510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k110215

B. Purpose for Submission:
New Device

C. Measurand:
Human ER alpha protein

D. Type of Test:
Qualitative, immunohistochemistry

E. Applicant:
Ventana Medical Systems, Inc.

F. Proprietary and Established Names:
CONFIRM anti-Estrogen Receptor (SP1) Rabbit Monoclonal Primary Antibody

G. Regulatory Information:

1. Regulation section:
   21 CFR § 864.1860 Immunohistochemistry reagents and kits
2. Classification:
   Class II
3. Product code:
   MYA, Immunohistochemistry antibody assay, Estrogen Receptor
4. Panel:
   Pathology 88

H. Intended Use:

1. Intended use(s):
   CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody is intended for laboratory use for the qualitative detection of estrogen receptor (ER) antigen in sections of formalin-fixed, paraffin-embedded breast tissue on a Ventana automated slide stainer with Ventana DAB detection chemistry and ancillary reagents. CONFIRM anti-ER (SP1) is directed against an epitope present on human ER alpha protein located in the nucleus of ER positive normal and neoplastic cells.

   This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

2. Indication(s) for use:
   CONFIRM anti-ER (SP1) is indicated as an aid in the management, prognosis, and prediction of hormone therapy for breast carcinoma.

3. Special conditions for use statement(s):
   None

4. Special instrument requirements:
   Ventana BenchMark XT or BenchMark ULTRA automated slide stainer
   Ventana *i*View Detection System or Ventana *ultraView* Detection System

I. Device Description:

The Ventana CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody is a rabbit monoclonal antibody produced as a cell culture supernatant. It is provided as a ready-to-use reagent for use only on the Ventana Benchmark XT and BenchMark ULTRA immunostainer platforms with the *i*View or *ultra*View DAB detection kits.

The antibody is diluted in 0.05 M Tris-HCl with 2% carrier protein, and 0.1% ProClin 300, a preservative. Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 1 µg/mL.

J. Substantial Equivalence Information:

The clearance of the Ventana CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody was based on clinical outcomes study data. The sponsor provided clinical study data that analyzed patient outcomes relative to the device performance on the BenchMark ULTRA stainer – The Calgary Cohort Study (2012). The device performance on the BenchMark XT was supported by data from an inter-platform comparison study between the BenchMark ULTRA and the BenchMark XT staining platforms.

K. Standard/Guidance Document Referenced (if applicable):

Guidance document: "FDA Guidance for Submission of Immunohistochemistry Applications to the FDA", Center for Devices and Radiologic Health.

L. Test Principle:

CONFIRM anti-ER (SP1) assay binds to human estrogen receptor alpha located in the nuclear region of normal and neoplastic cells. Automated immunohistochemistry staining is performed on formalin-fixed, paraffin-embedded tissue. The antigen in tissue sections is demonstrated through several steps. The specific antibody binds to the antigen present in tissue sections. The bound primary antibody is located by a biotin conjugated secondary antibody formulation which recognizes rabbit or mouse immunoglobulins (Ig). This step is followed by the addition of a streptavidin-enzyme conjugate which binds to the biotin on the secondary antibody. The primary antibody-secondary antibody-avidin enzyme complex is visualized by using a precipitating enzyme generated product. This is detected by light microscopy as

brown precipitate in the nucleus of normal and neoplastic cells.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*

Studies to assess the intra-run (within-run, intra-day) and inter-run (day-to-day) analytical precision of CONFIRM anti-ER (SP1) antibody was conducted using two Ventana IHC stainers: BenchMark XT and BenchMark Ultra instruments and the *i*View DAB Detection Kit. An acceptance criteria of at least 80% overall agreement for staining and 90% for background staining was set by the sponsor. In these studies all acceptance criteria were met.

Intra-run precision

Nine slides were stained with the CONFIRM anti-ER (SP1) antibody and one slide was stained with Negative Control Rabbit Ig antibody from each of the six individual breast carcinoma tissue blocks in this study. There were a total of sixty slides in this study. Of the six tissues, two were ER negative, two were ER low expression and two were ER high expression based on the following scoring criteria: <1% tumor cells staining for negative, 1-10% for low and >10% for high expression. This slide testing configuration was used for staining on both the BenchMark XT and BenchMark Ultra instruments. Slide to slide staining characteristics showed acceptable reproducibility with no variation in staining quality and patterns. There was 100% concordance for all slides for ER staining and the background staining was acceptable. All acceptance criteria for both instruments were met in this study.

Inter-run precision

Four slides from each case were stained with the CONFIRM anti-ER (SP1) antibody, and one slide from each case was stained with Negative Control Rabbit Ig antibody in five separate nonconsecutive runs on 5 days conducted over a 20-day period on the same BenchMark XT instrument. There were a total of 30 slides per run. The same testing configuration was also performed on a BenchMark ULTRA instrument. There was 100% concordance for all slides for ER staining and the background staining was acceptable. All acceptance criteria for both instruments were met in this study.

Intra-platform Reproducibility

Four slides from six cases were stained with CONFIRM anti-ER (SP1) antibody and one slide from each case was stained with Negative Control Rabbit Ig antibody on three separate BenchMark XT instruments. There were a total of thirty slides. The same testing configuration was used for the BenchMark ULTRA instrument. There was 100% concordance on all cases and the background staining was acceptable for both the BenchMark XT and BenchMark ULTRA instruments. All acceptance criteria for both instruments were met in this study.

Inter-Platform Reproducibility for ULTRA and XT

A cross platform comparison was performed to assess the reproducibility between the BenchMark ULTRA and the XT instruments. Three BenchMark XT and three BenchMark ULTRA instruments were used in this study. There were thirty slides stained per BenchMark XT instrument for a total of ninety slides in this study. The same testing configuration was used for the BenchMark ULTRA instrument for a total of ninety slides for the BenchMark ULTRA instrument. There was 100% concordance on all six cases and the acceptability rate for background staining exceeded the 80% acceptance criteria.

Detection Kit compatibility Study

This study utilized 199 individual breast cancer cases and three lots of the *i*View and the *ultraView* DAB Detection Kits. There were approximately 100 ER negative and 100 ER positive cases. The three lots were grouped into Group A instruments and reagents (one XT and one ULTRA using one lot of *i*VIEW DAB and *ultra*View DAB Detection). Similar configurations were used for Group B and Group C. The acceptance criteria of 90% for ER staining and 85% for background and morphology acceptability were met.

Assessment for ultraView Universal DAB Detection Kit versus *i*VIEW DAB Detection Kit on the BenchMark ULTRA Instrument

		***i*VIEW DAB Detection Kit**		
		Positive	**Negative**	**Total**
ultraView Universal DAB Detection Kit	**Positive**	108	3	111
	Negative	3	80	83
	Total	111	83	194

	n/N	% (95% CI)
Positive percent agreement	108/111	97.3 (92.4-99.1)
Negative percent agreement	80/83	96.4 (89.9-98.8)
Overall percent agreement	188/194	96.6 (93.4-98.6)

Assessment for ultraView Universal DAB Detection Kit versus *i*VIEW DAB Detection Kit on the BenchMark XT Instrument

		***i*VIEW DAB Detection Kit**		
		Positive	**Negative**	**Total**
ultraView Universal DAB Detection Kit	**Positive**	106	5	111
	Negative	2	79	81
	Total	108	84	192

	n/N	% (95% CI)
Positive percent agreement	106/108	98.1 (93.5-99.5)
Negative percent agreement	79/84	94.0 (86.8-97.4)
Overall percent agreement	185/192	96.4 (92.7-98.2)

Lot to Lot Reproducibility

Three lots of the CONFIRM anti-ER (SP1) antibody were used in this study. Ten breast cancer cases and 1 negative control slide for each case were stained with the three lots of CONFIRM anti-ER (SP1). The acceptance criteria of 90% for ER staining, background and morphology acceptability were met in this study.

b. *Linearity/assay reportable range:*
Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Positive and negative control slides should be stained with each staining run. The pathologist is responsible for assuring the proper performance of this test.

d. *Detection limit:*
Not applicable.

e. *Analytical specificity:*
A total of 90 formalin-fixed and paraffin-embedded tissues covering a wide range of normal human tissues types were tested with the CONFIRM anti-ER (SP1) antibody. The antibody demonstrated negative immunoreactivity in the appropriate tissues. Positive immunoreactivity was noted in breast, cervix/uterine, endometrium and prostate tissues as expected.

f. *Assay cut-off:*
A negative staining result is defined as <1% tumor cells staining and a positive staining result is defined as ≥ 1% tumor cells staining of any intensity.

2. Comparison studies:

a. Intra-Platform Comparison Studies

Comparison of BenchMark XT versus BenchMark ULTRA:
A randomized, multi-site, multi-reader study was conducted to compare the staining performance of the CONFIRM anti-ER (SP1) on the BenchMark ULTRA instrument versus the BenchMark XT instrument. 120 ER negative and 132 ER positive cases of breast cancer, representing the clinical range of the assay, were randomly assigned to three study sites such that each site received an equal number of cases and each site received cases representing each clinical assessment category. Each site stained its assigned cases with the CONFIRM anti-ER (SP1) antibody on a BenchMark ULTRA instrument and a CONFIRM anti-ER (SP1) antibody on a BenchMark XT instrument. The stained slides were evaluated by pathologists who determined the percentage of stained tumor cells. A case was considered ER positive if there was staining of the nucleus in at least ≥1% of invasive tumor cells. The acceptance criteria of 85% concordance for ER scoring and background staining set by the sponsor were met in these studies. Results of the study are summarized in table below:

Comparison of CONFIRM anti-ER (SP1) results on the BenchMark ULTRA Instrument and on the BenchMark XT Instrument

		BenchMark ULTRA Instrument		
		Positive	**Negative**	**Total**
BenchMark XT Instrument	**Positive**	99	8	107
	Negative	11	91	102
	Total	110	99	209

	n/N	% (95% CI)
Positive percent agreement	99/110	90.0 (83.0-94.3)
Negative percent agreement	91/99	91.9 (84.9-95.8)
Overall percent agreement	190/209	90.9 (86.2-94.1)

The morphology acceptability rates for all slides stained in this study were 100% (95% C.I. 98.5%-100%) for the BenchMark ULTRA instrument and 94.0% (95% C.I. 90.4% - 96.4%) for the BenchMark XT instrument. The background acceptability rates were 94.8% (95% C.I. 91.4% - 97.0%) for the BenchMark ULTRA instrument and 90.9% (95% C.I. 86.7%-93.8%) for the BenchMark XT instrument.

b. Method Comparison

Not applicable.

c. Matrix comparison:

Not applicable.

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not applicable.

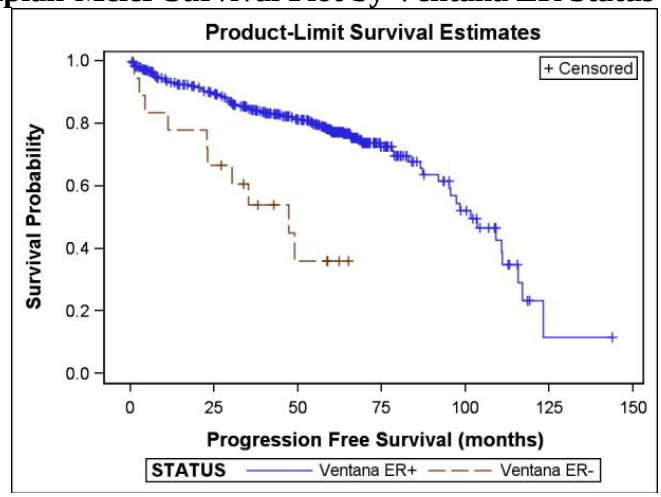
   b. *Clinical Specificity:*
      Not applicable.

   c. *Other clinical supportive data (when a. and b. are not applicable)*:
      Calgary Cohort Study: The sponsor performed this study to provide patient outcome data to support the performance of the CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody assay. The Calgary study leveraged a tamoxifen treatment cohort database and cohort tissue samples established by The Tom Baker Cancer Center at the Department of Oncology, University of Calgary, in Calgary, Alberta, Canada. The center had previously conducted a retrospective study in which a patient cohort was identified from the Calgary Tamoxifen Database consisting of 820 breast cancer patients identified as having received tamoxifen treatment between 1997 and 2003. The dataset consisted of 511 cases of primary tumors of invasive breast cancer, with the dates of occurrence spanning 1985-2000. Patients who were treated with tamoxifen, did not have a previous cancer diagnosis prior the cancer being examined and did not receive any other chemotherapy besides tamoxifen or any chemotherapy prior to collection of specimen were included in this study. This data set consisted of 459 cases.

      Sections were cut from these cases and stained with CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody using the BenchMark ULTRA stainer. Three pathologists (2 external and 1 internal) blinded to any previous ER-status independently evaluated the stained slides. All readers scored all cases included in the study in accordance with relevant package inserts and training provided by the sponsor. Cases were scored as ER-positive if 1% or more of the tumor cells in the core exhibited specific staining for ER. There were 441 cases with Ventana ER+ status and 18 cases with Ventana ER- status.

      A Kaplan-Meier survival plot by Ventana ER status showed strong separation between Ventana ER+ and ER– cases. As expected, ER+ patients had longer survival times than ER– patients when tamoxifen treatment was administered; the median survival times for ER+ and ER– patients were 101.6 (95% CI; 95.0 to 110.9) and 47.2 (95% CI: 22.8, not estimated) months, respectively. The log-rank test showed that this difference was statistically significant ($p < 0.001$). In addition, Cox regression analysis was performed with Ventana ER status and clinical covariates as age, tumor grade, tumor size and nodal status. The estimate of hazard ratio for Ventana ER status was 0.469 with 95% CI: 0.252-0.973 (p-value=0.026)

Kaplan-Meier Survival Plot by Ventana ER Status



4. Clinical cut-off**:**
   Same as assay cut-off

5. Expected values/Reference range:
   Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.