510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
INSTRUMENT ONLY TEMPLATE

A. 510(k) Number:

K050012

B. Purpose for Submission:
New product.

C. Manufacturer and Instrument Name:

TriPath Imaging, Inc.'s Ventana Image Analysis System (VIAS) for Estrogen and Progesterone Receptors

D. Type of Test or Tests Performed:

Computer-assisted image analyzer for estrogen/progesterone receptor immunohistochemistry (qualitative immunocytochemistry).

E. System Descriptions:

1. Device Description:

The Ventana Image Analysis System is an interactive, computer supported bright field microscopy system to assist the qualified pathologist in the consistent quantitative assessment of marker expression in immunohistochemically stained histological sections.

The system is comprised of a PC, flat panel LCD monitor **(1)**, keyboard **(2)**, mouse **(3),** interactive bar code reader (not shown in Fig. 1), printer access (not shown in Fig. 1), installed software, an interactive microscope of the type Zeiss Axioskop 2 Mot plus or equivalent with 20X magnification capabilities **(4),** with a 3CCD color camera **(5),** and a motorized stage **(6**), which can be operated automatically and interactively.

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.

2. Principles of Operation:

In this application, the VIAS is intended to aid a qualified pathologist in the acquisition and measurement of images to quantify the percentage of positively stained nuclei in paraffin embedded breast cancer tissue specimens immunohistochemically stained for the presence of estrogen receptor (ER) or progesterone receptor (PR) proteins using Ventana's ER and PR reagents as well as Ventana's DAB copper chromogen and nuclear hematoxylin.

During the course of an ER/PR 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, hematoxylin – blue) and the detection of cell nuclei in the brown (DAB) and blue (hematoxylin) images within the marked tumor areas.

The parameters for the dye characterization are stored in a slide type storage structure containing assay specific parameters to process ER and PR slides. The slide types for the ER and PR assays contain the name of the assay (ER or PR), Counterstain (Hematoxylin), Marker Stain (DAB), Marker Expression Localization (Nucleus) and the magnification of the objective used for quantitative analysis (20x). The ER/PR slide types are optimized for Ventana's ER and PR assays using Ventana's DAB copper chromogen and nuclear hematoxylin.

The calculation of the ER and PR percent positive score is based on the number of positive and negative nuclei detected in the brown (DAB) and blue (hematoxylin) images. The nuclei of positive tumor cells can be seen in both the blue and brown images. Nuclei of negative tumor cells – in the ideal case – have no brown nuclear image component. However, due to cytoplasmic staining this is only very rarely the case. As the cytoplasm of a cell covers its nucleus, cytoplasmic foreground stain makes a negative nucleus look positive. For the purpose of calculating the percent positive cells the VIAS system uses a score formula, which 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 the 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.

The final percent positive number is calculated by the VIAS system as a ratio of all detected nuclei determined as positive and accumulated over all fields selected by the

pathologist for a particular slide and the total number of detected nuclei (negative and positive) within these fields multiplied by 100%. To avoid an inflated denominator due to normal cell nuclei included in the count of negative nuclei in this ratio it is important to segment out normal nuclei. 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. 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 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.

Each laboratory can set the thresholds to the normal ranges preferred by their Pathologist for the ER and PR assays. Typical cut-off values are 1%, 5%, and 10% positive tumor cells. The principle of Operation for this device is well established.

3. Modes of Operation:

   Interactive with user

4. Specimen Identification:

   Specimen identification is by barcode applied to the slides manually

5. Specimen Sampling and Handling:

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

6. 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.

7. Quality Control (QC):

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

8. 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.

Prior to the present submission, the FDA has not reviewed TriPath's Hazard Analysis and Software Documentation for the Ventana Image Analysis System.

Yes_____ or No___X_____

Joseph Jorgens III has reviewed the software submission and found it to be acceptable for a moderate hazard level.

F. Regulatory Information:

1. Regulation section:

21 CFR 864.1860 Immunohistochemistry reagents and kits.

2. Classification:

Class II

3 Product code:

NQN (Microscope, Automated, Image Analysis, Immunohistochemistry, Operator Intervention, Nuclear Intensity and Percent Positivity)

4. Panel:

Pathology (88)

G. Intended Use:

1. 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 in the acquisition and measurement of images to quantify the percentage of positively stained nuclei in paraffin embedded breast cancer tissue specimens immunohistochemically stained for the presence of estrogen receptor (ER) or progesterone receptor (PR) proteins using Ventana's ER and PR reagents as well as Ventana's DAB copper chromogen and nuclear hematoxylin. It is indicated for use as an aid in the management, prognosis, and prediction of therapy outcomes of breast cancer when used with in vitro diagnostic reagents marketed for these indications.

2. Special Conditions for Use Statement(s):

   None

H. Substantial Equivalence Information:

1. Predicate Device Name(s) and 510(k) numbers:

   The ChromaVision Medical Systems, Inc., ACIS (Automated Cellular Imaging System) for Estrogen and Progesterone Receptors K012138

2. Comparison with Predicate Device:

DEVICE	PREDICATE
Similarities	
Histologic observation by a pathologist through a controlled microscope/ digital camera combination	Histologic observation by a pathologist through a controlled microscope/ digital camera combination
Aid to the pathologist in the classification and counting of cells of interest based on particular color, size and shape.	Aid to the pathologist in the classification and counting of cells of interest based on particular color, size and shape.
Colorimetric pattern recognition by microscopic examination of prepared cells by size, shape, hue, and intensity as observed by an automated computer controlled microscope and/or by visual observation by a health care professional.	Colorimetric pattern recognition by microscopic examination of prepared cells by size, shape, hue, and intensity as observed by an automated computer controlled microscope and/or by visual observation by a health care professional.
PC with Windows® based operating system	PC with Windows® based operating system

Examines formalin-fixed, paraffin-embedded specimens stained by immunohistochemistry such as breast cancer specimens stained for estrogen and progesterone receptor proteins	Examines formalin-fixed, paraffin-embedded specimens stained by immunohistochemistry such as breast cancer specimens stained for estrogen and progesterone receptor proteins
Keyboard and control panel	Keyboard and control panel
Color printer for reports	Color printer for reports
Halogen lamp light source	Halogen lamp light source
Microscope Objectives 10x, 20x, 40x	Microscope Objectives 10x, 20x, 40x
Color monitor for display of information	Color monitor for display of information
Ventana PR Assay	Ventana PR Assay

Differences		
Item	Device	Predicate
ER Assays Used	Ventana	DakoCytomation

I. Special Control/Guidance Document Referenced (if applicable):

None

J. Performance Characteristics*:* All of the following performance characteristics were generated using the Veridex CellSearch™ Circulating Tumor Cell Kit (Epithelial). (K050245)

1. Analytical Performance:
   a. Accuracy

   Two sets of 210 formalin-fixed, paraffin-embedded breast tissue specimens (one set for ER and one set for PR) were obtained from an outside source. They were immunohistochemically stained using Ventana's ER and PR reagents labeled with Ventana's DAB copper chromogen and nuclear hematoxylin for Estrogen Receptors (3 staining lots) or Progesterone Receptors (2 staining lots). 5 ER slides and 6 PR slides were excluded from the study due to missing tumor.

   Both sets of slides were manually read at different times in a blinded manner by three pathologists using the microscope of the same ***Ventana Image Analysis System***.
   Each pathologist estimated the percentage of positive cells for each slide (manual read) and selected at least 4 (or more) diagnostically significant fields of view which the system acquired and stored as digital images. The system automatically computed the percent positive cells based on the cumulative numbers of positive and negative cells derived from all 4 fields selected for a particular slide by one pathologist. The quantitative information was only displayed and retrieved from the system after the study was finished so as not to influence the manual read of the pathologists. Since different laboratories are known to use different cutoff thresholds, three examples are provided in the tables below.

Concordance for ER staining

ER			
Cutoff Threshold	**Pathologist – System Concordance [1] for Three Pathologists**	**Pathologist – Pathologist Concordance [2] between Three Pathologists**	**System – System Concordance [3]**
1%	94.6 – 97.6%	96.1 – 97.1%	95.1 – 97.1%
5%	98.0 – 98.5%	96.6 – 99.0%	97.1 – 97.6%
10%	96.6 – 98.0%	97.1 – 97.6%	96.6 – 98.0%

Table 6: Concordance ranges for ER staining

[1] Range of concordances seen between the system and each of three (3) pathologists
[2] Range of concordances seen between the three (3) different pathologists (manual call)
[3] Range of concordances seen between the three (3) different corresponding system calls based on the fields of view selected by each of the three (3) study pathologists. The pathologists did not see the system results

Column 2 shows the concordance ranges between the ***Ventana Image Analysis System*** and 3 pathologists, column 3 presents concordance ranges between the 3 pathologists and column 4 lists concordance ranges between the 3 corresponding system calls from fields independently identified by the pathologists.

Concordance for PR staining

PR			
Cutoff Threshold	**Pathologist – System Concordance [1] for Three Pathologists**	**Pathologist – Pathologist Concordance [2] between Three Pathologists**	**System – System Concordance [3]**
1%	90.2 – 92.6%	89.2 – 90.2%	88.7 – 92.6%
5%	91.2 – 94.1%	88.2 – 88.2%	91.2 – 92.6%
10%	88.2 – 91.2%	88.2 – 89.7%	89.7 – 94.6%

Table 7: Concordance ranges for PR staining

[1] Range of concordances seen between the system and each of three (3) pathologists
[2] Range of concordances seen between the three (3) different pathologists (manual call)
[3] Range of concordances seen between the three (3) different corresponding system calls based on the

fields of view selected by each of the three (3) study pathologists. The pathologists did not see the system results

Column 2 shows the concordance ranges between the ***Ventana Image Analysis System*** and 3 pathologists, column 3 presents concordance ranges between the 3 pathologists and column 4 lists concordance ranges between the 3 corresponding system calls from fields independently identified by the pathologists.

b. Precision/Reproducibility

Instrument Precision

To determine the precision of the ***Ventana Image Analysis System*** Intra- and Inter-Assay Reproducibility studies were conducted using a set of 4 ER and 4 PR slides. The slides consisted of formalin-fixed, paraffin-embedded tissue specimens immunohistochemically stained for Estrogen Receptors (ER) and Progesterone Receptors (PR) using one lot of Ventana's ER and PR assays labeled with Ventana's DAB copper chromogen and Ventana's nuclear hematoxylin. The slides were selected for their % tumor cell Positivity values falling in the ranges 0%, 1-10%, 11-50% and >50%. %Positivity stands for the percentage of positively stained nuclei measured within the selected field(s) of view of a slide.

The study results are presented in Table 1 through Table 5. For each slide, the mean, the standard deviation (StdDev), and the coefficient of variation (CV) of the instrument score %Pos readings were calculated. The number n of repeats per study is listed in the header of each table.

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

ER (n = 10)				**PR (n = 10)**			
Slide #	**Mean %Pos** [%]	**StdDev %Pos** [%]	**CV** [%]	**Slide #**	**Mean %Pos** [%]	**StdDev %Pos** [%]	**CV** [%]
1	0.00	0.00	N/A	**5**	0.00	0.00	N/A
2	3.35	0.11	3.23	**6**	7.71	0.15	1.98
3	37.58	0.57	1.53	**7**	37.81	1.34	3.54
4	94.64	0.67	0.71	**8**	97.62	0.39	0.40

Table 1: Results of the Intra-Assay (Within-Run) Precision Study

For each of the 4 ER and 4 PR slides of the study sample one (1) field of view was measured ten (10) 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 (10) values took approximately five (5) minutes. All measurements were performed by the same qualified operator.
Tables 2, 3, 4 describe the results of the Inter-Assay (Between Run) / Inter-System

Reproducibility. In this study one (1) field of view for each of the four (4) ER and four (4) PR slides were measured five (5) times on three (3) different ***Ventana Image Analysis Systems***. The three systems were calibrated by carefully adjusting the microscopes (see Microscope User's Guide) and setting up the slide types for ER and PR in an identical fashion (see ***Application Addendum – ER*** and ***Application Addendum – PR*** in the ***Application Specific Information*** section at the end of this appendix). 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 (see ***Acquiring reference images*** in ***Chapter 4: Screening a slide*** for more information).

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 (4) times on the same system. This resulted in 5 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 (Table 14), the study was repeated on system 2 (Table 15) and 3 (Table 16). 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-Instrument (System) Reproducibility
ER (n = 5) PR (n = 5)

ER (n = 5)				PR (n = 5)			
Slide #	**Mean %Pos** [%]	**SD %Pos** [%]	**%CV** [%]	**Slide #**	**Mean %Pos** [%]	**SD %Pos** [%]	**%CV** [%]
1	0.00	0.00	N/A	**5**	0.00	0.00	N/A
2	3.25	0.14	4.29	**6**	7.67	0.26	3.33
3	36.42	2.03	5.56	**7**	36.21	1.82	5.02
4	94.88	0.12	0.13	**8**	97.49	0.05	0.05

Table 2: Results of the Inter-Assay (Between Run) Precision Study - System 1

ER (n = 5)				PR (n = 5)			
Slide #	**Mean %Pos** [%]	**SD %Pos** [%]	**%CV** [%]	**Slide #**	**Mean %Pos** [%]	**SD %Pos** [%]	**%CV** [%]
1	0.00	0.00	N/A	**5**	0.00	0.00	N/A

2	3.24	0.26	7.87	**6**	7.73	0.20	2.63
3	36.80	1.12	3.05	**7**	36.72	1.09	2.98
4	95.12	0.65	0.68	**8**	98.29	0.64	0.65

Table 3: Results of the Inter-Assay (Between Run) Precision Study - System 2

R (n = 5)				**PR (n = 5)**			
Slide #	**Mean %Pos** [%]	**SD %Pos** [%]	**%CV** [%]	**Slide #**	**Mean %Pos** [%]	**SD %Pos** [%]	**%CV** [%]
1	0.00	0.00	N/A	**5**	0.00	0.00	N/A
2	3.30	0.48	14.65	**6**	7.34	0.16	2.21
3	36.11	1.25	3.45	**7**	36.63	0.58	2.57
4	95.10	0.66	0.70	**8**	98.23	0.69	0.70

Table 4: Results of the Inter-Assay (Between Run) Precision Study - System 3

ER (n = 3)				**PR (n = 3)**			
Slide #	**M Mean %Pos** [%]	**SD %Pos** [%]	**CV** [%]	**Slide #**	**M Mean %Pos** [%]	**SD %Pos** [%]	**CV** [%]
1	0.00	0.00	N/A	**5**	0.00	0.00	N/A
2	3.26	0.33	9.96	**6**	7.58	0.31	3.91
3	36.44	1.56	4.27	**7**	36.52	1.3	3.57
4	95.03	0.59	0.78	**8**	98.00	0.71	0.84

Table 5: Summary results of the Inter-System Precision Study – Systems 1, 2, 3

The columns labeled with M Mean show the mean values of the 3 mean instrument score %Pos values of system 1, 2, and 3. SD lists standard deviation values (degrees of freedom=14). The CV columns present the corresponding coefficients of variation (degrees of freedom=14). Both the SD and CV were calculated utilizing a propagation of variance formula which incorporates both intra- and inter-system variance calculations.

2. Other Supportive Instrument Performance Data Not Covered Above:

None

K. Proposed Labeling:

Extensive suggestions were made to the Operator's manual (labeling). The detailed suggestions can be seen in the 510(k) record.

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

L. Conclusion:

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

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