

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k051282

B. Purpose for Submission:

Adaptation of Ventana Image Analysis System (K050012) for Her2/neu assay

C. Measurand:

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

D. Type of Test:

Computer-assisted image analyzer for immunohistochemistry (immunocytochemistry)

E. Applicant:

TriPath Imaging, Inc.

F. Proprietary and Established Names:

Ventana Image Analysis System (VIAS) for Her2/neu application

G. Regulatory Information:

1. Regulation section:

21 CFR §864.1860 Immunohistochemistry reagents and kits

2. Classification:

Class II

3. Product code:

NOT (microscope, automated, image analysis, operator intervention)

4. Panel:

Pathology 88

H. Intended Use:

1. Intended use(s):

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 (HER-2/neu) in formalin-fixed, paraffin embedded normal and neoplastic tissue specimens immunohistochemically stained for the presence of HER-2/neu proteins using Ventana's HER-2/neu reagents as well as Ventana's DAB copper chromogen and nuclear hematoxylin.

This particular application is an accessory to the Ventana PATHWAY™ Her2 (clone CB11) (Ventana Medical Systems, Inc., Tucson, Arizona) and 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 to assure the validity of the VIAS-assisted HER2 score.

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™ Her2. The PATHWAY™ Her2 was compared to the DAKO HercepTest™ on an independent sample and found to provide acceptably concordant results. The actual correlation of PATHWAY™ Her2 to clinical outcome has not been established.

2. Indication(s) for use:
Same as intended use
3. Special conditions for use statement(s):
For prescription use only.
4. Special instrument requirements:
Ventana Image Analysis System (VIAS).

I. Device Description:

The Ventana Image Analysis System (VIAS) is an interactive histology imaging device that performs image processing using a microscope, digital color video camera, computer, and image analysis software and assist the qualified pathologist in the consistent quantitative assessment of marker expression in immunohistochemically stained histological sections. 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 which can be operated automatically and interactively, 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. The operating system used in the VIAS is MicroSoft Windows XP integrated with a proprietary user interface.

As an interactive system, the Ventana Image Analysis System device requires competent human intervention at all steps in the analysis 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 interpretations of the data.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Applied Imaging Ariol, Automated Cellular Imaging System (ACIS)
2. Predicate 510(k) number(s):
k031715, k032113
3. Comparison with predicate:

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

Similarities		
Item	Device	Predicate
Intended use	Aid to the pathologist in the classification and counting of cells of interest based on particular color, size and shape.	Same
Specimen type	Formalin-fixed, paraffin-embedded specimens stained by immunohistochemistry such as breast cancer specimens stained for Her2/neu proteins	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 health care professional.	Same
Hardware components	Computer, microscope, color monitor, keyboard, automatic storage of acquired images	Same

Differences		
Item	Device	Predicate
Assay used	Ventana PATHWAY™ Her2 (clone CB11) (Ventana Medical Systems, Inc., Tucson, Arizona)	DAKO Hercep Test™

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

FDA Standard for software verification and validation, FDA Guidance for off-the-shelf software use in medical devices

L. Test Principle:

The VIAS is intended to provide quantitative input to the Pathologist to supplement the qualitative interpretation of PATHWAY™ HER-2/*neu* slides. During the course of a HER-

2/*neu* slide evaluation the Pathologist performs the usual manual read of the HER-2/*neu* slides to assess the HER-2/*neu* expression as score on a scale (0, 1+, 2+, 3+) for the slide using the *VIAS* microscope. 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 HER-2/*neu* slide type is optimized for Ventana's *PATHWAY*TM HER-2/*neu* 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 fields of view selected by the pathologist for a slide.

The total cytoplasmic area is calculated as sum of the area estimates around all nuclei detected in the marked tumor regions in the hematoxylin components of the images, which were selected by the pathologist. The total membrane area is computed as the 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 final score value is derived from the normalized total membrane area computed 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 0, 1+, 2+, 3+ for HER-2/*neu* slides the system results' scale was adapted to the manual scoring scale. For this purpose the system results were derived from a training set of about 200 HER-2/*neu* 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 20. The result of the *VIAS* system quantification of the HER-2/*neu* over-expression is a continuous number ranging from 0 to 3.5. The manual scale, however, consists 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 Pathologist makes the final call based on both the qualitative and quantitative information. It is recommended that in this application of the *Ventana Image Analysis System* the user follow the appropriate instructions in the Ventana *PATHWAY*TM HER-2/*neu* product insert and associated scoring guide.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

To determine the precision of the *Ventana Image Analysis System* intra- and 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* protein expression using two lots of Ventana's *PATHWAY*TM HER-2/*neu* 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 the Intra-Assay (Within Run) Instrument (System) Reproducibility, one field of view for each of the eight HER-2/*neu* slides of the study sample was measured ten times in succession on the same *Ventana Image Analysis System* (System 1). The ten readings for each field of view were done without moving the corresponding slide. The measurement of each sequence of ten values took approximately five minutes. All measurements were performed by the same qualified operator.

Intra-Assay (Within-run) Instrument (System) Reproducibility

HER-2/ <i>neu</i> (n = 10)							
Slide #	Mean Score	StdDev Score	CV [%]	Slide #	Mean Score	StdDev Score	CV [%]
1	0.03	0.007	N/A	2	0.06	0.007	N/A
3	1.25	0.048	3.84	4	1.41	0.025	1.77
5	1.96	0.032	1.62	6	1.84	0.032	1.75
7	3.35	0.024	0.73	8	3.17	0.042	1.32

For the Inter-Assay (Between Run)/Inter-System Reproducibility, one field of view for each of the eight HER-2/*neu* slides was measured five times on three different *Ventana Image Analysis System*. The three systems were calibrated by carefully adjusting the microscopes and setting up the slide types for HER-2/*neu* 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 acquisition process by each system. 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. The studies were conducted within the same day. Each study took approximately 2 hours. All measurements were performed by the same qualified operator.

Inter-Assay (Between Run) / Inter-System Reproducibility

Slide #	System 1			System 2			System 3			Inter-System Systems 1, 2 & 3		
	Mean Score	SD Score	%CV [%]	Mean Score	SD Score	%CV [%]	Mean Score	SD Score	%CV [%]	Mean Score	SD Score	%CV [%]
1	0.03	0.016	N/A	0.03	0.008	N/A	0.03	0.004	N/A	0.03	0.011	N/A
2	0.05	0.009	N/A	0.01	0.005	N/A	0.03	0.008	N/A	0.03	0.021	N/A
3	1.34	0.061	4.51	1.27	0.035	2.78	1.35	0.029	2.12	1.32	0.062	4.66
4	1.34	0.063	4.66	1.31	0.061	4.67	1.42	0.037	2.59	1.36	0.079	5.86
5	2.09	0.063	3.02	2.06	0.038	1.83	1.95	0.052	2.65	2.03	0.091	4.44

Slide #	System 1			System 2			System 3			Inter-System Systems 1, 2 & 3		
6	1.82	0.069	3.79	1.85	0.051	2.77	1.83	0.062	3.4	1.83	0.063	3.45
7	3.28	0.129	3.94	3.34	0.066	1.98	3.36	0.107	3.17	3.33	0.112	3.38
8	3.23	0.043	1.34	3.21	0.067	2.10	3.16	0.085	2.68	3.2	0.076	2.4

b. Linearity/assay reportable range:

Not applicable

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

The analytical traceability of the system depends on the Ventana PATHWAY™ Her2 (clone CB11) kit. VIAS operating manual HER-2/neu application recommends the user to follow the package insert of Ventana PATHWAY™ Her2 for quality control procedures. Ventana PATHWAY™ Her2 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 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:

The analytical specificity of the test result is dependent on the analytical performance of the Ventana PATHWAY™ Her2 (clone CB11) kit.

f. Assay cut-off:

The assay cut-off of the test result is dependent on the analytical performance of the Ventana PATHWAY™ Her2 (clone CB11) kit.

2. Comparison studies:

a. Method comparison with predicate device:

The substantial equivalence studies were based on comparison to conventional manual microscopy performed in accordance with Ventana PATHWAY™ Her2 instructions for use.

Concordance was evaluated as the agreement between the manual Her2 scores and VIAS Her2 scores after they had been reviewed by a pathologist. A set of 201 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 (3 staining lots) labeled with Ventana's DAB copper chromogen and nuclear hematoxylin. 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. The images and the coordinates of their related slide locations were then copied to the databases of three different *Ventana Image Analysis*

Systems. Based on the manual score of the pathologist, 201 slides were grouped into four bins 0, 1+, 2+ and 3+, there were 41, 36, 57 and 67 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.

	Pathologist 1				
VIAS 1	0	1+	2+	3+	Total
0	37	1	0	0	38
1+	15	23	4	0	42
2+	0	7	33	9	49
3+	0	0	6	66	72
Total	52	31	43	75	201
<i>Concordance = 0.791 [95%CI = 0.730 – 0.842]</i>					

	Pathologist 2				
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VIAS 2	0	1+	2+	3+	Total
0	29	4	0	0	33
1+	6	28	7	0	41
2+	0	4	49	6	59
3+	0	0	16	52	68
Total	35	36	72	58	201
<i>Concordance = 0.786 [95%CI = 0.724 – 0.837]</i>					

	Pathologist 3				
VIAS 3	0	1+	2+	3+	Total
0	26	3	0	0	29
1+	14	18	4	0	36
2+	0	10	38	9	57
3+	0	0	11	68	79
Total	40	31	53	77	201
<i>Concordance = 0.746 [95%CI = 0.682 – 0.801]</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, Kappa values and the 95% confidence intervals of the Kappa values between the three different *Ventana Image Analysis System* – Pathologist pairs. Rows 8 to 10 show the concordance, Kappa values and 95% confidence intervals between the three corresponding system calls. Rows 13 to 15 show concordance, Kappa values and 95% confidence intervals between the three study pathologists.

HER-2/neu				
4 discrete bins 0, 1+, 2+, 3+				
Pathologist #	System #	Concordance	Kappa	CI 95%
1	1	0.791	0.715	0.634 – 0.796
2	2	0.786	0.708	0.626 – 0.789
3	3	0.746	0.647	0.564 – 0.729
System #	System #	Concordance	Kappa	CI 95%
1	2	0.896	0.858	0.776 – 0.939
1	3	0.846	0.787	0.706 – 0.869
2	3	0.871	0.821	0.739 – 0.904
Pathologist #	Pathologist #	Concordance	Kappa	CI 95%
1	2	0.751	0.665	0.587 – 0.744
1	3	0.771	0.685	0.603 – 0.766
2	3	0.801	0.728	0.648 – 0.809

The Concordance values between System and Pathologist (concordance range 0.746-0.791) was comparable to concordance values for the Pathologist to Pathologist read (concordance range 0.751 – 0.771) and the corresponding System to System read (concordance range 0.846 – 0.896).

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

The clinical sensitivity of the test result is dependent on the analytical performance of the Ventana PATHWAY™ Her2 (clone CB11) kit. The pathologist must follow the recommendations of the Ventana PATHWAY™ Her2 (clone CB11) kit.

b. Clinical specificity:

The clinical specificity of the test result is dependent on the analytical performance of the Ventana PATHWAY™ Her2 (clone CB11) kit. The pathologist must follow the recommendations of the Ventana PATHWAY™ Her2 (clone CB11) kit.

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

Not applicable

4. Clinical cut-off:

The clinical cut-off of the test result is dependent on the analytical performance of the Ventana PATHWAY™ Her2 (clone CB11) kit. The pathologist must follow the recommendations of the Ventana PATHWAY™ Her2 (clone CB11) kit.

5. Expected values/Reference range:

Not applicable

N. Instrument Name:

Ventana Image Analysis System for HER-2/neu application

O. System Descriptions:

1. Modes of Operation:
Interactive with user.
2. Software:
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
Yes ✓ or No
3. Specimen Identification:
Specimen identification is by barcode applied to the slides manually
4. Specimen Sampling and Handling:
Specimens for image analysis on the VIAS are specifically immunohistochemically stained slides containing human tissue pathology samples. The microscope slides to be examined are loaded on to the microscope stage manually one-at-a-time.
5. Calibration:
VIAS requires a black reference image at the beginning of every session (launching the Windows application). The black reference image is checked for low intensity and uniformity. If the black reference image is unacceptable (too bright or uneven illumination), the Pathologist is instructed to adjust the light level within tolerances to provide an acceptable black reference image. The system will not allow the Pathologist to acquire images without acquiring an acceptable black reference image.

VIAS also requires a white reference image before acquiring images for each slide. The white reference image is checked for high intensity, uniformity, and low saturation. If the white reference image is unacceptable (too dark, uneven illumination, over-saturated), the Pathologist is instructed to adjust the light level to within tolerances and provide an acceptable white reference image. The system will not allow the Pathologist to acquire images without first acquiring an acceptable white reference image. Together, the black and white reference images are used to calibrate the dynamic range and perform white balancing and shading correction for each slide. With the calibration above, VIAS assures that the light levels of the microscope are within the acceptable tolerance limits to generate valid quantitative results. Also, each assay is processed using an assay-specific Slide Type, incorporating algorithms and score formulas, developed for each specific assay. The assay-specific Slide Type is designed to handle the expected variation between staining runs using assay-specific staining controls.

6. Quality Control:
The accuracy of the system depends on the laboratory following the quality control instructions recommended in the labeling of the accessory immunohistochemistry (immunocytochemistry) kit associated with Ventana PATHWAY™ Her2.

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.