

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

**A. 510(k) Number:**

k111755

**B. Purpose for Submission:**

New device

**C. Manufacturer and Instrument Name:**

Ventana Medical Systems, Inc., Virtuoso System for IHC Ki67 (30-9)

**D. Type of Test or Tests Performed:**

Computer-assisted image analysis scoring and manual scoring of digital images of Ki67 immunohistochemistry stained slides.

**E. System Descriptions:**

1. Device Description:

The Virtuoso™ System is an instrument-plus-software system designed to assist the qualified pathologist in the consistent assessment of protein expression in immunohistochemically stained histologic sections from formalin-fixed, paraffin-embedded normal and neoplastic tissues.

The system consists of a slide scanner (iScan), computer, monitor, keyboard, mouse, image analysis algorithms for specific immunohistochemical markers, and software with a Windows web browser-based user interface. Virtuoso is a web-based, end-to-end, digital pathology software solution that allows pathology laboratories to acquire, manage, view, analyze, share, and report digital images of pathology specimens. Using the Virtuoso software, the pathologist can view digital images, add annotations, make measurements, perform image analysis, and generate reports.

The Digital Read option allows the pathologist to score the Ki67 stained slide digital image on a computer monitor. In the Image Analysis Application option, slides are scored by the Ki67 image analysis application. This score is then presented on the computer screen. The pathologist verifies this score and confirms it.

Hardware: The iScan slide scanning device captures digital images of formalin-fixed, paraffin-embedded tissues that are suitable for storage and viewing. The device includes a digital slide scanner, racks for loading glass slides, computer, scanner software, keyboard, mouse and monitor.

Software: The Virtuoso software is designed to complement the routine workflow of a qualified pathologist in the review of immunohistochemically stained histologic slides. It allows the user to select fields of view (FOVs) in the digital image for analysis and provides quantitative data on these FOVs to assist with interpretation. The software makes no independent interpretations of the data and requires competent human intervention for all steps in the analysis process.

2. Principles of Operation:

The Virtuoso System for Ki67 (30-9) employs image analysis and pre-defined

parameters to obtain Ki67 staining scores. The identification of the nucleus is carried out automatically by the image analysis algorithms. The steps involved in the analysis algorithms are:

- a. **Image Enhancement:** The image is enhanced by increasing the contrast to make it more suitable for analysis.
  - b. **Identification of the Epithelial Area:** The algorithm separates the tissue area from the background within the selected FOV such that only the tissue area is processed in the following steps.
  - c. **Identification of the Nucleus:** The nuclei in the epithelial area are identified.
  - d. **Classification:** Cells are classified based on the extent, intensity and thickness of nuclear staining.
  - e. **Scoring/Grading:** Based on the classification, an overall score for the image is computed using the numbers of stained cells, non-stained cells, and total cells for the calculations. The score assigned is based on the guidelines indicated in the package insert for Ki67 (30-9).
3. Modes of Operation:
- a. Manual scoring of immunohistochemically (IHC) Ki67 stained slide images on a computer monitor (digital read).
  - b. Computer scoring of IHC Ki67 stained slide images performed by Ki67 (30-9) Image Analysis Application. This score is verified by the pathologist.
4. Specimen Identification:  
Glass tissue slides are identified by slide label or barcode (if provided by the user) by scanning the whole slide including the label or barcode.
5. Specimen Sampling and Handling:  
IHC stained slides are manually loaded on to the iSCAN Coreo slide scanner individually or in slide racks. The slide racks hold a maximum of 160 slides. Under the default setting a thumbnail view of the slide and the area of interest (AOI) in the slide is scanned. The operator has the option of rescanning the slide after viewing the image on the computer monitor. Under the manual scanning option, the user has the ability to select the scan area for single or batched slides.
6. Calibration:  
The iSCAN Coreo contains a diagnostics module that can be run by the user. This application tests the scanner hardware components and functions. These tests must be run with nine custom slides calibrated for the module which can be obtained from the sponsor.
7. Quality Control:  
Quality control is performed by the operator before releasing the images to the pathologist for review. Slides with sub-optimal images will be rescanned.
8. Software:  
FDA has reviewed applicant's Hazard Analysis and Software Development processes for this line of product types:  
Yes  or No

## 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  
NOT - Microscope, Automated, Image Analysis, Operator Intervention  
OEO - Automated Digital Image Manual Interpretation Microscope
4. Panel:  
Pathology (88)

**G. Intended Use:**

1. Indication(s) for Use:  
The Virtuoso system provides automated digital slide creation, management, analysis, and viewing. It is intended for in vitro diagnostic use as an aid to the pathologist in the display, detection, counting, review and classification of tissues and cells of clinical interest based on particular morphology, color, intensity, size, pattern and shape.

The Virtuoso™ System for Ki67 (30-9) is for digital read and image analysis applications. This particular Virtuoso system is intended for use as an aid to the pathologist in the detection and semi-quantitative measurement of Ki67 (30-9) protein in formalin-fixed, paraffin-embedded normal and neoplastic tissue. This device is an accessory to the Ventana Medical Systems, Inc. CONFIRM™ anti-Ki67 (30-9) Rabbit Monoclonal Primary Antibody assay. The Ventana Medical Systems, Inc. CONFIRM™ anti-Ki67 (30-9) assay is indicated for use in assessing the proliferative activity of normal and neoplastic breast tissue. When used with this assay, the Virtuoso™ System for Ki67 (30-9) is indicated for use as an aid in the assessment of Ki-67 status in breast cancer patients (but is not the sole basis for treatment).

Note: The IHC Ki67 (30-9) Digital Read and Image Analysis applications are adjunctive computer-assisted methodologies for the qualified pathologist in the acquisition and measurement of images from microscope glass slides of breast cancer specimens stained for the presence of Ki67 protein. The pathologist should verify agreement with the Image Analysis software application score. The accuracy of the test results depends on 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 CONFIRM™ anti-Ki67 (30-9) Rabbit Monoclonal Primary Antibody assay to assure the validity of the Virtuoso™ System for Ki67 (30-9) Digital Read and Image Analysis scores. The actual correlation of CONFIRM™ anti-Ki67 (30-9) Rabbit Monoclonal Primary antibody assay to clinical outcome has not been established.

2. Special Conditions for Use Statement(s):  
For prescription use only  
  - \* A precautionary statement indicating that this device has not been tested, or its safety and effectiveness validated, when used with a personal computer (PC) from home was included in the Limitations section of the device package insert.

**H. Substantial Equivalence Information:**

1. Predicate Device Name(s) and 510(k) numbers:  
PATHIAM® System with iScan for p53 and Ki-67, k092333
2. Comparison with Predicate Device:

Similarities

Item	Device	Predicate K092333
Intended Use	This device is intended for in vitro diagnostic (IVD) use. The Virtuoso System provides automated digital slide creation, management, analysis, and viewing. It is intended for IVD use as an aid to the pathologist in the display, detection, counting, review and classification of tissues and cells of clinical interest based on particular morphology, color, size, intensity, pattern and shape.	This device is intended for in vitro diagnostic (IVD) use. The PATHIAM® System is intended as an aid to the pathologist to detect, count and classify cells of clinical interest based on recognition of cellular objects of particular color, size and shape using appropriate controls to assure the validity of the scores.
Sample type	Formalin-fixed, paraffin embedded tissue stained by immunohistochemical technique	Same
Device components	Automated digital slide scanner, computer, color monitor, and image analysis software and digital pathology information management software	Same

Differences

Item	Device	Predicate K092333
Primary Antibody (Assay) Reagent	Ventana CONFIRM™ Ki-67 (30-9)	Dako p53 and Dako Ki-67

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

None

**J. Performance Characteristics:**

1. Analytical Performance:

The performance of the Virtuoso™ System for Ki67 (30-9) was validated via two studies. The first study evaluated overall system performance in terms of: (1) agreement between the reference manual method (with a traditional microscope) and both the digital read (DR) and image analysis (IA) applications of the Virtuoso system, (2) intra-pathologist/inter-day reproducibility of DR and IA Virtuoso applications, and (3) inter-pathologist reproducibility of the DR and IA Virtuoso applications. These studies were conducted in 3 different sites.

In the second study, scanner precision was evaluated in an isolated fashion via a

cross-over design from the primary study. A subset of the clinical cases (n = 40) was scanned two more times with two different scanners at two separate locations. This study evaluated scanner precision of the IA application only for both inter-scanner precision and intra-scanner/inter-day precision. The IA generates an instrument-generated Ki-67 score that is not affected by memory bias as would be the case with human interpretations.

a. *Accuracy:*

This study was initially conducted in 3 sites with one pathologist at each site. There were 120 specimens included in this study. All pathologists read all the slides under the three different modes – manual, DR and IA scoring. There was a wash-out period of 7 days between reads with each different modality. The data were categorized as “negative” and “positive” using Ki-67 scoring criteria as follows: 0-10% of cells staining = Negative and >10% cells staining as Positive. The acceptance criteria of 75% or above agreement rates were set by the sponsor. Due to the inherent variability that is present in the manual method of scoring these slides, FDA requested that the sponsor perform the same study at an additional study site (site 4). This additional study site had one pathologist who read all the slides under the three different modes as above. All other study criteria were the same as above. In the study comparing DR scoring to manual read and in the study comparing IA scoring to the manual method the acceptance criteria were met in at least three sites. The percent agreements across the 4 sites with the 95% confidence intervals (CI) around the agreements are shown below.

**Agreement - Digital Read vs Manual (manual = true score)**

Confusion Matrix		Digital							
		Site 1 (n = 120)		Site 2 (n = 118)		Site 3 (n = 114)		Site 4 (n = 118)	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Manual	Neg ( $\leq 10\%$ )	37	10	43	0	46	3	59	5
	Pos ( $>10\%$ )	0	73	23	52	13	52	10	44
	% Agreement (95% CI)	92% (85% - 95%)		81% (72% - 87%)		86% (78% - 91%)		87% (80% - 92%)	
Negative % Agreement (95% CI)		79% (65% - 88%)		100% (92% - 100%)		94% (83% - 98%)		92% (83% - 97%)	
Positive % Agreement (95% CI)		100% (95% - 100%)		69% (58% - 79%)		80% (69% - 88%)		81% (69% - 90%)	

**Agreement - Image Analysis vs Manual (manual = true score)**

Confusion Matrix		Image Analysis							
		Site 1 (n = 120)		Site 2 (n = 117)		Site 3 (n = 114)		Site 4 (n = 117)	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
<b>Manual</b>	Neg (≤10%)	37	10	40	2	42	7	41	22
	Pos (>10%)	5	68	14	61	9	56	0	54
	% Agreement (95% CI)	88% (80% - 92%)		86% (79% - 91%)		86% (78% - 91%)		81% (73% - 87%)	
	Negative % Agreement (95% CI)	79% (65% - 88%)		95% (84% - 99%)		86% (73% - 93%)		65% (53% - 76%)	
	Positive % Agreement (95% CI)	93% (85% - 97%)		81% (71% - 89%)		86% (76% - 93%)		100% (93% - 100%)	

*b. Precision/Reproducibility:*

**Reproducibility**

Reproducibility of the device was assessed during slide reading sessions. A slide reading session consisted of pathologists conducting a digital read (DR) or image analysis (IA) of all 40 slides. There was a 7-day wash-out period between slide reading sessions. An agreement of  $\geq 75\%$  between each of the three reading sessions (Session 1 vs. Session 2, Session 1 vs. Session 3, and Session 2 vs. Session 3) for the Virtuoso digital read (DR) application was considered acceptable intra-pathologist/inter-day scoring performance (maximum n = 40). The same criterion was established for the Virtuoso image analysis application (IA) (maximum n = 40). Agreement was analyzed based upon the following score classification: 0 to 0.99% cells staining = Negative (0); 1 to 10% (1) and >10% (2) cells staining = Positive. The agreement rate set by the sponsor was met in each study.

**Intra-Pathologist/Inter-Day** (pair-wise comparisons, Session 1 vs. Session 2, Session 1 vs. Session 3, Session 2 vs. Session 3)

Confusion Matrix	Session 2		Session 3		Session 1	
	Neg	Pos	Neg	Pos	Neg	Pos
Session 1	2	38	1	1	1	21
Session 1	0	37	0	16	1	3
Session 2	3	34	0	17	0	27
Session 2	0	37	0	16	1	3
% Agreement (95% CI)	88%		95%		100%	
	(80% - 92%)		(87% - 100%)		(93% - 100%)	

Intra-Pathologist Image Analysis						
Confusion Matrix	Session 1		Session 2		Session 3	
	Neg	Pos	Neg	Pos	Neg	Pos
Session 1	5	3	7	1	7	1
Session 2	1	29	0	30	4	1
-- % Agreement		89%	97%	92%		
-- 95% CI		(79% - 96%)	(97% - 100%)	(79% - 97%)		

Reproducibility was also evaluated for the inter-pathologist variable, by comparing the agreement data in a pair-wise manner between Site 1 vs. Site 2, Site 1 vs. Site 3, and Site 2 vs. Site 3. This was done for both DR and IA, and used the same 75% acceptance criterion. The discrepancy in agreement in site 1 was noted to be for specimens around the cutoff value for the Ki-67 score.

- i. Inter-Pathologist (pair-wise comparisons, Pathologist 1 vs. Pathologist 2, Pathologist 1 vs. Pathologist 3, Pathologist 2 vs. Pathologist 3)

Inter-Pathologist Digital						
Confusion Matrix	Site 2		Site 3		Site 3	
	Neg	Pos	Neg	Pos	Neg	Pos
Site 1	37	35	0	30	3	55
Site 2	31	83	31	82	31	82
Site 3	46	55	54	10	4	62
-- % Agreement		74%	71%	85%		
-- 95% CI		(65% - 81%)	(62% - 78%)	(78% - 91%)		

Inter-Pathologist Image Analysis						
Confusion Matrix	Site 2		Site 3		Site 3	
	Neg	Pos	Neg	Pos	Neg	Pos
Site 1	42	37	2	36	1	63
Site 2	17	78	17	61	16	62
Site 3	24	62	48	4	4	39
-- % Agreement		84%	85%	93%		
-- 95% CI		(76% - 90%)	(78% - 91%)	(87% - 96%)		

### Scanner Precision

This study evaluated scanner precision of the image analysis application. The scanner precision study was designed to assess the performance of the Virtuoso system's scanner (iScan) in isolation from other variables. As the DR and IA images were derived from a single scanning "run," this study held other variables constant. This study utilized a randomly selected subset of 40 cases from the accuracy study. The clinical cases spanned the range of the Ki-67 scoring categories (0, 1 and 2) and the slides were stained with both universal DAB detection kits (*iVIEW* and *ultraView*). A subset of the clinical cases (n = 40) was scanned two more times on two different days with two different scanners at two separate locations. The acceptance criteria set by the sponsor of a minimum of 85% agreement rate was met in these studies.

### Inter-Scanner Agreement Rates

#### All FOVs

Image Analysis		Virtuoso Ki-67 (30-9) Results- Site 2			
Virtuoso Ki-67 (30-9) Results- Site 1		<1%	1-10%	>10%	Total
<1%	7	0	0	7	
1-10%	3	31	2	36	
>10%	0	2	74	76	
Total	10	33	76	119	
Overall Percent Agreement: 94.1% (112/119) 95% CI: (88.4% to 97.1%)					

Image Analysis		Virtuoso Ki-67 (30-9) Results- Site 3			
Virtuoso Ki-67 (30-9) Results- Site 1		<1%	1-10%	>10%	Total
<1%	7	0	0	7	
1-10%	3	32	1	36	
>10%	0	3	73	76	
Total	10	35	74	119	
Overall Percent Agreement: 94.1% (112/119) 95% CI: (88.4% to 97.1%)					

Image Analysis		Virtuoso Ki-67 (30-9) Results- Site 3			
Virtuoso Ki-67 (30-9) Results- Site 2		<1%	1-10%	>10%	Total
<1%	8	2	0	10	
1-10%	2	29	2	33	
>10%	0	4	73	77	
Total	10	35	75	120	
Overall Percent Agreement: 91.7% (110/120) 95% CI: (85.3% to 95.4%)					

Intra-Scanner/Inter-Day (Session) Agreement Rates

**All FOVs**

<b>Image Analysis</b>	<b>Virtuoso Ki-67 (30-9) Results- Session 2</b>			
<b>Virtuoso Ki-67 (30-9) Results- Session 1</b>	<1%	1-10%	>10%	Total
<1%	7	2	0	9
1-10%	0	32	0	32
>10%	0	2	75	77
Total	7	36	75	118
Overall Percent Agreement: 96.6% (114/118) 95% CI: (91.6% to 98.7%)				

<b>Image Analysis</b>	<b>Virtuoso Ki-67 (30-9) Results- Session 3</b>			
<b>Virtuoso Ki-67 (30-9) Results- Session 1</b>	<1%	1-10%	>10%	Total
<1%	6	4	0	10
1-10%	0	32	1	33
>10%	0	0	77	77
Total	6	36	78	120
Overall Percent Agreement: 95.8% (115/120) 95% CI: (90.6% to 98.2%)				

<b>Image Analysis</b>	<b>Virtuoso Ki-67 (30-9) Results- Session 3</b>			
<b>Virtuoso Ki-67 (30-9) Results- Session 2</b>	<1%	1-10%	>10%	Total
<1%	6	1	0	7
1-10%	0	33	3	36
>10%	0	0	75	75
Total	6	34	78	118
Overall Percent Agreement: 96.6% (114/118) 95% CI: (91.6% to 98.7%)				

c. *Linearity*

Not applicable

d. *Carryover*

Not applicable

e. *Interfering Substances:*

Not applicable

2. Other Supportive Instrument Performance Data Not Covered Above:

Not applicable

**K. Proposed Labeling:**

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.