Estrogen Receptor (SP1) Image Analysis Software
User Manual
Version 5.1

Ventana Medical Systems, Inc.
www.ventana.com

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Estrogen Receptor (SP1) Image Analysis Software User Manual

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 Estrogen Receptor (SP1) Image Analysis Software

Companion Algorithm Image Analysis Software

Welcome to the Estrogen Receptor (SP1) 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 Estrogen Receptor (SP1) Image Analysis Software assists the pathologist in the semi-quantitative measurement of ER in tissues stained with Ventana Medical Systems, Inc. CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody (CONFIRM anti-ER (SP1) assay). This application generates an ER 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 ER staining on breast cancer tissues.

The Virtuoso ER (SP1) Digital Read Application allows the pathologist to view ER 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 ER 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 IHC ER (SP1) 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 estrogen receptor (ER) protein in formalin-fixed, paraffin-embedded neoplastic tissue. This device is an accessory to the CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody Assay. The CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody is indicated for use as an aid in the assessment of breast cancer patients for whom endocrine treatment is being considered (but is not the sole basis for treatment).

Note: The IHC ER (SP1) 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 ER 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- Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody assay used to assure the validity of the Virtuoso System for IHC ER Digital Read and Image Analysis scores. The actual correlation of CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Antibody to clinical outcome has not been established. This device is intended for IHC slides stained on the BenchMark XT and BenchMark ULTRA stainers. For prescription use only.

Summary and Explanation

The Virtuoso software with the ER (SP1) 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) neoplastic tissues. The Virtuoso software 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 must validate the CONFIRM anti-ER (SP1) assay staining run by examination of the ER control images to verify that the expected staining results have been obtained before images from patient slides are analyzed. 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 software consists of image analysis algorithms and software with a user interface. VENTANA Virtuoso software 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 software with the ER (SP1) image analysis algorithm employs image analysis techniques to obtain ER scoring. Pre-defined parameters are used to obtain ER 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 anti-ER (SP1) assay package insert. For optimal image capture using the Virtuoso software, 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 slide scanner reference guide.

Procedure

Refer to Virtuoso Reference Guide.

Required Materials Not Provided

The Virtuoso software with the ER (SP1) image analysis algorithm requires use of the CONFIRM anti-ER (SP1) assay, and any additional material or supplies listed in the CONFIRM anti-ER (SP1) assay package insert, to stain tissues prior to analysis. The iScan Coreo scanner is required for scanning of the slides.

Results

The Virtuoso software with the ER (SP1) 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 image analysis software score. Refer to the Virtuoso Reference Guide for an example of a report.

Limitations

The algorithms are designed to work for CONFIRM anti-ER (SP1) assay 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 anti-ER (SP1) assay staining run by examination of the ER control images to verify that the expected results have been obtained before images from patient slides are acquired by the Virtuoso program. The pathologist must follow the manufacturer's recommendations for the CONFIRM anti-ER (SP1) assay 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 anti-ER (SP1) assay package insert for details about quality control recommendations.) The pathologist must follow the CONFIRM anti-ER (SP1) assay recommendations for surveying the entire breast cancer specimen to assess any heterogeneity in the CONFIRM anti-ER (SP1) assay monoclonal antibody staining, the degree of background staining, cytoplasmic staining, edge effect, etc. as recommended in the CONFIRM anti-ER (SP1) assay package insert (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 ER (SP1) 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 software 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 mobile hand-held devices and 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 anti-ER (SP1) assay package insert. See “Chapter 2: Estrogen Receptor Comparison and Reproducibility Studies” on page 7. for a description of the performance of the software.

Assay Cutoff

The performance of the Virtuoso software with the ER (SP1) image analysis algorithm was evaluated at the CAP/ASCO recommended clinical cutoffs: 0-0.99% was considered a negative test result and ≥1% were considered positive test results.

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Chapter 2: Estrogen Receptor Comparison and Reproducibility Studies

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

ER Marker Studies

Staining Procedure

Refer to the CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody package insert for the BenchMark XT and ULTRA instruments, *ultra*View, and *i*VIEW detection.

Performance with BenchMark XT Instrument

Study Devices and Samples

The ER (SP1) comparison and reproducibility studies for the Virtuoso Digital Read and Companion Algorithm Image Analysis software consisted of 120 de-identified archived breast carcinoma sections immunohistochemically stained with CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody on the BenchMark XT stainer. Study test samples covered the ranges of 0-0.99%, 1-10% and >10%, and were interpreted at three different sites by three 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 anti-ER (SP1) assay staining interpretation among three different sites:

1. Digital Read vs Manual Method.

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

		Digital					
		Site 1		Site 2		Site 3	
		(n = 114)		(n = 116)		(n = 114)	
		Neg	Pos	Neg	Pos	Neg	Pos
Manual	Neg	52	7	49	5	45	11
	Pos	0	55	2	60	2	56
Total % Agreement (95% CI)		94% (88% - 97%)		94% (88% - 97%)		89% (81% - 93%)	
Negative % Agreement (95% CI)		88% (77% - 94%)		91% (80% -96%)		80% (68% - 89%)	
Positive % Agreement (95% CI)		100% (93% - 100%)		97% (89% - 99%)		97% (88% - 99%)	

2. Image Analysis vs Manual Method.

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

		Image Analysis					
		Site 1		Site 2		Site 3	
		(n = 113)		(n = 118)		(n = 116)	
		Neg	Pos	Neg	Pos	Neg	Pos
Manual	Neg	56	2	56	0	52	3
	Pos	4	51	7	55	7	54
Total % Agreement (95% CI)		95% (89% - 98%)		94% (88% - 97%)		91% (85% - 95%)	
Negative % Agreement (95% CI)		97% (88% - 99%)		100% (94% - 100%)		95% (85% - 98%)	
Positive % Agreement (95% CI)		93% (83% - 97%)		89% (78% - 94%)		89% (78% - 94%)	

Intra-System and Inter-System Studies

The study was designed to demonstrate inter- and intra-Virtuoso software reproducibility for Virtuoso Digital Read and Virtuoso Image Analysis applications. A designated subset of 40 cases that span the range of the ER scoring categories (0-0.99%, negative, 1-10%, positive, and >10%, positive) were used.

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

Table 2-3. Intra-Pathologist Digital Read.

			Intra-Pathologist Digital					
			Session 2		Session 3		Session 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			18	20	17	22	17	22
Session 1	Neg	18	18	0	17	1		
	Pos	22	0	20	0	21		
Session 2	Neg	18					17	1
	Pos	20					0	20
% Agreement			100%		97%		97%	
(95% CI)			(91% - 100%)		(87% - 100%)		(87% - 100%)	

2. For Intra-Pathologist Image Analysis.

Table 2-4. Intra-Pathologist Image Analysis.

			Intra-Pathologist Image Analysis					
			Session 2		Session 3		Session 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			20	20	19	21	19	21
Session 1	Neg	20	19	1	19	1		
	Pos	20	1	19	0	20		
Session 2	Neg	20					19	1
	Pos	20					0	20
% Agreement			95%		98%		98%	
(95% CI)			(83% - 99%)		(87% - 100%)		(87% - 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.

			Inter-Pathologist Digital					
			Site 2		Site 3		Site 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			51	65	47	67	47	67
Site 1	Neg	52	48	3	44	6		
	Pos	63	3	60	2	58		
Site 2	Neg	51					41	8
	Pos	65					5	58
% Agreement			95%		93%		88%	
(95% CI)			(89% - 98%)		(86% - 96%)		(81% - 93%)	

4. Inter-Pathologist Image Analysis.

Table 2-6. Inter-Pathologist Image Analysis.

			Inter-Pathologist Image Analysis					
			Site 2		Site 3		Site 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			63	55	59	57	59	57
Site 1	Neg	60	59	1	55	4		
	Pos	54	2	52	3	51		
Site 2	Neg	63					57	4
	Pos	55					2	53
% Agreement			97%		94%		95%	
(95% CI)			(93% - 99%)		(88% - 97%)		(89% - 98%)	

Scanner Precision Studies

Forty (40) cases representing the useful categories of <1%, 1-10%, and 10% positive staining for ER 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 (sessions, intra-scanner). The precision tables are found below.

ER Inter-Scanner Agreement Rates (Site to Site)

2x2 Tables

Table 2-7. ER Inter-Scanner Agreement Rates (Site to Site)

Image Analysis	Virtuoso ER (SP1) Results- Site 2		
Virtuoso ER (SP1) Results- Site 1	Negative <1%	Positive ≥1%	Total
Negative <1%	49	5	54
Positive ≥1%	0	66	66
Total	49	71	120
Overall Percent Agreement % (n/N) (95% CI)	95.8% (115/120) (90.6-98.2)		
Average Positive Agreement % (n/N) (95% CI)	96.4% (132/137) (92.8-99.2)		
Average Negative Agreement % (n/N) (95% CI)	95.1% (98/103) (90.2-99.0)		

Image Analysis	Virtuoso ER (SP1) Results- Site 3		
Virtuoso ER (SP1) Results- Site 1	Negative <1%	Positive ≥1%	Total
Negative <1%	50	4	54
Positive ≥1%	2	61	63
Total	52	65	117
Overall Percent Agreement % (n/N) (95% CI)	94.9% (111/117) (89.3-97.6)		
Average Positive Agreement % (n/N) (95% CI)	95.3% (122/128) (91.2-98.5)		
Average Negative Agreement % (n/N) (95% CI)	94.3% (100/106) (89.3-98.3)		

Image Analysis	Virtuoso ER (SP1) Results- Site 3		
Virtuoso ER (SP1) Results- Site 2	Negative <1%	Positive ≥1%	Total
Negative <1%	49	0	49
Positive ≥1%	3	65	68
Total	52	65	117
Overall Percent Agreement % (n/N) (95% CI)	97.4% (114/117) (92.7-99.1)		
Average Positive Agreement % (n/N) (95% CI)	97.7% (130/133) (94.7-100.0)		
Average Negative Agreement % (n/N) (95% CI)	97.0% (98/101) (93.0-100.0)		

Conclusion (ER Inter-scanner)

Overall inter-scanner percent agreements for the 3 scoring bins categorization ranged from 94.9% to 97.4% for all FOVs combined.

3x3 Tables

Table 2-8. ER Inter-Scanner Agreement Rates (Site to Site)

Image Analysis	Virtuoso ER (SP1) Results- Site 2			
Virtuoso ER (SP1) Results- Site 1	<1%	1-10%	>10%	Total
<1%	49	5	0	54
1-10%	0	20	0	20
>10%	0	0	46	46
Total	49	25	46	120
Overall Percent Agreement % (n/N) (95% CI)	95.8% (115/120) (90.6 to 98.2)			

Image Analysis	Virtuoso ER (SP1) Results- Site 3			
Virtuoso ER (SP1) Results- Site 1	<1%	1-10%	>10%	Total
<1%	50	3	1	54
1-10%	2	16	2	20
>10%	0	0	43	43
Total	52	19	46	117
Overall Percent Agreement % (n/N) (95% CI)	93.2% (109/117) (87.1-96.5)			

Image Analysis	Virtuoso ER (SP1) Results- Site 3			
Virtuoso ER (SP1) Results- Site 2	<1%	1-10%	>10%	Total
<1%	49	0	0	49
1-10%	3	19	3	25
>10%	0	0	43	43
Total	52	19	46	117
Overall Percent Agreement % (n/N) (95% CI)	94.9% (111/117) (89.3-97.6)			

Conclusion (ER Inter-scanner)

Overall inter-scanner percent agreements for the positive/negative categorization ranged from 93.2% to 95.8% for all FOVs combined.

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

2x2 Tables

Table 2-9. ER Intra-Scanner/Inter-Day Agreement Rates (Session-to-Session)

Image Analysis	Virtuoso ER (SP1) Results- Session 2		
Virtuoso ER (SP1) Results - Session 1	Negative <1%	Positive ≥1%	Total
Negative <1%	49	0	49
Positive ≥1%	1	65	66
Total	50	65	115
Overall Percent Agreement: 99.1% (114/115) 95% CI: (95.2% to 99.8%)			

Image Analysis	Virtuoso ER (SP1) Results- Session 3		
Virtuoso ER (SP1) Results - Session 1	Negative <1%	Positive ≥1%	Total
Negative <1%	46	0	46
Positive ≥1%	0	71	71
Total	46	71	117
Overall Percent Agreement: 100.0% (117/117) 95% CI: (96.8% to 100.0%)			

Image Analysis	Virtuoso ER (SP1) Results- Session 3		
Virtuoso ER (SP1) Results - Session 2	Negative <1%	Positive ≥1%	Total
Negative <1%	46	1	47
Positive ≥1%	0	65	65
Total	46	66	112
Overall Percent Agreement: 99.1% (111/112) 95% CI: (95.1% to 99.8%)			

Conclusion (ER Intra-scanner/Inter-day)

Overall percent agreements for intra-scanner precision for the positive/negative categorization ranged 99.1% to 100.0% for all FOVs combined.

3x3 Tables

Table 2-10. ER Intra-Scanner/Inter-Day Agreement Rates (Session-to-Session)

Image Analysis	Virtuoso ER (SP1) Results- Session 2			
Virtuoso ER (SP1) Results- Session 1	<1%	1-10%	>10%	Total
<1%	49	0	0	49
1-10%	1	24	0	25
>10%	0	0	41	41
Total	50	24	41	115
Overall Percent Agreement: 99.1% (114/115) 95% CI: (95.2% to 99.8%)				

Image Analysis	Virtuoso ER (SP1) Results- Session 3			
Virtuoso ER (SP1) Results- Session 1	<1%	1-10%	>10%	Total
<1%	46	0	0	46
1-10%	0	23	2	25
>10%	0	0	46	46
Total	46	23	48	117
Overall Percent Agreement: 98.3% (115/117) 95% CI: (94.0% to 99.5%)				

Image Analysis	Virtuoso ER (SP1) Results- Session 3			
Virtuoso ER (SP1) Results- Session 2	<1%	1-10%	>10%	Total
<1%	46	1	0	47
1-10%	0	22	2	24
>10%	0	0	41	41
Total	46	23	43	112
Overall Percent Agreement: 97.3% (109/112) 95% CI: (92.4% to 99.1%)				

Conclusion (ER Intra-scanner/Inter-day)

Overall percent agreements for intra-scanner precision for the 3 scoring bins categorization ranged 97.3% to 99.1% for all FOVs combined.

Performance with BenchMark ULTRA

These ER (SP1) comparison studies for the Virtuoso Digital Read and Companion Algorithm Image Analysis software consisted of 120 de-identified archived breast carcinoma sections immunohistochemically stained with CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody on the BenchMark ULTRA stainer. Study test samples covered the ranges of 0-0.99%, 1-10% and >10%, and were interpreted by one pathologist at one site. All test slides were scanned at 20X magnification and all images were output in bif file format. 111 of 120 cases were able to be evaluated and were included in the analyses below.

The table below shows the concordance results for CONFIRM anti-ER (SP1) assay stained on the BenchMark ULTRA instrument.

Digital Read

Table 2-11 Agreement (ULTRA Stainer) - Digital Read vs Manual

	Manual Microscopic Read		
Digital Read	**Positive**	**Negative**	**Total**
Positive	69	1	70
Negative	1	40	41
Total	70	41	111
Positive Percent Agreement (PPA) n/N (%) (95% CI)	69/70 (98.6) (92.3-99.7)		
Negative Percent Agreement (NPA) n/N (%) (95% CI)	40/41 (97.6) (87.4-99.6)		
Overall Percent Agreement (OPA) n/N (%) (95% CI)	109/111 (98.2) (93.7-99.5)		

Image Analysis

Table 2-12 Agreement (ULTRA Stainer) - Image Analysis vs Manual

	Manual Microscopic Read		
Image Analysis Read	**Positive**	**Negative**	**Total**
Positive	64	4	68
Negative	6	37	43
Total	70	41	111
Positive Percent Agreement (PPA) n/N (%) (95% CI)	64/70 (91.4) (82.5-96.0)		
Negative Percent Agreement (NPA) n/N (%) (95% CI)	37/41 (90.2) (77.5-96.1)		
Overall Percent Agreement (OPA) n/N (%) (95% CI)	101/111 (91.0) (84.2-95.0)		

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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-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody package insert.

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