Ki-67 (30-9) Image Analysis Software User Manual Version 5.1

Ventana Medical Systems, Inc.
www.ventana.com

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Ki-67 (30-9) Image Analysis Software User Manual

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Open Source and Commercial Software

Refer to the Virtuoso Reference Guide for information on Open Source and Commercial Software programs.

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About the Ki-67 (30-9) Image Analysis Software

Companion Algorithm Image Analysis Software

Welcome to the Ki-67 (30-9) Image Analysis Software User Manual.

Who Should Read this Manual

System Administrators should read this user manual and use it for reference while operating the VENTANA Virtuoso software.

Introduction

The Companion Algorithm Ki-67 (30-9) Image Analysis Software assists the pathologist in the semi-quantitative measurement of Ki-67 in tissues stained with Ventana Medical Systems, Inc. CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody (CONFIRM Ki-67 (30-9)). This application generates an Ki-67 score that can be reviewed and accepted by the pathologist, or if necessary, overridden by the pathologist. The image analysis application is an assist to the pathologist in the scoring and interpretation of Ki-67 staining on breast cancer tissues.

The Virtuoso Ki-67 (30-9) Digital Read Application allows the pathologist to view Ki-67 stained slides as images on a computer monitor, similar to what can be viewed under a microscope. While reviewing the image, the pathologist may change magnification and move freely about the image.

Related Document

For additional information on the Virtuoso software, see the following document:

- Virtuoso Reference Guide (PL-000091-US)

Technical Support

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Connection Requirements

Refer to the Virtuoso Reference Guide for information on connection requirements.

Cyber Security

Any device that is connected to a network (internally or externally) has the potential to be compromised by unauthorized access or viruses. As with most devices, the software is designed to run on a computer utilizing Microsoft Windows and virus protection software which requires the validation and implementation of the appropriate patches.

Some of the potential cyber security hazards are:

- Malicious software that alters the device software (such as viruses)
- Unauthorized access to the system that could compromise data safety
- Security of data transmitted over the internet

Cyber security involves protecting data by preventing, detecting, and responding to malicious cyber attacks. Cyber attacks could involve computer viruses which can completely erase data or hackers who alter files or even use the device as a host to attack other devices. As serious as these hazards are, steps can be taken to maximize cyber security.

User Authorization

All software users must login with a valid user name and password. The user name and password are securely transmitted in encrypted form over the Internet or Intranet. Once a user has logged in, the user remains active in the application until the user explicitly logs out, closes the browser, or because the application closes after a period of inactivity.

Securing Networks and Servers

Network security consists of the provisions made in the computer network infrastructure, policies adopted by the network administrator to protect the network, and the network resources that prevent unauthorized access.

The following are critical steps for securing a network server:

- Physical security (servers and network infrastructure behind locked doors)
- Use of robust passwords
- System and data backups (at regular intervals)
- Data protection
- Terminating unused services
- Restricting access to used services

The following are critical steps and methodologies used to secure network and servers:

- Data protection
- Data backups (at regular intervals)
- Refusal of automatic updates from off-the-shelf software
- Antivirus software for computers and servers

Protecting Data

Establishment of a network firewall and protection of the network against viruses using anti-virus software are effective methods to protect data. Virus definitions should be kept up to date and regular scans of computers for spyware should be performed using a legitimate anti-spyware application. If viruses or spyware are found, remove them immediately.

Evaluate Your Software Settings

The default settings of most software enable all available functionality. However, hackers may be able to take advantage of this functionality to access devices. It is especially important to check the settings for software that connects to the internet (browsers, email clients, etc.). Apply the highest level of security available that still provides needed functionality.

Backup and Recovery

In order to develop a successful backup and recovery plan, comprehension of data accessibility needs and the potential impact of data loss is essential. Automatic backup procedures need to be adopted using a data backup utility.

Chapter 1: Intended Use and Indications for Use

This chapter shows comparison and reproducibility studies for the Ki-67 marker.

Intended Use and Indications 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 Ki-67 (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 Ki-67 (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-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody assay. The Ventana Medical Systems, Inc. CONFIRM anti-Ki-67 (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 Ki-67 (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: **Note: The IHC Ki-67 (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 Ki-67 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-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody assay to assure the validity of the Virtuoso System for Ki-67 (30-9) Digital Read and Image Analysis scores. The actual correlation of CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary antibody assay to clinical outcome has not been established.**

Summary and Explanation

The Virtuoso system with the Ki-67 (30-9) algorithm is a software system designed to assist the qualified pathologist in the consistent quantitative assessment of protein expression in immunohistochemically (IHC) stained histologic sections from formalin-fixed, paraffin-embedded (FFPE) normal and neoplastic tissues. The Virtuoso system can be used for review of digitized images of histologic sections with image analysis algorithms (Companion Algorithm Image Analysis applications), or without image analysis algorithms (Virtuoso Digital Read applications).

Digital Read applications present images on the computer screen in the same manner as one would see with a manual microscope, inclusive of the pathologist's ability to select any areas of interest and the option of various magnification levels. For the Companion Algorithm Image Analysis applications, the pathologist may use the system software to select and outline one or several field of views (FOVs), and each FOV may be viewed at various magnifications and then analyzed by the software; a count of the total number of target cells and the number interpreted by the algorithm as positive and negative is generated. The pathologist can accept the score provided by the algorithm, or may override the score with a pathologist score. The system requires competent human intervention at all steps in the analysis process, and the software makes no independent interpretations of the data.

The Virtuoso system consists of image analysis algorithms and software with a user interface. Virtuoso is an end-to-end digital pathology software solution that allows pathology laboratories to acquire, manage, view, analyze, share, and report on digital images of pathology specimens. Using the Virtuoso software, the pathologist can view digital images at various magnifications (as previously stated), add annotations, make measurements, perform image analysis, and generate reports.

Test Principles

The Virtuoso system for with the Ki-67 (30-9) image analysis algorithm employs image analysis techniques to obtain Ki-67 scoring. Pre-defined parameters are used to obtain Ki-67 scores. The identification of the nucleus is carried out automatically by the image analysis algorithms. The steps involved in the analysis algorithms are:

1. Enhancing the image. This process increases the contrast to make the image more suitable for analysis.
2. Identifying the epithelial area. The epithelial area is the region of the image where there is the possibility of epithelial cells being present.
3. Identifying the nucleus.
4. Classifying the cells based on extent, intensity, and thickness of nuclear staining.
5. Computing the score.

Warnings and Precautions

For *in vitro* diagnostic (IVD) use.

It is important that glass slides with acceptable staining quality be used.

Pre-Analytical Variables

Tissue preparation and staining should follow the recommendations provided in the CONFIRM Ki-67 (30-9) package insert. For optimal image capture using the Virtuoso system, it is recommended that the tissue be free of folds and be placed on the slide with a minimum of 2 mm boundary from the edge on all sides. The cover slip and slide label (if present) should not overhang the edges of the slide. For further information on scanning, please refer to the appropriate iScan Reference Guide.

Procedure

Refer to the Virtuoso Reference Guide.

Required Materials Not Provided

The Virtuoso system with the Ki-67 (30-9) image analysis algorithm requires use of the CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody, and any additional material or supplies listed in the package insert, to stain tissues prior to analysis. The iScan Coreo scanner is required for scanning of the slides.

Results

The Virtuoso system with the Ki-67 (30-9) image analysis algorithm produces a staining score. The pathologist views the image and the instrument score, makes an assessment, and reports a score which may not be the same as the instrument score. Refer to the Virtuoso Reference Guide for an example of a report.

Limitations

The algorithms are designed to work for CONFIRM Ki-67 (30-9) cell nuclei staining. The test results are only as good as the quality and accuracy of the immunohistochemistry slide that is imaged, and the subsequent image that is analyzed. The pathologist must validate the CONFIRM Ki-67 (30-9) staining run by manual microscopic examination of the Ki-67 control slides to verify that the expected results have been obtained before images from patient slides are acquired by the Virtuoso software. The pathologist must follow the manufacturer's recommendations for the CONFIRM Ki-67 (30-9) including using all the positive and negative quality control materials for each staining run. If the control slides are not acceptable with manual microscopic examination, the patient tissues need to be re-stained with acceptable results. (See the CONFIRM Ki-67 (30-9 package insert for details about quality control recommendations.) The pathologist must follow the recommendations for surveying the entire breast cancer specimen to assess any heterogeneity in the CONFIRM Ki-67 (30-9) staining, the degree of background staining, cytoplasmic staining, edge effect, etc. as recommended in the Ki-67 user manual (available at www.ventana.com). If the images captured have different staining (nuclear, cytoplasm, etc.), incorrect results will be generated. Incorrect results will also be generated if the image quality cannot be analyzed. The software algorithms determine whether the quality of an image can be analyzed, based on pre-defined parameters. Refer to the Virtuoso Reference Guide for more information.

The Ki-67 (30-9) image analysis algorithm will reject nuclei that are elongated regardless of the overall shape of the cell. For this reason, tumors containing large numbers of cells with elongated nuclei may need to be evaluated manually. In addition, performance of the Virtuoso system with the following types of breast cancers has not been evaluated: carcinoma in situ, carcinosarcoma, comedo carcinoma, cystosarcoma phylloides, medullary carcinoma of the breast, mucinous variants of breast cancer, and spindle cell carcinoma.

This device has not been tested, or its safety and effectiveness validated, when used with a personal computer (PC) from home.

According to the 1988 Clinical Laboratory Improvement Amendments (CLIA '88), each laboratory that introduces an FDA cleared system must demonstrate that it can obtain performance specifications comparable to those established by the manufacturer. Please see “Performance Characteristics” below to review those specifications.

As with any change in diagnostic methodology, and especially one that relies on visual interpretation of complex images, a transition from conventional microscopy to digital microscopy presents the possibility of unintended, but systematic changes in diagnostic performance. Users should be aware that their IHC categorizations may be biased when switching from conventional to digital microscopy and as such, training beyond self study should be undertaken as needed to assure concordance before clinical adoption of the device. The laboratory is responsible for ensuring that concordance goals are reached and maintained.

Performance Characteristics

Performance of the staining agent is described in the CONFIRM Ki-67 (30-9) package insert. See “Chapter 2: Ki-67 Comparison and Reproducibility Studies” on page 7. for a description of the performance of the software.

Assay Cutoff

Clinical cutoffs used for the assessment of Ki-67 varies between laboratories. The performance of the Virtuoso system with Ki-67 (30-9) image analysis algorithm was evaluated at these commonly used clinical cutoffs: ≤10% was considered a negative test result and >10% were considered positive test results.

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Chapter 2: Ki-67 Comparison and Reproducibility Studies

This chapter shows comparison and reproducibility studies for the Ki-67 marker. For these studies, scanning was performed using the iScan Coreo scanner. Scanner settings were as follows: Magnification: 20x, Focus Approach: Routine Scan, Scan Approach: Regular, AOI Detect Approach: Standard.

Ki-67 Marker Studies

Staining Procedure

Refer to the CONFIRM Ki-67 (30-9) package insert for the Benchmark XT and BenchMark ULTRA instruments, *ultra*View, and *i*VIEW detection.

Performance with BenchMark XT Stainer

Study Devices and Samples

The Ki-67 comparison and reproducibility studies for the Virtuoso Digital Read and Companion Algorithm Image Analysis consisted of 120 de-identified archived breast carcinoma sections immunohistochemically stained with CONFIRM Ki-67 (30-9) on the BenchMark XT stainer. Study test samples covered the ranges of ≤10% and >10% were interpreted at four different sites by four different pathologists. All test slides were scanned at 20X magnification and all images were output in bif file format.

The table below shows the concordance results for CONFIRM Ki-67 (30-9) staining interpretation among four different sites:.

1. Digital Read vs Manual Method.

Table 2-1. Agreement - Digital Read vs Manual (manual = true score).

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

2. Image Analysis vs Manual Method.

Table 2-2. Agreement - Image Analysis vs Manual (manual = true score).

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

Intra-System and Inter-System Studies

The study was designed to demonstrate inter- and intra-Virtuoso system reproducibility for Virtuoso Digital Read and Companion Algorithm Image Analysis applications at three sites. For the intra-system study only, a designated subset of 40 cases that span the range of the Ki-67 scoring categories (≤10%, negative and >10% positive) were used.

1. Intra-pathologist/Inter-Day (pair-wise comparisons, Session1 vs Session 2, Session1 vs Session 3, Session 2 vs Session 3).

Table 2-3. Intra- Pathologist Digital Read.

Confusion Matrix			Intra-Pathologist Digital					
			Session 2		Session 3		Session 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			9	31	7	31	7	31
Session 1	Neg	14	9	5	7	5		
	Pos	26	0	26	0	26		
Session 2	Neg	9					6	2
	Pos	31					1	29
% Agreement			88%		87%		92%	
(95% CI)			(74% - 95%)		(73% - 94%)		(79% - 97%)	

2. For Intra-Pathologist Image Analysis.

Table 2-4. Intra-Pathologist Image Analysis.

Confusion Matrix			Intra-Pathologist Image Analysis					
			Session2		Session 3		Session 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			17	21	17	21	17	21
Session 1	Neg	19	17	2	17	2		
	Pos	19	0	19	0	19		
Session 2	Neg	17					17	0
	Pos	21					0	21
% Agreement			95%		95%		100%	
(95% CI)			(83% - 99%)		(83% - 99%)		(91% - 100%)	

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

Table 2-5. Inter-Pathologist Digital Read.

Confusion Matrix			Inter-Pathologist Digital					
			Site 2		Site 3		Site 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			66	52	61	55	61	55
Site 1	Neg	37	35	0	30	3		
	Pos	83	31	52	31	52		
Site 2	Neg	66					54	10
	Pos	52					7	45
% Agreement			74%		71%		85%	
(95% CI)			(65% - 81%)		(62% - 78%)		(78% - 91%)	

4. Inter-Pathologist Image Analysis.

Table 2-6. Inter-Pathologist Image Analysis.

Confusion Matrix			Inter-Pathologist Image Analysis					
			Site 2		Site 3		Site 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			54	63	52	63	52	63
Site 1	Neg	42	37	2	36	1		
	Pos	78	17	61	16	62		
Site 2	Neg	54					48	4
	Pos	63					4	59
% Agreement			84%		85%		93%	
(95% CI)			(76% - 89%)		(78% - 91%)		(87% - 96%)	

Scanner Precision Studies

Forty (40) cases representing the useful categories of 0-0.99%, 1-10%, and >10% positive staining for Ki-67 were scanned on three different scanners at three different sites to assess inter-scanner precision, and the same three FOVs (total = 120) were captured and evaluated each time by the image analysis application. Limiting the study to image analysis only ensured that only scanner precision was under evaluation, as all other factors were kept constant. Similarly, these same 40 cases and three FOVs per case were scanned on three different days by the same scanner to assess intra-scanner/inter-day precision.

Pairwise comparisons were performed between each of the three sites (inter-scanner), and between each of the three days (intra-scanner). The precision tables are found below.

Inter-Scanner

Table 2-7. Inter-Scanner Site 1 vs Site 2.

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% - 97.1%)				

Table 2-8. Inter-Scanner Site 1 vs Site 3.

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%)				

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Table 2-9. Inter-Scanner Site 2 vs Site 3.

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%)				

Overall inter-scanner percent agreements ranged from 91.7% to 94.1% for all FOVs combined.

Table 2-10. Intra-Scanner/Inter-Day.

Image Analysis	Virtuoso Ki-67 (30-9) Results- Session 2			
Virtuoso Ki-67 (30-9) Results- Session 1	<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%)				

Table 2-11. Intra-Scanner/Inter-Day.

Image Analysis	Virtuoso Ki-67 (30-9) Results- Session 3			
Virtuoso Ki-67 (30-9) Results- Session 1	<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%)				

Table 2-12. Intra-Scanner/Inter-Day.

Image Analysis	Virtuoso Ki-67 (30-9) Results- Session 3			
Virtuoso Ki-67 (30-9) Results- Session 2	<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%)				

Overall intra-scanner/inter-day percent agreements ranged 95.8% to 96.6% for all FOVs combined.

The precision also underwent analysis for percent coefficient of variation (%CV); this could be achieved as the system provides a quantitative output. The results from the inter-scanner percent %CV analyses are presented in the table below. The %CVs were derived for each source of variability as the standard deviation (SD) for that source, divided by the mean, multiplied by 100%. Across all 359 evaluable observations, the mean percent positivity for Ki-67 was 30.75%. The %CV for “sites” measures precision of the site-to-site scanning and, at 4.63%, demonstrates that scanning results were reproducible between sites. The %CV for “case” represents between-case biological variability, and the %CV for the residual term represents within-case, between-field biological variability, and as such, these sources of variability are outside the scope of scanner performance.

Table 2-13. Inter-Scanner %CV Analyses.

Parameter	Statistic	All FOVs
Percent Positivity (%)	N	359
	Mean	30.750
	Site (Scanner) SD	1.425
	Site (Scanner) %CV	4.63
	Case SD	27.857
	Case %CV	90.59
	Residual SD	11.702
	Residual %CV	38.05

Results of the intra-scanner/ inter-day %CV analyses are presented in the table below. Here, the %CV for "day" is shown to be 0.00%. The software that is used to estimate the variance components in the random effects model can obtain slightly negative estimates. Since it is impossible for a variance to be negative, the model sets the variance component to zero in those cases. Thus, a %CV of 0 should not be interpreted as a complete absence of variability for that particular source, but rather as variability that is negligible in magnitude. Thus, for the one site that repeated measurements on multiple days, reproducibility between days was shown to be extremely high. As before, the %CVs for "case" and for the residual term reflects the between-case and between-field biological heterogeneity, factors that are outside the scope of scanner performance.

Table 2-14. Intra-Scanner, Inter-Day %CV Analyses.

Parameter	Statistic	All FOVs
Percent Positivity (%)	N	358
	Mean	29.111
	Day SD	0.000
	Day %CV	0.00
	Case SD	27.932
	Case %CV	95.95
	Residual SD	8.354
	Residual %CV	28.70

Performance with BenchMark ULTRA

Digital Read

The Virtuoso System for IHC Ki-67 (30-9) with the BenchMark ULTRA stainer was clinically validated for digital reading (DR) via a concordance study where 120 cases were evaluated by the manual and DR methods by one pathologist at one site. Each case was scored manually with a routine microscope and as a digital image. The manual score (reference result) was compared to the digital read result.

The data were evaluated as positive or negative for Ki-67 status using 0% to 10% as negative status, and >10% as positive status.

Table 2-15. Digital Read vs Manual (manual = true score) (BenchMark ULTRA stainer). Negative= 0-10%; Positive= >10%

Digital Read	Manual Microscopic Read		
	Positive	Negative	Total
Positive	52	12	64
Negative	0	56	56
Total	52	68	120
Positive Percent Agreement (PPA) n/N (%) (95% CI)	52/52 (100.0%) (93.1-100)		
Negative Percent Agreement (NPA) n/N (%) (95% CI)	56/68 (82.4%) (71.6-89.6)		
Overall Percent Agreement (OPA) n/N (%) (95% CI)	108/120 (90.0%) (83.3-94.2)		

Image Analysis

The Virtuoso System for IHC Ki-67 (30-9) with the BenchMark ULTRA stainer was clinically validated for image analysis (IA) via a concordance study where 120 cases were evaluated by the manual and IA methods by three pathologists at three sites. Each case was scored manually with a routine microscope and by using the Virtuoso IA application. The manual score (reference result) was compared to the IA result.

The data were evaluated as positive or negative for Ki-67 status using 0% to 10% as negative status, and >10% as positive status.

Table 2-16. Agreement: Site 1 Image Analysis vs Manual (manual = true score) (BenchMark ULTRA stainer) Negative= 0-10%; Positive= >10%

Image Analysis	Manual Microscopic Read		
	Positive	Negative	Total
Positive	52	1	53
Negative	25	40	65
Total	77	41	118
Positive Percent Agreement (PPA) n/N (%) (95% CI)	52/77 (67.5%) (56.5-76.9)		
Negative Percent Agreement (NPA) n/N (%) (95% CI)	40/41 (97.6%) (87.4-99.6)		
Overall Percent Agreement (OPA) n/N (%) (95% CI)	92/118 (78.0%) (69.7-84.5)		

Table 2-17. Agreement: Site 2 Image Analysis vs Manual (manual = true score) (BenchMark ULTRA stainer). Negative= 0-10%; Positive= >10%

Image Analysis	Manual Microscopic Read		
	Positive	Negative	Total
Positive: >10%	54	2	56
Negative: 0-10%	13	43	56
Total	67	45	112
Positive Percent Agreement (PPA) n/N (%) (95% CI)	54/67 (80.6%) (69.6-88.3)		
Negative Percent Agreement (NPA) n/N (%) (95% CI)	43/45 (95.6%) (85.2-98.8)		
Overall Percent Agreement (OPA) n/N (%) (95% CI)	97/112 (86.6%) (79.1-91.7)		

Table 2-18. Agreement: Site 3 Image Analysis vs Manual (manual = true score) (BenchMark ULTRA stainer). Negative= 0-10%; Positive= >10%

Image Analysis	Manual Microscopic Read		
	Positive	Negative	Total
Positive: >10%	59	1	60
Negative: 0 – 10%	10	49	59
Total	69	50	119
Positive Percent Agreement (PPA) n/N (%) (95% CI)	59/69 (85.5%) (75.3-91.9)		
Negative Percent Agreement (NPA) n/N (%) (95% CI)	49/50 (98.0%) (89.5-99.6)		
Overall Percent Agreement (OPA) n/N (%) (95% CI)	108/119 (90.8%) (84.2-94.8)		

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Appendix A: Reagents (Antibody) Package Inserts

Reagents (Antibody) Package Inserts

Refer to the www.ventana.com website or contact Ventana Medical Systems, Inc. at (520) 887-2155 or 1-800-227-2155 (US) to obtain the CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody package insert.

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