510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k122556

B. Purpose for Submission:
Addition of Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ on the Bond III staining platform to the previously cleared Leica BioSystems Estrogen Receptor Clone 6F11 – manual method.

C. Measurand:
Human Estrogen receptor alpha protein

D. Type of Test:
Qualitative, immunohistochemistry

E. Applicant:
Leica Biosystems

F. Proprietary and Established Names:
Estrogen Receptor Clone 6F11 (ER 6F11)
- Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™;
- Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™

G. Regulatory Information:
1. Regulation section:
   21 CFR 864.1860 Immunohistochemistry Reagents and Kits
2. Classification:
   Class II
3. Product code:
   MYA – Estrogen receptor immunohistochemistry antibody assay
4. Panel:
   Pathology (88)

H. Intended Use:
1. Intended use(s):
   Estrogen Receptor Clone 6F11 (ER 6F11) Mouse Monoclonal antibody is intended for laboratory use to qualitatively identify estrogen receptor (ER) antigen in sections of formalin fixed, paraffin embedded breast cancer tissue by immunohistochemistry methods. Estrogen Receptor Clone 6F11 specifically binds to the ER antigen located in the nucleus of ER positive normal and neoplastic cells.

   Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and the

Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ are optimized for use on the Leica Biosystems Bond III staining platform using the Bond Polymer Refine Detection Kit.

2. Indication(s) for use:
   ER 6F11 is indicated as an aid in the management, prognosis and predication of therapy outcome of breast cancer. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   Bond III staining system
   Leica DS9800 Bond Polymer Refine Detection Kit

I. Device Description:

Estrogen Receptor clone 6F11 is a mouse anti-human monoclonal antibody produced as a tissue culture supernatant and supplied in Tris buffered saline with carrier protein, containing 0.35% ProClin™ 950 as a preservative. This antibody is utilized to perform a qualitative immunohistochemical (IHC) assay to identify estrogen receptor (ER) expression in human breast cancer tissue routinely processed and paraffin-embedded for histological examination.

Estrogen Receptor clone 6F11 primary antibody is provided in a Ready-to-Use and liquid concentrate format and is optimally diluted for use on the automated Bond III staining platform in combination with Bond Polymer Refine Detection (DS9800). The total protein concentration is approximately 3.8g/L. The total antibody concentration in the reagent is approximately 75 mg/L.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Leica BioSystems Estrogen Receptor Clone 6F1 I (ER6F11) [formerly known as Vision BioSystems Estrogen Receptor Clone 6F1 I (ER6F11)]

2. Predicate 510(k) number(s):
   K060227

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Qualitative detection of human estrogen receptor alpha protein	Same

Similarities		
Item	Device	Predicate
Antibody Type	Mouse monoclonal	Same
Isotype	IgG1	Same
ER Clone	6F11	Same
Immunogen	Alpha form of the human estrogen receptor molecule	Same
Technology	Immunohistochemistry	Same
Tissue Type	Formalin-fixed paraffin-embedded breast cancer	Same
Staining Pattern	Nuclear	Same

Differences		
Item	Device	Predicate
Staining Method	Used on the Bond III automated staining platform	Manual staining method
Interpretation of Results	Positive: ≥1% of tumor cells with stained nuclei of any intensity	Positive: ≥10% of tumor cells with stained nuclei of any intensity

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

None

L. Test Principle:

Estrogen Receptor Clone 6F11 (ER 6F11) is recommended for use in an immunohistochemical procedure, which allows the qualitative identification by light microscopy of antigens in sections of formalin-fixed, paraffin-embedded tissue, via sequential steps with interposed washing steps. Prior to staining, endogenous peroxidase activity is blocked and sections are subjected to epitope retrieval. The tissue section is subsequently incubated with the mouse primary antibody that binds with the human tissue antigen. A polymeric enzyme-conjugated secondary antibody that recognizes mouse immunoglobulins is used to detect the primary antibody. Sections are further incubated with the substrate/chromogen, 3,3'-diaminobenzidine (DAB) and DAB Substrate Buffer. Reaction with the peroxidase produces a visible brown precipitate at the antigen site. Sections are counterstained with hematoxylin and coverslipped. Results are interpreted using a light microscope.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

   Intra-run precision

   Intra-run precision testing of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (RTU) was evaluated at a single site on FFPE breast cancer tissue on the Bond III staining platform. Similarly the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (concentrate) was

also evaluated at this site on FFPE breast cancer tissue on the Bond III staining platform. The following testing configuration was used in each of the above studies: Testing was conducted using 3 slides mounted with tissue micro arrays (TMAs) of human breast carcinomas samples. Each slide contained 13 TMA cores as follows: 3 cores each of high, medium and low positive ER staining and 3 cores of negative ER staining breast cancer tissue and 1 core of normal breast (non-neoplastic) tissue serving as a control tissue. In addition, 1 test slide with negative control antibody and 1 assay control slide were also used. Staining was performed three times over three different days (3 slides x 3 tests = 9 TMA slides incorporating 108 breast cancer specimens). Two cores were unevaluable due to tissue loss. All test slides were blinded, randomized and assessed by a single observer. The scoring was performed per the ASCO/CAP scoring method [American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Arch Pathol Lab Med. 2010;134:e48–e72), i.e. positive for ER if finding of ≥ 1% of tumor cell nuclei are immunoreactive and negative for ER if finding of < 1% of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER (positive intrinsic controls are seen)]. In addition the staining intensity was also assessed. The acceptance criteria set by the sponsor was 85% agreement (overall, positive and negative) at the lower bound of a two-sided 95% confidence interval. The acceptance criteria were met in these studies.

Inter-run/inter-day precision

Inter-run precision of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (RTU) was evaluated at a single site on FFPE breast cancer tissue on the Bond III staining platform. Similarly the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (concentrate) was also evaluated at this site on FFPE breast cancer tissue on the Bond III staining platform. The following testing configuration was used in each of the above studies: Testing was conducted using 3 slides mounted with TMAs of human breast carcinomas samples. Each slide contained 13 TMA cores as follows: 3 cores each of high, medium and low positive ER staining and 3 cores of negative ER staining breast cancer tissue and 1 core of normal breast (non-neoplastic) tissue serving as a control tissue. In addition, 1 test slide with negative control antibody and 1 assay control slide were also used. Staining was performed five times over five different days performed over a twenty (20) day period (3 slides x 5 tests = 15 TMA slides incorporating 180 breast cancer specimens). All test slides were blinded, randomized and assessed by a single observer. Four cores were unevaluable due to tissue loss. The scoring criteria were the same as above. The acceptance criteria were the same as above. The acceptance criteria were met in these studies.

Within Instrument Precision Study (intra-instrument)

Within-instrument precision testing of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (RTU) was evaluated on FFPE breast cancer tissue on three Bond III staining platforms. Similarly the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (concentrate) was also

evaluated on FFPE breast cancer tissue on three Bond III staining platforms. Three slides – one TMA slide (12 breast cancer cores as described in 1a above), 1 with negative control antibody and 1 assay control slide, were stained three times over three different days (3 slides x 3 runs x3 instruments, 108 test data points) for each combination of the antibody format and the staining platform. Samples spanned the range of ER expression. All test slides were blinded, randomized and assessed by a single observer. Five cores were unevaluable due to tissue loss. The scoring criteria were the same as above. The acceptance criteria were the same as above and were met in these studies.

Inter-Site Reproducibility

Inter-site reproducibility testing of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ was evaluated at 3 investigational sites on whole tissue sections using the Bond III stainer. The test cohort consisted of 18 cases. Samples spanned the range of ER expression. Testing was performed over a span of 5 non-consecutive days (Day1, Day3, Day 5), with each site staining a full set of cases on each day. This provided 9 replicates of 18 cases. The scoring criteria were the same as above. In addition, all aspects of staining quality was assessed and reported for each case i.e. staining intensity, proportion of cells staining, background staining, tissue morphology, staining of benign ductal cells, stromal cells, endothelial cells and lymphocytes for each study case. The acceptance criteria set by the sponsor was 85% agreement (overall, positive and negative) at the lower bound of a 95% confidence interval. The table below shows the data for overall agreement:

		Positive	Negative
ER(6F11) RTU - Bond III	Positive	96	6
	Negative	3	57
Overall Percent Agreement		94.44% (89.72-97.43)	
Positive Percent Agreement		96.97% (91.40-99.37)	
Negative Percent Agreement		90.48% (80.41-96.42)	
		Positive	Negative
ER(6F11) Concentrate - Bond III	Positive	111	0
	Negative	6	45
Overall Percent Agreement		96.30% (92.11-98.63)	
Positive Percent Agreement		94.87% (89.17-98.10)	
Negative Percent Agreement		100% (93.56-100)	

Lot to Lot Reproducibility

Lot to Lot reproducibility testing of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ on the Bond III platform was evaluated at a single site on whole tissue sections. The test cohort consisted of 18 cases. All cases were stained using three (3) independently manufactured reagent lots. The scoring criteria were the same as above. In addition, all aspects of staining quality

was assessed and reported for each case i.e. staining intensity, proportion of cells staining, background staining, tissue morphology, staining of benign ductal cells, stromal cells, endothelial cells and lymphocytes for each study case. The acceptance criteria set by the sponsor was 85% agreement (overall, positive and negative) at the lower bound of a 95% confidence interval. The table below shows the study data:

		Positive	Negative
ER(6F11) RTU - Bond III	Positive	32	1
	Negative	1	20
Overall Percent Agreement		96.30% (87.25-99.55)	
Positive Percent Agreement		96.97% (84.24-99.92)	
Negative Percent Agreement		95.24% (76.18-99.88)	
ER(6F11) Concentrate – Bond III	Positive	32	0
	Negative	1	21
Overall Percent Agreement		98.15% (90.11-99.95)	
Positive Percent Agreement		96.97% (84.24-99.92)	
Negative Percent Agreement		100% (86.71-100)	

Inter-Observer Reproducibility
Inter-observer reproducibility of Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ was evaluated between 3 observers at one site. Twenty (20) whole section breast cancer cases consisting of 5 strong ER expression, 5 medium ER expression, 5 weak ER expression and 5 negative ER expression breast carcinoma were used. The same testing configuration was used to assess the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™.

Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™: Inter-observer agreement between observer 1 and 2 and observer 1and 3 was 95% (19/20). Inter-observer agreement between observer 2 and 3 was 100% (20/20). Overall inter-observer agreement was 98.33% (59/60).

Inter-observer reproducibility of Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ was also evaluated in a similar manner as above. Inter-observer agreement between observer 1 and 2 was 89.47% (17/19). One case was omitted as it was reported by observer 1 as invasive tumor, while observer 2 reported this as ductal carcinoma *in situ* (DCIS). Inter-observer agreement between observer 1 and observer 3 was 94.74% (18/19). One case was omitted as it was reported by observer 1 as invasive tumor, while observer 2 reported this as DCIS. Inter-observer agreement between observer 2 and observer 3 was 94.74% (18/19). One case was omitted as both observers reported a single case as DCIS.

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.

The stability of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ was assessed by subjecting the device to temperature extremes and the intended use storage conditions. Testing was performed on breast cancer tissue sections that expressed estrogen receptor at a high, medium, low intensity and negative ER expression and on normal breast tissue. Testing was performed at specified intervals. Acceptance criteria were as follows: Test case passes if the stain intensity score and proportion score (≥ 1% of tumor cell nuclei staining = Positive; < 1% of tumor cell nuclei staining = Negative) are identical for each of the test estrogen expressive breast cancer cases used when compared to a control score. Based on the studies the shelf-life of the device is 18 months.

d. Detection limit:
Not applicable

e. Analytical specificity:
Please refer to analytical specificity in k060227 which tested the same antibody clone as the current device.

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. A study was performed to comparison the performance of Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (Concentrate) on the Bond III to Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (RTU) on the Bond III platform. This study was performed at 3 independent sites using formalin fixed paraffin embedded whole tissue sections from 306 unique invasive breast cancer cases. These cases spanned the range of ER expression. Appropriate positive and negative controls were performed to validate assay performance. The scoring was performed per the ASCO/CAP scoring method. In addition, the Allred scoring method was used as a secondary evaluation method. All aspects of staining quality were assessed and reported for each case, i.e. staining intensity, proportion of cells staining, background staining, tissue morphology, staining of benign ductal cells, stromal cells, endothelial cells and lymphocytes. Blinding and randomization was used throughout the evaluation to reduce bias. Screeners were blinded to the test used. The data are shown below:

		ER(6F11) RTU - Bond III	
		Positive	Negative
ER(6F11) Concentrate – Bond III	Positive	241	6
	Negative	4	55
Overall Percent Agreement (95% CI)		96.73% (94.07-98.42)	
Positive Percent Agreement		98.37% (95.87-99.55)	
Negative Percent Agreement		90.16% (79.81-96.30)	

b. Method comparison with predicate device:
Not applicable

c. Matrix comparison:
Not applicable

3. Clinical studies:

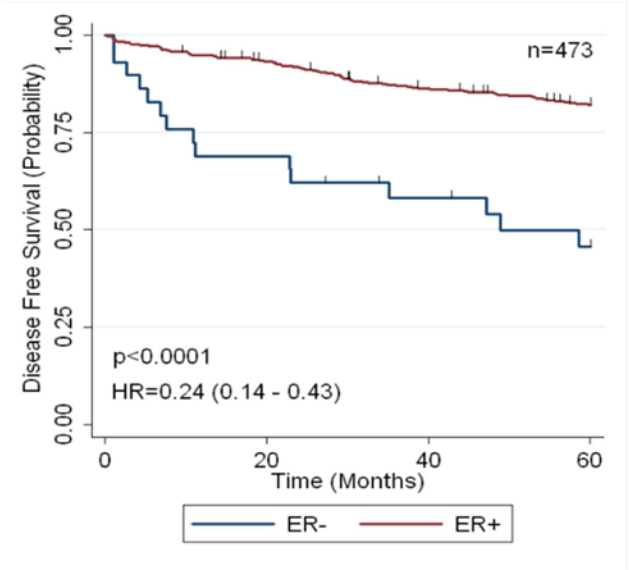
a. Clinical Sensitivity:
Not applicable

b. Clinical specificity:
Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*
The test device Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and the staining platform Bond III using the DS9800 Bond Polymer Refine Detection kit was tested in an independent clinical outcome study. This study used a retrospective patient cohort (n=532) composed of breast cancer patients diagnosed between 1985 and 2000 in the Tom Baker Cancer Center in Calgary, who were treated with primary adjuvant tamoxifen regardless of their ER and PR status. This cohort had a greater than 5 years of follow-up, it was enriched for events to increase its statistical power and it included ER negative patients so as to remove treatment selection bias. There were 473 samples available for evaluation with the test device.

A Kaplan-Meier survival plot by Leica ER (6F11) status showed strong separation between Leica ER positive and ER negative cases. As expected, ER positive patients had longer survival times than ER negative patients when tamoxifen treatment was administered.

Univariate Kaplan-Meier and Multivariate Cox survival analysis showing patients dichotomized to survival groups:



Multivariate Cox models were analyzed along with lymph node status, tumor grade, tumor size and HER2 status. The model is shown in figure below:

	Leica Device (n=363)		
	HR	95% CI	p-value
ER Status	0.39	(0.19 – 0.78)	0.008
Lymph Node Status	3.18	(1.88 – 5.37)	<0.001
Tumor Grade	3.15	(1.84 – 5.38)	<0.001
Tumor Size	1.67	(0.94 – 2.98)	0.083
HER2 Status	1.13	(0.38 – 3.33)	0.823

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.