. Modes of Operation:

Interactive with user

2. Software:

The operating system used in the VIAS is Microsoft Windows XP integrated with a proprietary user interface. The VIAS system interfaces with Microsoft SQL Server. The VIAS does not interface with a laboratory information system. It is a stand-alone system and does not communicate with other systems in this application.

FDA has reviewed applicants Hazard Analysis and software development processes for this line of product types:

Yes X or No

Joseph Jorgens III has reviewed the original software submission in support of the predicate device (k051282) Ventana Image Analysis system-HER-2/neu and found it to be acceptable for a moderate hazard level.

3. Specimen Identification:

Specimen identification is by barcode applied to the slides manually.

4. Specimen Sampling and Handling:

The microscope slides to be examined are loaded onto the microscope stage manually one-at-a-time.

5. Calibration:

The VIAS software calculates an internal control. As the cytoplasm of a cell covers its nucleus, cytoplasmic foreground stain makes a negative nucleus look positive. For the purpose of calculating the output (percent positive cells) the VIAS system uses a score formula that automatically corrects for potential cytoplasmic foreground stain. This formula determines the percentage of nuclei that exhibit specific positive staining. The positive/negative threshold calculation contained in the formula is a function of the noise level indicated by the measured mean intensity of DAB in cell's cytoplasm. The minimum value of the threshold is 0.02, establishing a reasonable lower bound for the cytoplasmic staining noise level. This threshold value increases as the cytoplasmic staining noise level rises above the minimum value, allowing the system to look for the appropriate level of specific staining in the nucleus, relative to the staining detected in the cytoplasm.

6. Quality Control:

The quality of the result depends on the laboratory following the quality control instructions recommended in the labeling of the accessory immunohistochemistry (IHC) assay kit used with the VIAS.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The Performance Characteristics Section above:

None

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.