

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k053520

B. Purpose for Submission:

New application on approved system.

C. Measurand:

Ki-67, a nuclear protein expressed in proliferation cells. During the cell cycle Ki-67 is present in the G1, S, G2 and M phases, but absent in the G0 (quiescent) phase.

D. Type of Test:

Computer-assisted image analyzer for Ki-67 nuclear protein immunohistochemistry (qualitative immunocytochemistry).

E. Applicant:

TriPath Imaging, Inc.

F. Proprietary and Established Names:

Ventana Image Analysis System, Ki-67 Application

G. 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)

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 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 Ki-67 proteins using Ventana's reagents and nuclear hematoxylin. It is indicated for use in assessing the proliferative activity of normal and neoplastic breast tissue when used with in vitro diagnostic reagents marketed for these indications.

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 Ki-67 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 Ki-67 kit to assure the validity of the VIAS-assisted Ki-67 assessment.

2. Indication(s) for use:

It is indicated for use in assessing the proliferative activity of normal and neoplastic breast tissue when used with in vitro diagnostic reagents marketed for these indications

3. Special conditions for use statement(s):

None

4. Special instrument requirements:

Ventana Image Analysis System (VIAS).

I. Device Description:

The Ventana Image Analysis System (VIAS) 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 **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.

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 Ki-67 histology slides. As result of the quantitative analysis of these images the system presents the percent of Ki-67 positive nuclei detected within the selected fields on a scale of 0% - 100%.

This device is intended to provide quantitative input to aid a pathologist in the qualitative interpretation of Ki-67 stained immunohistochemistry (IHC) slides. For this application, it is recommended that the user follow the appropriate instructions in the Ventana Ki-67 kit product insert (Ventana Catalogue Number 790-2910) to stain and interpret the test slides. The pathologist then performs the usual manual read of the Ki-67 slides to assess the Ki-67 expression as score on a scale (0% to 100% positive stained tumor cells) for the slide using the VIAS microscope. The pathologist then has the opportunity to select multiple fields of view using the VIAS microscope and computer for quantitative analysis. The VIAS device processes the user-selected color images to assess the per cent of Ki-67 expressing tumor cells using a software algorithm that is the mathematical equivalent to the Pathologist's qualitative read. The Pathologist makes the final call based on both the manual qualitative and VIAS quantitative interpretation.

As an interactive system, the Ventana Image Analysis System 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):
Ventana Image analysis System (VIAS) – ER/PR Application
2. Predicate 510(k) number(s):
k050012
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Hardware and Software	VIAS System	VIAS System
Specimen	Formalin-fixed paraffin-embedded breast cancer specimens stained by immunohistochemical (IHC) technique	Formalin-fixed paraffin-embedded breast cancer specimens stained by immunohistochemical (IHC) technique
Localization of IHC positive stain	nuclear	nuclear
Interpretation	By the pathologist. The VIAS aids the pathologist in the interpretation of the specimen.	By the pathologist. The VIAS aids the pathologist in the interpretation of the specimen.

Differences		
Item	Device	Predicate
IHC Antigen Detected	Ki-67	Estrogen and Progesterone

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

FDA’s Guidance for Submission of Immunohistochemistry Applications to the FDA

L. Test Principle:

After the specimen on the slide is stained immunohistochemically for the nuclear antigen, Ki-67, on a Ventana Automated slide stainer according to the directions in the Ventana Ki-67 package insert (790-2910), 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 Ki-67 slides.

The slide type for the Ki-67 assay contains the name of the assay (Ki-67), Counterstain (Hematoxylin), Marker Stain (DAB), Marker Expression Localization (Nucleus) and the magnification of the objective used for quantitative analysis (20x). The Ki-67 slide type is

optimized for Ventana's Ki-67 assay using Ventana's DAB copper chromogen and nuclear hematoxylin.

The calculation of the Ki-67 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 stain 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.

Establishing the System Score Formula

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%.

Interactive Region Correction

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 (see *Operator's Manual, Chapter 4, Defining regions on the field*). 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 *Operator's Manual, Chapter 4, Defining regions on the field*). 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 threshold to the normal range preferred by their Pathologist for the Ki-67 assay. Typical cut-off values are 1%, 5%, and 10% positive tumor cells [1) *Brown et al.*; 2) *Keshgegian et al.*; 3) *Molino et al.*; 4) *Railo, et al.*; 5) *Sahin et al.*].

The principle of staining slides immunohistochemically has been used for at least 20 years. The limitations of IHC methodology are well described. The VIAS has been cleared in the past for two additional applications: Her-2/neu and Estrogen (ER) and progesterone (PR) Nuclear Receptors. The ER/PR application is very similar to the Ki-67 application. Both

tests look at the IHC stained nucleus and determine the percent positive cells. The software and hardware are identical. The only difference is in the antigen detected by the Ventana antibody test.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Instrument Precision

To evaluate the between run precision of the *Ventana Image Analysis System* one (1) selected field of view on each of the nine (9) study slides was measured once before repeating the same sequence another four (4) times. This resulted in five (5) 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 1), the study was repeated on system 2 (Table 2) and 3 (Table 3).

For the study a set of nine (9) Ki-67 slides was used. The slides consisted of formalin-fixed, paraffin-embedded tissue specimens immunohistochemically stained for Ki-67 protein expression using Ventana’s Ki-67 assay labeled with Ventana’s iVIEW™ DAB chromogen and Ventana’s nuclear hematoxylin. The study slides were selected with mean per cent positive Ki-67 tumor cells of around 0, 1, 5, 10, 20, 30, 50, 70 and 90 per cent.

The study results are presented in Table 1 through Table 3. For each slide, the mean, the standard deviation (StdDev), and the coefficient of variation (CV) of the instrument score readings were calculated. The number n of repeats per study is listed in the header of each table.

Ki-67 (n = 5)							
Slide #	Mean Score	SD Score	%CV [%]	Slide #	Mean Score	SD Score	%CV [%]
1	0.0	0.00	N/A	2	1.0	0.00	0.00
3	5.0	0.0	0.0	4	10.4	0.55	5.27
5	21.4	0.89	4.18	6	28.8	0.84	2.91
7	49.4	0.55	1.11	8	69.6	0.55	0.79
9	90.0	0.71	0.79				

Table 1: Results of the Between Run Precision Study - System 1

Ki-67 (n = 5)							
Slide #	Mean Score	SD Score	%CV [%]	Slide #	Mean Score	SD Score	%CV [%]
1	0.0	0.00	N/A	2	1.0	0.00	0.00
3	5.2	0.45	8.6	4	10.6	0.55	5.17
5	22.0	0.71	3.21	6	28.8	0.45	1.55
7	50.4	1.14	2.26	8	69.4	0.89	1.29
9	89.8	1.10	1.22				

Table 2: Results of the Between Run Precision Study - System 2

Ki-67 (n = 5)							
Slide #	Mean Score	SD Score	%CV [%]	Slide #	Mean Score	SD Score	%CV [%]
1	0.0	0.00	N/A	2	1.0	0.00	0.00
3	5.2	0.45	8.60	4	10.2	0.45	4.38
5	21.0	0.71	3.37	6	29.4	0.55	1.86
7	51.0	0.71	1.39	8	69.2	0.45	0.65
9	89.8	0.84	0.93				

Table 3: Results of the Between Run Precision Study - System 3

Between Run/ Inter-Instrument (System) Reproducibility

Ki-67 (n = 3)							
Slide #	M Mean Score	SD* Score	CV* [%]	Slide #	M Mean Score	SD* Score	CV* [%]
1	0.0	0.00	N/A	2	1.0	0.00	0.00
3	5.13	0.38	7.37	4	10.4	0.55	5.32
5	21.47	0.92	4.31	6	29.0	0.72	2.49
7	50.27	1.16	2.31	8	69.4	0.69	0.99
9	89.87	0.90	1.00				

Table 4: Summary results of the (Between Run) Inter-System Reproducibility Study – Systems 1, 2, 3

Table 1 through Table 4 describes the results of the Between Run / Inter-System Reproducibility study. In this study one (1) field of view for each of the nine (9)

Ki-67 slides was 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 Ki-67 in an identical fashion (see *Application Addendum – Ki-67* 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 *VIAS Operator's Manual: Acquiring reference images* in *Chapter 4: Screening a slide* for more information).

Table 4 shows the summary results of the Inter-System Reproducibility study based on systems 1, 2 and 3. The columns labeled with M Mean present the mean values of the 3 mean instrument score values of system 1, 2, and 3. SD* lists standard deviation values. The CV* columns present the corresponding coefficients of variation. Both the SD* and CV* were calculated utilizing a propagation of variance formula which incorporates both intra- and inter-system variance calculations.

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

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):

Ventana Medical Systems, Inc., the manufacturer of the Class I IHC reagents used in this application, is responsible for good manufacturing practices that assure the stability of the reagents.

Following the FDA's Guidance for Submission of Immunohistochemistry Applications to the FDA, the Ventana package insert for Ki-67 describes the positive and negative controls to be assembled by each laboratory for use to control the assay. They are not traceable to a higher standard. Each laboratory assigns their own values to control materials used.

Due to the nature of the test (qualitative immunohistochemical), there are no calibrators.

d. Detection limit:

Taken from the package insert of the Ventana Confirm™ anti-Ki-67 (K-2) Primary Antibody: "The sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage that alters antigenicity weakens Ki67 detection by CONFIRM anti-Ki67 (K-2) and may generate false negative results."

e. Analytical specificity:

Ventana Medical Systems, the manufacturer of the reagents, followed the instructions in the FDA's Guidance for Submission of Immunohistochemistry Applications to the FDA. Taken from the package insert of the Ventana Confirm™ anti-Ki-67 (K-2)

Primary Antibody: “Specificity of CONFIRM anti-Ki67 (K-2) was demonstrated by testing formalin-fixed, paraffin-embedded normal and neoplastic tissues. For each normal tissue type three cases were stained from three different sources on the BenchMark automated slide stainer. Slides were stained in triplicate with a negative control. The staining results of the normal tissues are as follows: focal positive staining was observed in breast, thyroid, prostate, testis, ovary, uterus, pancreas, lung, kidney, intestine, and skin tissues. Negative staining was observed in adrenal, brain, and heart. Several tumor types were screened using a tumor multi-tissue block. The slides were stained in duplicate. The following results were observed: for liver, kidney, lung, pancreas, stomach, ovarian, thyroid, breast, prostate, colon, and undifferentiated carcinomas, along with sarcoma, melanoma, carcinoids, and lymphoma all stained positive.

f. *Assay cut-off:*

Each laboratory can set the threshold for positivity preferred by their pathologist for the Ki-67 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. The following 5 literature references were provided to demonstrate that different laboratories use different cutoffs with this product.

- 1) Brown RW, Allred DC, Clark GM, *et al.* Prognostic value of Ki-67 compared to S-phase fraction in axillary node-negative breast cancer. *Clin Cancer Res* 1996;2:585–92
- 2) Keshgegian AA, Cnaan A. Proliferation Markers in Breast Carcinoma. *A.J.C.P.* 1995; 104-1:42-49
- 3) Molino A, Micciolo R, *et al.* Ki-67 Immunostaining in 322 Primary Breast Cancers: Associations with Clinical and Pathological Variables and Prognosis. *Int. J. Cancer* 1997; 74, 433-437
- 4) Railo M, Lundin J, Haglund C, *et al.* Ki-67, p53, Er-receptors, ploidy and S-phase as prognostic factors in T1 node negative breast cancer. *Acta Oncol* 1997;36:369–74
- 5) Sahin AA, Blick MB, Ordonez NG, Smith TL, Ayala AG. Ki-67 Immunostaining in Node-Negative Stage I/II Breast Carcinoma. *Cancer* 1991; 68: 549-557

2. Comparison studies:

a. *Method comparison with predicate device:*

Study Design: The predicate device for the comparison of the automated image analysis was the manual method performed by the pathologist on the same set of 207 formalin-fixed, paraffin-embedded breast tissue specimens obtained from an outside source. They were immunohistochemically stained using Ventana’s KI-67 reagents (3 staining lots) labeled with Ventana’s DAB copper chromogen and nuclear hematoxylin. The slides were selected in such a way that they represented the three

histological grades of infiltrating ductal carcinoma (approximately 50 slides of each grade, ≈75% of total sample size) using the Scarf-Bloom-Richardson grading system and also included approximately 50 lobular carcinoma with no designated subtypes (≈25% of total sample size).

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 (3) and six (6) 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 6 slides were excluded from the initial sample by the pathologist for various reasons (e.g. bad fixation, bad staining). An additional 2 slides were identified and excluded as duplicates of cases which were already included in the study set.

The images and the coordinates of their related slide locations were then copied to the databases of three (3) different *Ventana Image Analysis Systems*.

During the comparison study three (3) different board-certified pathologists performed a manual read in a blinded manner of each slide of the study sample by having the preselected fields of interest automatically relocated underneath the microscope of one *Ventana Image Analysis System*. Each pathologist was using the microscope of a different system (e.g. pathologist 1 used system 1, pathologist 2 used system 2, pathologist 3 used 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.

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 preselected 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.

If only a sub region of a field was taken into account for the evaluation, such as for example a small gland, the pathologist marked this area in the digital image with the drawing tool as being either included or excluded from the evaluation.

Based on the recaptured images the system automatically computed the %positivity scores for each slide. The slide score results were later retrieved from the system and used in the subsequent data analysis.

Since different laboratories are known to use different cutoff thresholds, three examples are provided in the tables below.

Concordance Results for Ki-67 staining

Ki-67			
Cutoff Threshold	1. Pathologist – System Concordance¹ for Three Pathologist – System pairs	2. Pathologist – Pathologist Concordance² between Three Pathologists	3. System – System Concordance³ for three VIAS systems
1%	97.0 %	97.0 – 98.0%	100%
5%	93.0 – 94.0%	92.5 - 94.5%	98.0 – 99.0%
10%	88.4 – 95.5%	87.9 – 91.0%	99.0 – 100%

Table 6: Concordance ranges for Ki-67 staining

¹ Range of concordances seen between the three (3) system – pathologist pairs

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

Table 6 shows the concordance range for the manual scores of the three study pathologists with the corresponding system scores (column two in table 6), the concordance range between the system scores (column three in table 6) and the concordance range between the manual scores of the three (3) study pathologists (column four in table 6). The concordance ranges are given for the three example cutoff thresholds of $\text{pos} \geq 1\%$, $\text{pos} \geq 5\%$, and $\text{pos} \geq 10\%$ positive stained tumor cells.

Conclusion: Expected Results

The concordance values seen in column 1 between the ***Ventana Image Analysis System*** readings and manual readings by three pathologists (88.4 – 97.0% for cutoff values 1%, 5% and 10%) are comparable to the manual readings of three pathologists at three different sites seen in column 2 (87.1 – 98.0% for the same cutoff values).

These data demonstrate that the likelihood of the ***Ventana Image Analysis System*** to produce a score comparable to the reference manual reading on a given slide is just as likely as the pathologists are to agree with each other’s manual readings. These results are based on each pathologist and each system reading the same preselected fields of view. Column three shows that the corresponding System to System readings (98.0 – 100.0% for the same cutoff values) are actually more reproducible than the pathologists reference manual readings.

b. *Matrix comparison:*

Not applicable. There is only one matrix: the formalin-fixed paraffin-embedded tissue section stained slide.

3. Clinical studies:
 - a. *Clinical Sensitivity:*
No clinical Studies were undertaken
 - b. *Clinical specificity:*
No clinical studies were undertaken
 - c. *Other clinical supportive data (when a. and b. are not applicable):*
No clinical studies were undertaken. See the report of the comparison studies above.
4. Clinical cut-off:
Same as assay cut-off.
5. Expected values/Reference range:
The CONFIRM™ anti-Ki67 (K-2) Primary Antibody 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 applicant's Hazard Analysis and software development processes for this line of product types:
Yes or No
Joseph Jorgens III has reviewed the software submission 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.