

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

A. 510(k) Number:

k111872

B. Purpose for Submission:

New device

C. Manufacturer and Instrument Name:

Ventana Medical Systems, Inc., Virtuoso™ System for IHC p53 (DO-7)

D. Type of Test or Tests Performed:

Computer-assisted image analysis (IA) scoring and manual scoring of digital images (DR) of p53 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.

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 aid the qualified pathologist in the review of immunohistochemically stained histologic slides. It 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 specimen slides. Using the Virtuoso software, the pathologist can view digital images, add annotations, make measurements, perform image analysis, and generate reports. 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. Results are reported by a qualified pathologist as semi-quantitative scores. The software makes no independent interpretations of the data and requires competent human intervention for all steps in the analysis process.

- a. The Virtuoso™ System for IHC p53 (DO-7) consists of a slide scanner (iScan Coreo Au), computer, monitor, keyboard, mouse, image analysis algorithms for IHC p53 (DO-7) and software with a Windows web browser-based user

interface. The Digital Read option allows the pathologist to score the p53 stained slide digital image on a computer monitor. In the Image Analysis Application option, slides are scored by the p53 image analysis application and presented on the computer screen. The pathologist verifies this score and confirms it.

2. Principles of Operation:

The Virtuoso™ System for p53 (DO-7) employs image analysis and pre-defined parameters to obtain p53 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 identifies the epithelial area which is the area where there is a possibility of epithelial cells being present. This area is used in the subsequent analysis 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:** 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. Based on this score, test results are classified as negative ($\leq 10\%$ of cells staining) or positive ($> 10\%$ cells staining).

3. Modes of Operation:

- a. Manual scoring of immunohistochemically (IHC) p53 stained slide images on a computer monitor (digital read).
- b. Computer scoring of IHC p53 stained slide images performed by p53 (DO-7) 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:

Breast cancer tissue section slides that are stained with the anti-p53 antibody IHC stain on the Benchmark XT IHC stainer using either the *iView* or the *UltraView* detection system are analyzed on this system. p53 IHC stained slides are manually loaded on to the iScan Coreo Au 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 Au 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:
The slides selected for analysis on this device must have optimum IHC staining quality such as optimal staining intensity, minimal to no background staining, etc. Slides with sub optimal staining should be restained.

Quality control is performed on slide images by the operator. Slides with sub-optimal images (out of focus images, etc.) should be rescanned. The image quality assessment module also performs an image quality assessment of the areas in the digital images marked by the pathologist for analysis. Measures of color, contrast and sharpness of images are used to report the quality of the images as good, average or poor to the user. Analysis of the images is performed regardless of the image quality. Images with poor quality can be automatically rejected by the pathologist through customer-specific settings. In all cases, the pathologist is provided with feedback from the quality algorithm, and if the pathologist determines that the results of the analysis are reasonable, he/she can accept it or reject it. If rejected, alternate areas on the slide or a new slide may be selected for analysis.

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 p53 (DO-7) 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 p53 (DO-7) protein in formalin-fixed, paraffin-embedded normal and neoplastic tissue. This device is an accessory to the Ventana Medical Systems, Inc. CONFIRM™ anti-p53 (DO-7) Mouse Monoclonal Primary Antibody assay. The Ventana Medical Systems, Inc. CONFIRM™ anti- p53 (DO-7) assay is indicated for use in assessment of p53 protein where mutations have been linked to tumor proliferation. When used with this assay, the Virtuoso™ System for p53 (DO-7) is indicated for use as an aid in the assessment of p53 status in breast cancer patients (but is not the sole basis for treatment).

Note: The IHC p53 (DO-7) 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 p53 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- p53 (DO-7) Mouse Monoclonal Primary Antibody assay to assure the validity of the Virtuoso™ System for p53 (DO-7) Digital Read and Image Analysis scores. The actual correlation of CONFIRM™ anti- p53 (DO-7) Mouse 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™ p53 (DO-7)	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 p53 (DO-7) 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 p53 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. Slides were excluded from analysis for the following reasons: out of focus image, staining artifacts and scant or no invasive carcinoma. 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 p53 scoring criteria as follows: $\leq 10\%$ of cells staining as 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)

		Digital Read							
		Site 1 (n = 119)		Site 2 (n = 119)		Site 3 (n = 117)		Site 4 (n = 114)	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Manual Read	Neg ($\leq 10\%$)	73	0	82	6	71	4	56	20
	Pos ($> 10\%$)	8	38	0	31	3	39	1	37
	% Agreement (95% CI)	93% (87% - 97%)		95% (89% - 98%)		94% (88% - 97%)		82% (73% - 88%)	
Negative % Agreement (95% CI)		100% (95% - 100%)		93% (86% - 97%)		95% (87% - 98%)		74% (63% - 82%)	
Positive % Agreement (95% CI)		83% (69% - 91%)		100% (89% - 100%)		93% (81% - 98%)		97% (87% - 100%)	

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

		Image Analysis							
		Site 1 (n = 119)		Site 2 (n = 119)		Site 3 (n = 117)		Site 4 (n = 105)	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Manual Read	Neg ($\leq 10\%$)	72	1	84	4	71	4	61	6
	Pos ($> 10\%$)	9	37	0	31	7	35	4	34
	% Agreement (95% CI)	92% (85% - 95%)		97% (92% - 99%)		91% (84% - 95%)		90% (83% - 95%)	
Negative % Agreement (95% CI)		99% (93% - 100%)		95% (89% - 98%)		95% (87% - 98%)		91% (82% - 96%)	
Positive % Agreement (95% CI)		80% (67% - 89%)		100% (89% - 100%)		83% (69% - 92%)		89% (76% - 96%)	

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 set by the sponsor for the intra-pathologist/inter-day scoring performance. The same criterion was established for the Virtuoso image analysis application (IA). Agreement was analyzed based upon the following score classification: $\leq 10\%$ of cells staining = Negative and $> 10\%$ cells staining as Positive. The agreement rate set by the sponsor was met in each study.

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

p53 Intra-Pathologist Digital								
			Session 2		Session 3		Session 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			26	14	25	15	25	15
Session 1	Neg	27	26	1	25	2		
	Pos	13	0	13	0	13		
Session 2	Neg	26					24	2
	Pos	14					1	13
% Agreement			98%		95%		93%	
(95% CI)			(87% - 100%)		(83% - 99%)		(80% - 97%)	

p53 Intra-Pathologist Image Analysis								
			Session 2		Session 3		Session 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			26	14	25	15	25	15
Session 1	Neg	25	24	1	24	1		
	Pos	15	2	13	1	14		
Session 2	Neg	26					25	1
	Pos	14					0	14
% Agreement			93%		95%		98%	
(95% CI)			(80% - 97%)		(83% - 99%)		(87% - 100%)	

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.

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

Inter-Pathologist Digital								
			Site 2		Site 3		Site 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			82	37	75	43	75	43
Site 1	Neg	81	81	0	74	6		
	Pos	38	1	37	1	37		
Site 2	Neg	82					75	6
	Pos	37					0	37
% Agreement			99%		94%		95%	
(95% CI)			(95% - 100%)		(88% - 97%)		(89% - 98%)	

Inter-Pathologist Image Analysis								
			Site 2		Site 3		Site 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			84	35	79	39	79	39
Site 1	Neg	81	81	0	76	4		
	Pos	38	3	35	3	35		
Site 2	Neg	84					79	4
	Pos	35					0	35
% Agreement			97%		94%		97%	
(95% CI)			(93% - 99%)		(88% - 97%)		(92% - 99%)	

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. This study utilized a randomly selected subset of 40 cases from the accuracy study. The study cases included the p53 staining of 0-0.99%, 1-10%, and >10% of cells staining positive. The slides were stained with both universal DAB detection kits (*iVIEW* and *ultraView*). The same three FOV's were scanned and analyzed each time by the IA application. These 40 cases were scanned two more times on two different days with two different scanners at two separate locations to assess intra-scanner/inter-day precision. The acceptance criterion 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 p53 (DO-7) Results- Site 2			
Virtuoso p53 (DO-7) Results- Site 1	<1%	1-10%	>10%	Total
<1%	28	2	0	30
1-10%	2	40	1	43
>10%	0	1	46	47
Total	30	43	47	120
Overall Percent Agreement: 95.0% (114/120) 95% CI: (89.5% to 97.7%)				

Image Analysis	Virtuoso p53 (DO-7) Results- Site 3			
Virtuoso p53 (DO-7) Results- Site 1	<1%	1-10%	>10%	Total
<1%	30	0	0	30
1-10%	7	36	0	43
>10%	0	2	45	47
Total	37	38	45	120
Overall Percent Agreement: 92.5% (111/120) 95% CI: (86.4% to 96.1%)				

Image Analysis	Virtuoso p53 (DO-7) Results- Site 3			
Virtuoso p53 (DO-7) Results- Site 2	<1%	1-10%	>10%	Total
<1%	30	0	0	30
1-10%	7	36	0	43
>10%	0	2	45	47
Total	37	38	45	120
Overall Percent Agreement: 92.5% (111/120) 95% CI: (86.4% to 96.1%)				

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

All FOVs

Image Analysis	Virtuoso p53 (DO-7) Results- Session 2			
Virtuoso p53 (DO-7) Results- Session 1	<1%	1-10%	>10%	Total
<1%	27	3	0	30
1-10%	1	41	1	43
>10%	0	0	47	47
Total	28	44	48	120
Overall Percent Agreement: 95.6% (115/120) 95% CI: (90.6% to 98.2%)				

Image Analysis	Virtuoso p53 (DO-7) Results- Session 3			
Virtuoso p53 (DO-7) Results- Session 1	<1%	1-10%	>10%	Total
<1%	27	3	0	30
1-10%	2	39	2	43
>10%	0	0	47	47
Total	29	42	49	120
Overall Percent Agreement: 94.2% (113/120) 95% CI: (88.4% to 97.1%)				

Image Analysis	Virtuoso p53 (DO-7) Results- Session 3			
Virtuoso p53 (DO-7) Results- Session 2	<1%	1-10%	>10%	Total
<1%	26	2	0	28
1-10%	3	40	1	44
>10%	0	0	48	48
Total	29	42	49	120
Overall Percent Agreement: 95.0% (114/120) 95% CI: (89.5% to 97.7%)				

- 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.