Virtuoso System for IHC PR (1E2) Using the VENTANA iScan HT Slide Scanner User Manual

Ventana Medical Systems, Inc.
www.ventana.com

Part No.: 101480300 Rev. A
August 2015

IVD

Virtuoso System for IHC PR (1E2) Using the VENTANA iScan HT User Manual Copyright

Trademarks

Open Source and Commercial Software

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

Contact Information

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, AZ 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.ventana.com

Ventana Medical Systems, Inc. 203
Ravendale Drive Mountain View, CA
94043 USA

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+49 621 7590

Contents

INTENTIONALLY BLANK

About the PR (1E2) Digital Read Application

Welcome to the Progesterone Receptor (PR) (1E2) Digital Read Application User Manual.

Who Should Read this Manual

Any user of the system should read this user manual and use it for reference while operating the VENTANA Virtuoso software.

Introduction

The Virtuoso PR (1E2) Digital Read Application allows the pathologist to view PR 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 and the iScan slide scanners, see the following documents:

- Virtuoso Reference Guide (click Help button in Virtuoso user interface to download)
- VENTANA iScan HT slide scanner reference guide (click Help button in scanner user interface and select Reference Guide on menu)

Technical Support

Ventana Medical Systems, Inc. **1910 E. Innovation Park Drive** **Tucson, AZ 85755** **USA**	**Tel (USA): +1 800 227 2155; for Technical Support, press 1** **Tel: +1 520 887 2155; for Technical Support, press 1**

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 PR 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 PR (1E2) using VENTANA iScan HT is for the digital read application. This particular Virtuoso system is intended for use as an aid to the pathologist in the qualitative detection of progesterone receptor (PR) protein in formalin-fixed, paraffin-embedded normal and neoplastic tissue. This device is an accessory to Ventana Medical Systems, Inc. CONFIRM anti-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody assay. The CONFIRM anti-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody assay 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 PR (1E2) Digital Read application is an adjunctive computer-assisted methodology for the qualified pathologist in the acquisition and interpretation of images from microscope glass slides of breast cancer specimens stained for the presence of PR protein. 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-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody assay used to assure the validity of the Virtuoso system for IHC PR Digital Read. The actual correlation of CONFIRM anti-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary 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 system can be used for review of digitized images of histologic sections.

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. The system requires competent human intervention at all steps in the process and the software makes no independent interpretations of the data.

The Virtuoso system consists of software with a user interface. Virtuoso software is an end-to-end digital pathology software solution that allows pathology laboratories to acquire, manage, view, 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 and generate reports.

For *in vitro* diagnostic (IVD) use.

Warnings and Precautions

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-PR (1E2) package insert. For optimal image capture using the Virtuoso system, it is recommended that the tissue be free of folds. 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 VENTANA iScan HT slide scanner Reference Guide.

Procedure

Refer to Virtuoso Reference Guide.

Required Materials Not Provided

The Virtuoso system with the PR (1E2) digital read requires use of the CONFIRM anti-PR (1E2) monoclonal antibody, and any additional material or supplies listed in the CONFIRM anti-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody package insert, to stain tissues prior to analysis. The VENTANA iScan HT scanner is required for scanning of the slides.

Monitor Requirements

Table 1-1 Monitor Requirements

Specification	Minimum Requirement
Resolution	1920x1080 24-bit color (16.7 million colors)
Display technology	LCD TFT
Physical size of the display available for image visualization	17 inches (measured diagonally) or greater
Backlight type	LED preferred; CCFL acceptable
Pixel array, pitch and pattern	The pixel array (resolution) must be 1920 (horizontal) by 1080 (vertical) or greater. As long as the monitor matches or exceeds the resolution and the physical size of the display specifications, there is no separate requirement for pixel pitch.The requirement for the pixel pattern is that there should be one red, one green and one blue component for each pixel.
Sub-pixel and color driving techniques	The color driving technology must provide 24-bit color or the equivalent.
Video bandwidth	The video bandwidth must be sufficient to refresh the display at 60 Hz or faster.
QC procedures	The display should be inspected before use and regularly during use, for visually obvious problems such as dead pixels and non-uniformity of display intensity.

Results

The pathologist views the image, makes an assessment based on viewing the whole slide image and reports a score. Refer to the Virtuoso Reference Guide for an example of a report.

Limitations

The image quality is only as good as the quality and accuracy of the immunohistochemistry slide that is scanned. The pathologist must validate the CONFIRM anti-PR (1E2) antibody staining run by examination of the PR control images to verify that the expected results have been obtained before images from patient slides are reviewed. The pathologist must follow the manufacturer's recommendations for the CONFIRM anti-PR (1E2) antibody 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-PR (1E2) antibody staining package insert for details about quality control recommendations.)) The pathologist must follow the CONFIRM anti-PR (1E2) antibody recommendations for surveying the entire breast cancer specimen to assess any heterogeneity in the CONFIRM anti-PR (1E2) antibody staining, the degree of background staining, cytoplasmic staining, edge effect, etc. as recommended in the CONFIRM anti-PR (1E2) antibody assay package insert (available at www.ventana.com).

This device has not been tested, or its safety and effectiveness validated, when used with mobile hand-held devices and/or a personal computer (PC).

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.

Device performance has been tested at 20X magnification and one z plane (volume scan not supported) only. Device performance at 40X has not been determined.

Performance Characteristics

Performance of the staining agent is described in the CONFIRM anti-PR (IE2) antibody package insert. See “Chapter 2 PR Comparison and Reproducibility Studies” for a description of the performance of the software.

Assay Cutoff

The performance of the Virtuoso system with the PR (1E2) digital read application was evaluated at the CAP/ASCO recommended clinical cutoffs: 0-0.99% was considered a negative test result and 1-10% and >10% were considered positive test results.

INTENTIONALLY BLANK

Chapter 2: PR Comparison and Reproducibility Studies

Staining Procedure

Refer to the CONFIRM anti-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody package insert for the BenchMark XT and BenchMark ULTRA instruments, *ultra*View, and *i*VIEW detection.

Performance with BenchMark XT and BenchMark ULTRA Instruments and VENTANA iScan HT Slide Scanner

This section shows comparison and reproducibility studies for the PR marker. For these slides, scanning was performed using the VENTANA iScan HT scanner. Scanner settings were as follows: Magnification: 20X, Focus Approach: Routine Scan, Scan Approach: Regular, AOI Detect. Assay positive and negative controls were scanned at 20X for the study.

The PR (1E2) comparison and reproducibility studies for the Virtuoso Digital Read software consisted of approximately 160 de-identified archived primary breast carcinoma sections immunohistochemically stained with CONFIRM anti- Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody on the BenchMark XT and BenchMark ULTRA stainers. 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-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody staining interpretation among three different sites:

1. Digital Read vs Manual Method

Table 2-1 Digital Read for PR.

Confusion Matrix		Digital					
		Site 1		Site 2		Site 3	
		(n = 159)		(n = 162)		(n = 156)	
		Neg	Pos	Neg	Pos	Neg	Pos
Manual	Neg (0-0.99%)	50	1	72	7	82	0
	Pos (1-10%)	1	107	11	72	6	68
	% Agreement	98.7%		88.9%		96.2%	
	(95% CI)	(95.5% - 99.7%)		(83.1%- 92.9%)		(91.9% - 98.2%)	
Negative % Agreement		98.0%		91.1%		100.0%	
(95% CI)		(89.7% - 99.7%)		(82.8% - 95.6%)		(95.5% - 100.0%)	
Positive % Agreement		99.1%		86.7%		91.9%	
(95% CI)		(94.9% - 99.8%)		(77.8% - 92.4%)		(83.4% - 96.2%)	

Intra-System and Inter-System Studies

The study was designed to demonstrate inter- and intra-Virtuoso system reproducibility for Virtuoso Digital Read. For intra-system studies, a randomly selected subset of approximately 40 cases that span the range of the PR scoring categories (0-0.99%, negative, 1-10% 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 2-2 Intra-Pathologist Digital Read.

Confusion Matrix			Intra-Pathologist Digital Read					
			Session 2		Session 3		Session 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			20	19	19	20	19	20
Session 1	Neg	19	19	0	17	2		
	Pos	20	1	19	2	18		
Session 2	Neg	20					18	2
	Pos	19					1	18
% Agreement			97.4%		89.7%		92.3%	
(95% CI)			(86.8% - 99.5%)		76.4% - 95.9%)		79.7% - 97.3%)	

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

Table 2-3 Inter-Pathologist Digital Read.

Confusion Matrix			Inter-Pathologist Digital Read					
			Site 2		Site 3		Site 3	
			Neg	Pos	Neg	Pos	Neg	Pos
			79	80	85	70	88	70
Site 1	Neg	51	50	1	50	0		
	Pos	108	29	79	35	70		
Site 1	Neg	82					77	5
	Pos	76					11	65
% Agreement			81.1%		77.4%		89.9%	
(95% CI)			(74.3% - 86.5%)		(70.2%-83.3%)		(84.2% - 93.7%)	

VENTANA iScan HT Scanner Precision Studies

Forty (40) cases representing the useful categories of <1%, 1-10%, and 10% positive staining for PR 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. Similarly, these same 40 and three FOVs per case were scanned on three different days by the same scanner and the same three FOVs were evaluated 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.

Table 2-4 PR Inter-Scanner Agreement Rates (Site to Site)

	Virtuoso PR (1E2) Results - Site 2			
Virtuoso PR (1E2) Results-Site 1	<1%	1-10%	>10%	Total
<1%	181	10	0	191
1-10%	15	30	3	48
>10%	0	8	113	121
Total	196	48	116	360
Overall Percent Agreement: 90.0% (324/360) 95% CI: (86.5% - 92.7%)				

	Virtuoso PR (1E2) Results - Site 3			
Virtuoso PR (1E2) Results-Site 1	<1%	1-10%	>10%	Total
<1%	184	7	0	191
1-10%	10	34	4	48
>10%	0	2	119	121
Total	194	43	123	360
Overall Percent Agreement: 93.6% (337/360) 95% CI: (90.6% - 95.7%)				

	Virtuoso PR (1E2) Results - Site 3			
Virtuoso PR (1E2) Results-Site 2	<1%	1-10%	>10%	Total
<1%	182	14	0	196
1-10%	12	28	8	48
>10%	0	1	115	116
Total	194	43	123	360
Overall Percent Agreement: 90.3% (325/360) 95% CI: (86.8% - 92.9%)				

Conclusion (PR Inter-scanner)

Overall inter-scanner percent agreements for the three categories ranged from 90.0% to 93.6% for all FOVs combined.

Table 2-5 PR Intra-Scanner/Inter-Day Agreement Rates (Session-to-Session)

	Virtuoso PR (1E2) Results - Session 2			
Virtuoso PR (1E2) Results-Session 1	<1%	1-10%	>10%	Total
<1%	182	11	1	194
1-10%	11	33	1	45
>10%	0	4	117	121
Total	193	48	119	360
Overall Percent Agreement: 92.2% (332/360) 95% CI: (89.0% - 94.6%)				

	Virtuoso PR (1E2) Results - Session 3			
Virtuoso PR (1E2) Results-Session 1	<1%	1-10%	>10%	Total
<1%	180	14	0	194
1-10%	14	29	2	45
>10%	0	3	118	121
Total	194	46	120	360
Overall Percent Agreement: 90.8% (327/360) 95% CI: (87.4% - 93.4%)				

	Virtuoso PR (1E2) Results - Session 3			
Virtuoso PR (1E2) Results-Session 2	<1%	1-10%	>10%	Total
<1%	181	12	0	193
1-10%	12	31	5	48
>10%	1	3	115	119
Total	194	46	120	360
Overall Percent Agreement: 90.8% (327/360) 95% CI: (87.4% - 93.4%)				

Conclusion (PR Intra-scanner/Inter-day)

Overall percent agreements for the three categories ranged 90.8% to 92.2% for all FOVs combined.

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-Progesterone Receptor (PR) (1E2) rabbit monoclonal primary antibody package insert.

INTENTIONALLY BLANK

Index