510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I. Background Information:

A. 510(k) Number

K193393

B. Applicant

Leica Biosystems Newcastle Ltd.

C. Proprietary and Established Names

BOND Ready-to-Use Primary Antibody Progesterone Receptor (16), Novocastra Liquid Mouse Monoclonal Antibody Progesterone Receptor Clone 16 (Concentrated Liquid Antibody Format)

D. Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MXZ	Class II	21 CFR 864.1860 - Immunohistochemistry Reagents and Kits	

II. Submission/Device Overview:

A. Purpose for Submission:

Leica Biosystems Newcastle Ltd., is submitting this traditional 510(k) notification to request modifications to their BOND™ Ready-to-Use (RTU) Primary Antibody Progesterone Receptor (16) and Concentrated Liquid Mouse Monoclonal Antibody (Novocastra™), previously cleared under K062615, K171753 and K183102 for the BOND-MAX instrument. The proposed modifications are for adding the following to the existing device:

- BOND-III System (staining platform)
- BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) (30mL)

B. Measurand:

Human Progesterone Receptor in formalin-fixed, paraffin embedded breast cancer tissue.

C. Type of Test:

Immunohistochemistry (IHC)

III. Intended Use/Indications for Use:

A. Intended Use(s):
See Indications for Use below.

B. Indication(s) for Use:

Ready-to-Use Format

For *in vitro* diagnostic use.

BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) is a monoclonal antibody intended to be used for the qualitative identification by light microscopy of human progesterone receptor in formalin-fixed, paraffin-embedded tissue by immunohistochemical staining using the automated BOND-MAX or BOND-III systems. Progesterone Receptor Clone (16) specifically binds to the progesterone receptor antigen located in the nucleus of progesterone receptor positive normal and neoplastic cells.

Progesterne Receptor Clone (16) is indicated as an aid in the management, prognosis and prediction 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.

Progesterone Receptor Clone (16) is optimized for use on the Leica Biosystems automated BOND-MAX or BOND-III systems using the BOND Polymer Refine Detection kit.

Concentrated Liquid Antibody Format

For *in vitro* diagnostic use.

Novocastra Liquid Mouse Monoclonal Antibody Progesterone Receptor Clone (16) (Concentrated Liquid Antibody Format) is intended to be used for the qualitative identification by light microscopy of human progesterone receptor in formalin-fixed, paraffin-embedded tissue by immunohistochemical staining. Progesterone Receptor Clone (16) specifically binds to the progesterone receptor antigen located in the nucleus of progesterone receptor positive normal and neoplastic cells.

Progesterone Receptor Clone (16) Monoclonal Antibody (Concentrated Liquid Antibody Format) is indicated as an aid in the management, prognosis and prediction 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.

C. Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D. Special Instrument Requirements:
BOND-MAX and BOND-III staining platforms.

IV. Device/System Characteristics:

A. Device Description:

BOND Ready-to-Use Primary Antibody Progesterone Receptor (16), Novocastra Liquid Mouse Monoclonal Antibody Progesterone Receptor Clone 16 (Concentrated Liquid Antibody Format)

- Progesterone Receptor Clone (16) or PR (16) 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 preservative. This antibody is utilized to perform a qualitative IHC assay to identify Progesterone Receptor (PR) expression in human breast cancer tissue routinely processed and paraffin-embedded for histological examination. There are two configurations of the RTU antibody: 7 mL and 30 mL.
- PR (16) Primary Antibody is provided in a Ready-to- Use (RTU) and a concentrated liquid format. The RTU format is supplied in Tris buffered saline with carrier protein, containing 0.35% ProClin™ 950 as a preservative and is provided in two volumes (7 mL and 30 mL). The total protein concentration is approximately 10 mg/mL and the total antibody concentration is greater than or equal to 1 mg/L as determined by ELISA. The RTU format is optimally diluted for use on the automated BOND-MAX and BOND-III instrument staining platforms in combination with BOND Polymer Refine Detection kit. The concentrated liquid format is for manual staining protocols.
- The concentrated liquid antibody format is a liquid tissue culture supernatant containing 15 mM sodium azide as a preservative. The total protein concentration is determined on a per batch basis and is described on the vial label, and the antibody concentration is greater than or equal to 324.0 mg/L as determined by ELISA.

BOND-III Instrument

- The BOND-MAX and BOND-III instruments are fully automated slide stainers that perform automated deparaffinization (dewaxing), antigen retrieval, IHC staining/*in situ* hybridization (ISH) staining, and counterstaining. The major components of the BOND staining platforms are the processing module, computer (BOND controller), handheld ID scanner, and slide label printer. The BOND staining platforms are composed of a number of discrete software components including the BOND

application software, BOND instrument/processing module software, BOND service software, and Laboratory interface system - integration package (LIS-IP).

B. Principle of Operation:

Immunohistochemical techniques are used to demonstrate the presence of antigens in tissue and cells. The recommended staining protocol for PR (16) primary antibody is IHC Protocol F: Heat induced epitope retrieval is recommended using Bond Epitope Retrieval Solution 2 for 20 minutes. Bond Polymer Refine Detection utilizes a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates.

Bond Polymer Refine Detection works as follows:

- The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity.
- Bond RTU Primary Antibody PR (16) is applied.
- A post primary antibody solution enhances penetration of the subsequent polymer reagent
- A poly-HRP anti-mouse/rabbit IgG reagent localizes the primary antibody.
- The substrate chromogen, 3,3'- diaminobenzidine (DAB), visualizes the complex via a brown precipitate.
- Hematoxylin (blue) counterstaining allows the visualization of cell nuclei using Bond Polymer Refine Detection in combination with the automated BOND-MAX system reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Table 1: PR IHC Staining Protocol F on BOND-III and BOND-MAX Instruments

BOND Protocol	Step No	Reagent	Temperature (°C)	Incubation Time (min:sec)
IHC Protocol F	1	Peroxide Block	Ambient	05:00
	2	BOND Wash Solution	Ambient	00:00
	3	BOND Wash Solution	Ambient	00:00
	4	BOND Wash Solution	Ambient	00:00
	5	PRIMARY Antibody (PGR)	Ambient	15:00
	6	BOND Wash Solution	Ambient	00:00
	7	BOND Wash Solution	Ambient	00:00
	8	BOND Wash Solution	Ambient	00:00
	9	Post Primary	Ambient	08:00
	10	BOND Wash Solution	Ambient	02:00
	11	BOND Wash Solution	Ambient	02:00
	12	BOND Wash Solution	Ambient	02:00
	13	Polymer Detection	Ambient	08:00
	14	BOND Wash Solution	Ambient	02:00
	15	BOND Wash Solution	Ambient	02:00

BOND Protocol	Step No	Reagent	Temperature (°C)	Incubation Time (min:sec)
	16	Deionized Water	Ambient	00:00
	17	Mixed DAB Refine	Ambient	00:00
	18	Mixed DAB Refine	Ambient	10:00
	19	Deionized Water	Ambient	00:00
	20	Deionized Water	Ambient	00:00
	21	Deionized Water	Ambient	00:00
	22	Hematoxylin	Ambient	05:00
	23	Deionized Water	Ambient	00:00
	24	BOND Wash Solution	Ambient	00:00
	25	Deionized Water	Ambient	00:00

C. Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☒	☐
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

1. Instrument Name:
   Leica BOND-III staining instrument.

2. Specimen Identification:
   Specimen identification is performed through the BOND Application Software which is the primary interface to the BOND instrument.

3. Specimen Sampling and Handling:
   Tissue sections (glass slides) are prepared from formalin-fixed, paraffin-embedded (FFPE) breast biopsy specimens. Slides are placed on the BOND-III staining instrument for staining with the BOND RTU Primary Antibody PR (16).

4. Calibration:
   The initial calibration of the staining instrument is performed by Leica Biosystems Melbourne Pty Ltd. manufacturing facility before shipping to the customer site. Leica field service engineers perform calibration and service through the BOND Service software.

5. Quality Control:

Positive (endometrium or weakly positive breast carcinoma) and negative (tonsil) controls should be performed with each staining run. The pathologist is responsible for assuring that the assay is performing appropriately per instructions for use.

V. Substantial Equivalence Information:

A. Predicate Device Name(s):
BOND Ready-to-Use Primary Antibody Progesterone Receptor (16), Novocastra Liquid Mouse Monoclonal Antibody Progesterone Receptor Clone 16

B. Predicate 510(k) Number(s):
K171753

C. Comparison with Predicate(s):

Device & Predicate Device(s):	K193393	K171753
Device Trade Name	BOND Ready-to-Use Primary Antibody Progesterone Receptor (16), Novocastra Liquid Mouse Monoclonal Antibody Progesterone Receptor Clone 16 (Concentrated Liquid Antibody Format)	BOND Ready-to-Use Primary Antibody Progesterone Receptor (16), Novocastra Liquid Mouse Monoclonal Antibody Progesterone Receptor Clone 16
General Device Characteristic Similarities		
Intended Use/Indications for Use	Qualitative identification of human Progesterone Receptor in breast cancer patients	Same
Antibody Type	Mouse monoclonal	Same
Isotype	IgG1	Same
PR Clone	16	Same
Immunogen	A prokaryotic recombinant protein corresponding to the N-terminal region of the A form of the human progesterone receptor	Same
Storage	2-8^0 C	Same
Technology	Immunohistochemistry	Same
Tissue Type	Formalin-fixed paraffin-embedded breast cancer tissue	Same

Staining Pattern	Nuclear	Same
Staining Protocol	IHC Protocol F	Same
General Device Characteristic Differences		
Staining Instrument	BOND-III & BOND-MAX	BOND-MAX
RTU Antibody Progesterone Receptor (16) Configuration	7 mL & 30 mL	7 mL

VI. Standards/Guidance Documents Referenced:

21 CFR 864.1860 – Immunohistochemistry reagents and kits

VII. Performance Characteristics (if/when applicable):

A. Analytical Performance:

1. Precision/Reproducibility:

The sponsor conducted repeatability and reproducibility studies to support the performance of the device.

Precision (Repeatability)

The objective of this study was to evaluate the precision (repeatability) of the Leica BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) on BOND-III instrument.

a. Within-run repeatability

The within-run study involved testing of sectioned slides from 6 unique FFPE breast tumor tissue specimens. The slides consisted of 3 PR negative specimens and 3 PR positive specimens including 1 PR staining around the cut-off (1-10% staining). The study was conducted using one reagent lot on one BOND-III instrument at one laboratory testing site. Three replicates from each of the 6 unique breast tumor tissue cases plus a tissue control slide were stained.

Acceptance Criteria:

All PR negative cases must be scored negative using the recommended retrieval protocol conditions.

All PR low positive cases must be scored low positive using the recommended retrieval protocol conditions.

All PR high positive cases must be scored high positive using the recommended retrieval protocol conditions.

Table 2: Within-run Repeatability Study Results

	PPA		NPA		OPA	
	Estimate (count)	95% CI	Estimate (count)	95% CI	Estimate (count)	95% CI

Run 1	94.4% (17/18)	[74.2 – 99.0%]	88.9% (8/9)	[56.5 – 98.0%]	92.6% (25/27*)	[76.6 - 97.9%]
Run 2	100% (9/9)	[70.1 – 100%]	100% (17/17)	[81.6 – 100%]	100% (26/26)	[87.1 – 100%]
Overall	96.3% (26/27)	[81.7 - 99.3%]	96.2% (25/26)	[81.1 - 99.3%]	96.2% (51/53*)	[87.2 – 99.0%]

*1 slide could not be assessed by the pathologist due to background staining obscuring the nuclei.
PPA: Positive percent agreement; NPA: Negative percent agreement; OPA: Overall percent agreement

Results:
As shown in Table 2, the Overall Percent Agreement (OPA) was 96.2% (51/53; 95% CI: 87.2% to 99.0%), with Positive Percent Agreement (PPA) of 96.3% (26/27; 95% CI: 81.7% – 99.3%), and Negative Percent Agreement (NPA) of 96.2% (25/26; 95% CI: 81.1% – 99.3%). The study results are acceptable.

b. Between-day repeatability
The between-day study involved testing of sectioned slides from 27 unique FFPE breast tumor tissue specimens. The slides consisted of 13 PR negative specimens, 14 PR positive specimens including 5 PR low positive tissue specimens (1-10% staining). The study was conducted using three BOND-III instruments at one laboratory-testing site. One sectioned slide from each unique breast tumor tissue case (total 27) and three tissue control slides, one per Slide Staining Assembly (SSA) were stained with Lot 1 on Days 1 and 2, Lot 2 on Days 3 and 4, and Lot 3 on Days 5 and 6 on a single BOND-III instrument. Each unique breast tissue case was also stained using 2 additional instruments such that the lots and days tested were varied. BOND-III instrument has 30 slide positions allowing for 9 unique cases and a tissue control to be run on each SSA and for all 27 cases to be stained in a single run. Each day a single case was stained 3 times, once on each of the three instruments. In total, using 3 SSAs per instrument and 3 instruments, 486 measurements were generated (3 instruments x 3 SSAs x 9 slides x 6 days) .
Acceptance Criteria for between-days, between-instruments, between lots, between pathologists and between laboratories studies are as follows:
The Overall Agreement (OA), Positive Agreement (PA) and Negative Agreement (NA), shall be 85% of the lower bound of the two-sided 95% CI when the following sources of potential variability are studied: between-days, between-instruments, between-lots, between-pathologists and between-laboratories.
Note: samples used in between-day, between-instrument and between-lot repeatability studies were pre-screened, pre-characterized and scored by an independent scorer.

Table 3: Between-day Repeatability Study Results

	PPA		NPA		OPA	
	Estimate (count)	95% CI	Estimate (count)	95% CI	Estimate (count)	95% CI
Day1	100% (33/33)	[89.6 – 100%]	91.5% (43/47)	[80.1 - 96.6%]	95% (76/80*)	[87.8 – 98.0%]
Day2	100% (33/33)	[89.6 – 100%]	95.8% (46/48)	[86.0 - 98.8%]	97.5% (79/81)	[91.4 - 99.3%]
Day3	100% (33/33)	[89.6 – 100%]	100% (48/48)	[92.6 – 100%]	100% (81/81)	[95.5 – 100%]
Day4	100% (33/33)	[89.6 – 100%]	100% (48/48)	[92.6 – 100%]	100% (81/81)	[95.5 – 100%]

Day5	100% (33/33)	[89.6 – 100%]	100% (48/48)	[92.6 – 100%]	100% (81/81)	[95.5 – 100%]
Day6	100% (33/33)	[89.6 – 100%]	100% (48/48)	[92.6 – 100%]	100% (81/81)	[95.5 – 100%]
Overall	100% (198/198)	[98.1 – 100%]	97.9% (281/287)	[95.5 – 99.0%]	98.8% (479/485*)	[97.3 - 99.4%]

*1 slide could not be assessed by the pathologist due to poor fixation.
PPA: Positive percent agreement; NPA: Negative percent agreement; OPA: Overall percent agreement

Results:
As shown in Table 3, the OPA was 98.8% (479/485; 95% CI: 97.3% - 99.4%), with PPA of 100% (198/198; 95% CI: 98.1% – 100%), and NPA of 97.9% (281/287; 95% CI: 95.5% – 99.0%). The study results are acceptable.

c. Between-instrument repeatability
The between-instrument study involved testing of sectioned slides from 27 unique FFPE breast tumor tissue specimens. The slides consisted of 13 PR negatives, 14 PR positives including 5 PR low positive tissue specimens (1-10% staining). The study was conducted using three BOND-III instruments at one laboratory-testing site. One sectioned slide from each unique breast tumor tissue case (total 27) and three tissue control slides (one per SSA) were stained with Lot 1 on Days 1 and 2, Lot 2 on Days 3 and 4, and Lot 3 on Days 5 and 6 on a single BOND-III instrument. Each unique breast tissue case was also stained using 2 additional instruments such that lots and days tested varied. BOND-III instrument has 30 slide positions allowing for 9 unique cases and a tissue control to be run on each SSA and for all 27 cases to be stained in a single run. Each day a single case was stained 3 times, once on each of three instruments. In total, using 3 SSAs per instrument and 3 instruments, 486 measurements were generated (3 instruments x 3 SSAs x 9 slides x 6 days).

Table 4: Between-instrument Repeatability Study Results

	PPA		NPA		OPA	
	Estimate (count)	95% CI	Estimate (count)	95% CI	Estimate (count)	95% CI
Instrument #1	100% (66/66)	[94.5 – 100%]	97.9% (93/95)	[92.6 - 99.4%]	98.8% (159/161*)	[95.6 - 99.7%]
Instrument #2	100% (66/66)	[94.5 – 100%]	96.9% (93/96)	[91.2 - 98.9%]	98.1% (159/162)	[94.7 - 99.4%]
Instrument #3	100% (66/66)	[94.5 – 100%]	99.0% (95/96)	[94.3 - 99.8%]	99.4% (161/162)	[96.6 - 99.9%]
Overall	100% (198/198)	[98.1 – 100%]	97.9% (281/287)	[95.5 – 99.0%]	98.8% (479/485*)	[97.3 - 99.4%]

*1 slide could not be assessed by the pathologist due to poor fixation.
PPA: Positive percent agreement; NPA: Negative percent agreement; OPA: Overall percent agreement

Results:
As shown in Table 4, the OPA was 98.8% (479/485; 95% CI: 97.3% - 99.4%), with PPA of 100% (198/198; 95% CI: 98.1% – 100%), and NPA of 97.9% (281/287; 95% CI: 95.5% – 99.0%). The study results are acceptable.

d. Between-lot repeatability

The between-lot study involved testing of sectioned slides from 27 unique FFPE breast tumor tissue specimens. The slides consisted pf 13 PR negatives, 14 PR positives (>10% staining) including 5 PR low positive tissue specimens (1-10% staining). The study was conducted using three BOND-III instruments at one laboratory-testing site. One sectioned slide from each unique breast tumor tissue case (total 27) and three tissue control slides (one per SSA) were stained with Lot 1 on Days 1 and 2, Lot 2 on Days 3 and 4, and Lot 3 on Days 5 and 6 on a single BOND-III instrument. Each unique breast tissue case was also stained using 2 additional instruments such that lots and days tested varied. BOND-III instrument has 30 slide positions allowing for 9 unique cases and a tissue control to be run on each SSA and for all 27 cases to be stained in a single run. Each day a single case was stained 3 times, once on each of three instruments. In total, using 3 SSAs per instrument and 3 instruments, 486 measurements were generated (3 instruments x 3 SSAs x 9 slides x 6 days).

Table 5: Between-lot Repeatability Study Results

	PPA		NPA		OPA	
	Estimate (count)	95% CI	Estimate (count)	95% CI	Estimate (count)	95% CI
Lot# 1	100% (66/66)	[94.5 – 100%]	97.9% (93/95)	[92.6 - 99.4%]	98.8% (159/161)	[95.6 - 99.7%]
Lot# 2	100% (66/66)	[94.5 – 100%]	96.9% (93/96)	[91.2 - 98.9%]	98.1% (159/162)	[94.7 - 99.4%]
Lot# 3	100% (66/66)	[94.5 – 100%]	99.0% (95/96)	[94.3 - 99.8%]	99.4% (161/162)	[96.6 - 99.9%]
Over all	100% (198/198)	[98.1 – 100%]	97.9% (281/287)	[95.5 – 99.0%]	98.8% (479/485)	[97.3 - 99.4%]
*1 slide (RT-3542, sample id: 2017/E896) could not be assessed by the pathologist due to poor fixation. PPA: Positive percent agreement; NPA: Negative percent agreement; OPA: Overall percent agreement						

Results:

As shown in Table 5, the OPA was 98.8% (479/485; 95% CI: 97.3% - 99.4%), with PPA of 100% (198/198; 95% CI: 98.1% – 100%), and NPA of 97.9% (281/287; 95% CI: 95.5% – 99.0%). The study results are acceptable.

Reproducibility Studies

The purpose of the reproducibility study was to generate data in support of the validation of the Leica BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) on the BOND-III instrument at one internal laboratory-testing site and two external laboratory-testing sites in the US. The study assessed the following potential sources of assay variability for the BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) on the BOND-III Instrument:

- Between-pathologist reproducibility
- Between-laboratory reproducibility

The reproducibility panel consisted of slides from 135 cases. Each site stained 1 slide from each of the 135 cases, totaling 405 slides combined. Each slide was read by each of 3 pathologists, totaling 1215 readings combined. Each pathologist (1 per site), read slides from all 3 sites (i.e., each pathologist read a slide from the same case 3 times).

- Inter-pathologist study: The concordance between pairs of pathologists (Pathologist 1 vs. 2, 1 vs. 3 and 2 vs. 3) was calculated by Overall Percent Agreement (OPA) and Average Negative Agreement (ANA). Negative and positive percent agreements (NPA, PPA), were weighted as per their marginal totals. Nonparametric bootstrap methods were used to calculate the confidence interval for APA and ANA to preserve the correlation structure of the pair.
- Inter-laboratory study: A similar concordance analysis between pairs of laboratory-testing sites (Site 1 vs. 2, 1 vs. 3 and 2 vs. 3) was carried out for 135 cases with 3 slides per case totaling 405 slides combined.

Table 6: Reproducibility Study: Between-Pathologist Agreement of PGR Status by PGR B-RTU Antibody on BOND-III – Primary Analyses:

Pathologist	Measure	Number of Agreements	Number of Pairs	% Agreement	95 % CI
Pathologist 1 vs 2	APA	196	209	93.8%	[91.3%-96.0%]
	ANA	182	195	93.3%	[90.6%-95.7%]
	AOA	378	404	93.6%	[91.1%-95.8%]
Pathologist 1 vs 3	APA	210	220	95.5%	[93.3%-97.3%]
	ANA	174	184	94.6%	[92.0%-96.7%]
	AOA	384	404	95.0%	[92.8%-97.0%]
Pathologist 2 vs 3	APA	196	211	92.9%	[90.2%-95.3%]
	ANA	178	193	92.2%	[89.2%-94.8%]
	AOA	374	404	92.6%	[89.9%-95.0%]
All pathologists	APA	602	640	94.1%	[92.0%-95.8%]
	ANA	534	572	93.4%	[91.1%-95.3%]
	AOA	1136	1212	93.7%	[91.7%-95.5%]
Note: Number of pairs for APA and ANA were averaged between the comparison groups (average of row and column sums) since neither one is the reference.					

Results:
As shown in Table 6, the average positive, negative, and overall percent agreements were 94.1%, 93.4% and 93.7%, respectively. The results met the study acceptance criteria.

Table 7: Reproducibility Study: Between-laboratory Agreement of PGR Status by PGR B-RTU Antibody on BOND-III – Primary Analyses:

Laboratories	Measure	Number of Agreements	Number of Pairs	% Agreement	95 % CI
Lab 1 vs 2	APA	203	211	96.2%	[94.2%-98.0%]
	ANA	183	191	95.8%	[93.6%-97.7%]
	AOA	386	402	96.0%	[94.0%-97.8%]
Lab 1 vs 3	APA	206	215	95.8%	[93.7%-97.6%]
	ANA	181	190	95.3%	[92.9%-97.3%]
	AOA	387	405	95.6%	[93.6%-97.5%]
Lab 2 vs 3	APA	206	213.5	96.5%	[94.6%-98.2%}
	ANA	181	188.5	96.0%	[93.9%-97.9%]
	AOA	387	402	96.3%	[94.3%-98.0%]

All Labs	APA	615	693.5	96.2%	[94.5%-97.6%]
	ANA	545	569.5	95.7%	[93.9%-97.3%]
	AOA	1160	1209	95.9%	[94.3%-97.4%]
Note: Number of pairs for APA and ANA were averaged between the comparison groups (average of row and column sums) since neither one is the reference.					

Results:
As shown in Table 7, the average positive, negative, and overall percent agreements were 96.2%, 95.7%, and 95.9%, respectively. These results met the study acceptance criteria.

2. Linearity:
Not applicable.

3. Analytical Specificity/Interference:
Analytical specificity and interference studies were provided in the original 510k notification. Please refer to K062615 for more details.

4. Assay Reportable Range:
Not applicable.

5. Stability, Expected Values (Controls, Calibrators, or Methods):
The original stability studies on BOND Ready-to-Use Primary Antibody Progesterone Receptor (16), 7 mL, were submitted in K062615. The sponsor submitted the continued stability studies on the existing 7 mL, and the additional stability studies and justification on the new 30 mL configuration, including real-time, transport, in use and cut section stability studies in Section 14 of the K193393:
Three batches (lots) of antibody in immediate packaging (batches 1, 2 and 3) were manufactured and then stored under recommended storage conditions (4^0C). All three lots were used in the Real-Time Stability Test to determine the shelf-life of the BOND Ready-to-Use Primary Antibody Progesterone Receptor (16). In addition to Real-Time Stability testing, all units of batch 1 underwent a transport simulation test. Following the completion of the transport simulation test, units from lot 1 were used for the In-Use test and the Accelerated Stability Testing. Stability studies were performed on both BOND-MAX and BOND-III systems.

Results and Conclusion:
- Stability study data support the claim for 7 mL Bond RTU Primary Antibody Progesterone Receptor (16), for shelf life of 18 months (545 days) from the point of manufacture when stored at 2-8°C.
- Stability study data support the claim for 30 mL Bond RTU Primary Antibody Progesterone Receptor (16), for shelf life of 18 months (545 days) from the point of manufacture when stored at 2-8°C.

6. Detection Limit:
Not applicable.

7. Assay Cut-Off:

Scoring: Test results are considered as positive when ≥1% of tumor nuclei are immunoreactive (positive staining) for progesterone receptor and negative when <1% of tumor cells are immunoreactive for progesterone receptor.

8. Accuracy (Instrument):
   Not applicable.

9. Carry-Over:
   Not applicable.

B. Comparison Studies:

a. Method Comparison with the Predicate Device:

The objective of the method comparison study was to evaluate the concordance of results obtained using BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) on BOND-III (subject device), with results obtained using BOND Ready-to-Use Primary Antibody Progesterone Receptor (16), (K171753), on BOND-MAX (predicate device, K062615). The study was conducted at three laboratory-testing sites. Each site tested 152 of 456 unique FFPE breast whole tissue sections on the BOND-III instrument, and 152 on the BOND-MAX (two unstained slides were received per each of 152 specimens). One trained pathologist interpreted the stained slides at each site. Positive (endometrium) and negative (tonsil) tissue controls were used in the study.
Scoring:
Tissue is considered "PR Positive" when ≥1% of tumor nuclei are immunoreactive for PR. When <1% of tumor cells are immunoreactive for PR, the tissue is considered "PR Negative".
Acceptance criteria:
The lower bounds of the 2-sided 95% confidence interval BOND-III of the OPA, PPA and NPA shall be ≥ 85% when using BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) on BOND-III compared to BOND-MAX.

Table 8: Method Comparison Study: Percent Agreement Between PR Status by BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) on BOND-III and BOND Ready-to-Use Primary Antibody Progesterone Receptor (16) on BOND-MAX – Primary Analysis:

Measure	Number of Agreements	Number of Pairs	% Agreement	95% CI
PPA	213	223	95.5%	[91.9% - 97.5%]
NPA	222	232	95.7%	[92.2% - 97.6%]
OPA	435	455	95.6%	[93.3% - 97.1%]

Table 9: Method Comparison Study: 2 by 2 Contingency Table – Primary Analysis

		BOND-MAX		
		Negative	Positive	Total
BOND-III	Negative	222	10	232

	Positive	10	213	223
	Total	**232**	**223**	**455**

Results:
As shown in Table 8, the positive, negative and overall percent agreement were 95.5%, 95.7% and 95.6%, respectively. These results indicate PR (16) staining using the BOND-III is comparable to PR (16) staining using the BOND-MAX.

b. Matrix Comparison:
Not applicable.

C. Clinical Studies:

a. Clinical Sensitivity:
Not applicable.

b. Clinical Specificity:
Not applicable.

c. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
Not applicable.

D. Clinical Cut-Off:
Tissue is considered as positive" when ≥1% of tumor nuclei are immunoreactive (positive staining), and negative when <1% of tumor cells are immunoreactive for progesterone receptor.

E. Expected Values/Reference Range:
Not applicable.

F. Other Supportive Instrument Performance Characteristics Data:
Not applicable.

VIII. Proposed Labeling:

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

IX. Conclusion:

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