February 21, 2023

Leica Biosystems Newcastle, Ltd.
Christine Kishi
Staff Regulatory Affairs Specialist
Balliol Business Park West, Benton Lane
Newcastle Upon Tyne, NE12 8EW
United Kingdom

Re: K213348
Trade/Device Name: BOND MMR Antibody Panel
Regulation Number: 21 CFR 864.1866
Regulation Name: Lynch Syndrome Test Systems
Regulatory Class: Class II
Product Code: PZJ
Dated: November 10, 2022
Received: November 23, 2022

Dear Christine Kishi:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Zivana Tezak-fragale -S

Zivana Tezak, PhD
Branch Chief
Division of Molecular Genetics
and Pathology
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K213348

Device Name
BOND MMR Antibody Panel

Indications for Use *(Describe)*
The BOND MMR Antibody Panel is intended to be used for the qualitative identification by light microscopy of human mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 in formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue sections by immunohistochemical staining. The BOND MMR Antibody Panel includes BOND Ready-to-Use Primary Antibody MLH1 (Mismatch Repair Protein) (ES05), BOND Ready-to-Use Primary Antibody MSH2 (Mismatch Repair Protein) (79H11), BOND Ready-to-Use Primary Antibody MSH6 (Mismatch Repair Protein) (EP49) and BOND Ready-to-Use Primary Antibody PMS2 (Mismatch Repair Protein) (EP51). The BOND MMR Antibody Panel is intended for use on the BOND-III or BOND-MAX fully automated systems with BOND Polymer Refine Detection.

The BOND MMR Antibody Panel is indicated for the detection of MMR protein deficiency as an aid in the identification of potential hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch Syndrome in patients diagnosed with CRC. Patients with "MMR Loss" results should receive additional diagnostic testing consistent with clinical practice guidelines for diagnosis of Lynch syndrome. The BOND MMR Antibody Panel is not intended for use in indications other than CRC. This test should not be used for diagnosis of CRC.

The clinical interpretation of any staining or its absence when using the BOND MMR Antibody Panel should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

The clinical performance of this device to guide treatment of MMR deficient patients has not been established.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

1. COMPANY AND CONTACT INFORMATION

Company Name:	Leica Biosystems Newcastle Ltd.
Address:	Balliol Business Park Benton Lane Newcastle upon Tyne UK NE12 8EW
Telephone:	(+44)-191-215-0567
Contact Person:	Christine Kishi Staff Regulatory Affairs Specialist
Date of Submission:	September 30, 2021

2. DEVICE IDENTIFICATION

Trade (Proprietary) Name:	Leica Biosystems BOND MMR Antibody Panel
Common (Usual) Name:	BOND MMR Antibody Panel
Classification Name:	Lynch Syndrome Test System
Product code:	PZJ
Regulation Number:	21 CFR Part 864.1866
Regulatory Class:	Class II
Panel:	88 - Pathology

3. Predicate Device

VENTANA MMR IHC Panel

4. Device Description

4.1 BOND MMR Antibody Panel

The BOND MMR Antibody Panel [subject device] consists of the following BOND Ready-to-Use (RTU) Primary Antibody (PA) products:

- MLH1 (Mismatch Repair Protein) (ES05) (PA0988-U)
- MSH2 (Mismatch Repair Protein) (79H11) (PA0989-U)
- MSH6 (Mismatch Repair Protein) (EP49) (PA0990-U)
- PMS2 (Mismatch Repair Protein) (EP51) (PA0991-U)

The BOND MMR Antibody Panel is intended for use on the BOND-III or BOND-MAX fully automated systems with BOND Polymer Refine Detection (DS9800). The BOND MMR Antibody Panel is indicated for the detection of mismatch repair protein deficiency as an aid in the identification of potential Hereditary Non-Polyposis Colorectal Cancer (HPNCC)/Lynch Syndrome in patients diagnosed with CRC.

MLH1 (Mismatch Repair Protein) (ES05) 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 and in a total volume of 7 mL. The antibody is optimally diluted for use on the automated BOND-MAX or BOND-III instrument staining platforms in combination with BOND Polymer Refine Detection (DS9800).

MSH2 (Mismatch Repair Protein) (79H11) 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 and in a total volume of 7ml. The antibody is optimally diluted for use on the automated BOND-MAX or BOND-III instrument staining platforms in combination with BOND Polymer Refine Detection (DS9800).

MSH6 (Mismatch Repair Protein) (EP49) is a rabbit anti-human monoclonal antibody produced as an affinity-purified tissue culture supernatant, and supplied in Tris buffered saline with carrier protein, containing 0.35 % ProClin™ 950 as a preservative and in a total volume of 7ml. The antibody is optimally diluted for use on the automated BOND-MAX or BOND-III instrument staining platforms in combination with BOND Polymer Refine Detection (DS9800).

PMS2 (Mismatch Repair Protein) (EP51) is a rabbit anti-human monoclonal antibody produced as an affinity purified tissue culture supernatant, and supplied in Tris buffered saline with carrier protein, containing 0.35 % ProClin™ 950 as a preservative and in a total volume of 7ml. The antibody is optimally diluted for use on the automated BOND-MAX or BOND-III instrument staining platforms in combination with BOND Polymer Refine Detection (DS9800).

Instrument and Software: The BOND-MAX and BOND-III instruments are fully automated slide stainers that perform automated deparaffinization (dewaxing), antigen retrieval, immunohistochemistry (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).

4.2 Test Principle

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells. MLH1 (Mismatch Repair Protein) (ES05), MSH2 (Mismatch Repair Protein) (79H11), MSH6 (Mismatch Repair Protein) (EP49) and PMS2 (Mismatch Repair Protein) (EP51) primary antibodies are Ready-to-Use products that have been specifically optimized for use on the automated BOND-MAX or BOND-III systems in combination with BOND Polymer Refine Detection.

The BOND Polymer Refine Detection Process is as follows:

- The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity.
- The BOND Ready- to-Use Primary Antibody 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.

4.3 Summary and Explination

Colorectal cancer (CRC) is the third most common cancer with 1.8 million new cases diagnosed globally in 2018.[1] While histopathologic features provide primary indications of pathogenesis, a more detailed understanding of the genetic drivers of this disease have emerged in recent years. A significant factor in approximately 15% of colorectal cancer cases is the presence of defects in the mismatch repair (MMR) system that result in microsatellite instability (MSI).[2,3,4] MSI has both prognostic and predictive significance and in about 12% of colorectal cancers it arises from epigenetic silencing or somatic mutations of MMR genes. The remaining 3% of cases are often referred to as hereditary non-polyposis colorectal cancer (HNPCC) and are associated with Lynch Syndrome (LS), one of the most common cancer susceptibility syndromes.[3]

Lynch Syndrome is caused by germline mutations in at least one of the four DNA MMR genes - MLH1, MSH2, MSH6 and PMS2.[2,3] The MMR proteins encoded by these genes play a vital role in maintaining genetic integrity during the cell cycle. Disruption of their function leads to a failure in the repair of errors made during DNA replication, an accumulation of which can lead to MSI and promote carcinogenesis. Lynch syndrome is associated with a 50-70% lifetime risk of colorectal cancer and an increased risk of other malignancies. Identification of such high risk individuals is vital so that surveillance and screening programs for family members can be initiated.[2,3,4]

4.4 System Components

4.4.1 System Overview

The Test Components/Accessories of the BOND MMR Antibody Panel System are summarized below:

Components of the BOND MMR Antibody Panel System			
BOND Ready-to-Use (RTU) Primary Antibodies	**BOND Detection System**	**BOND Ancillary Reagents/BOND Consumables**	**BOND Platforms**

• **BOND MMR Antibody Panel**
  ○ **MLH1 (Mismatch Repair Protein) (ES05) (PA0988-U)**
  ○ **MSH2 (Mismatch Repair Protein) (79H11) (PA0989-U)**
  ○ **MSH6 (Mismatch Repair Protein) (EP49) (PA0990-U)**
  ○ **PMS2 (Mismatch Repair Protein) (EP51) (PA0991-U)**
• **Negative (Mouse) BOND Ready-to-Use Negative Control (PA0996)**
• **Negative (Rabbit) BOND Ready-to-Use Negative Control (PA0777)**
	• **BOND Polymer Refine Detection (DS9800)**	• **BOND Wash Solution 10X (AR9590) Concentrate**
		• **BOND Dewax Solution (AR9222)**
		• **BOND Epitope Retrieval Solution 1 (AR9961)**
		• **BOND Epitope Retrieval Solution 2 (AR9640)**
		• **BOND Universal Covertile (S21.4611 & S21.2001)**
			• **BOND-MAX Instrument**
			• **BOND-III Instrument**

4.4.2 Materials Provided with the BOND MMR Antibody Panel

The BOND MMR Antibody Panel consists of the following BOND Ready-to-Use (RTU) Primary Antibody (PA) products:

- MLH1 (Mismatch Repair Protein) (ES05) (PA0988-U)
- MSH2 (Mismatch Repair Protein) (79H11) (PA0989-U)
- MSH6 (Mismatch Repair Protein) (EP49) (PA0990-U)
- PMS2 (Mismatch Repair Protein) (EP51) (PA0991-U)

4.4.3 Materials Not provided with the BOND MMR Antibody Panel

Reagents required but not provided with the BOND MMR Antibody Panel are listed below:

- BOND Ready-to-Use Negative Control Negative (Mouse) (Catalog No. PA0996)
- BOND Ready-to-Use Negative Control Negative (Rabbit) (Catalog No.

PA0777)
- BOND Polymer Refine Detection (Catalog No. DS9800)
- BOND Dewax Solution (Catalog No. AR9222)
- BOND Epitope Retrieval Solution 1 (Catalog No. AR9961)
- BOND Epitope Retrieval Solution 2 (Catalog No. AR9640)
- BOND Wash Solution 10X Concentrate (Catalog No. AR9590)

4.5 Controls

4.5.1 Internal Positive Tissue Control

Normal epithelial cells in the vicinity of the tumor or infiltrating lymphocytic cells, fibroblasts, nerves and muscle integral to the patient specimen can serve as an internal positive tissue control for MLH1 (Mismatch Repair Protein) (ES05) as they should exhibit expression of MLH1 even if the tumor in the patient specimen is deficient for the protein. The presence of staining elicited by MLH1 (Mismatch Repair Protein) (ES05) in an internal positive tissue control indicates the following:

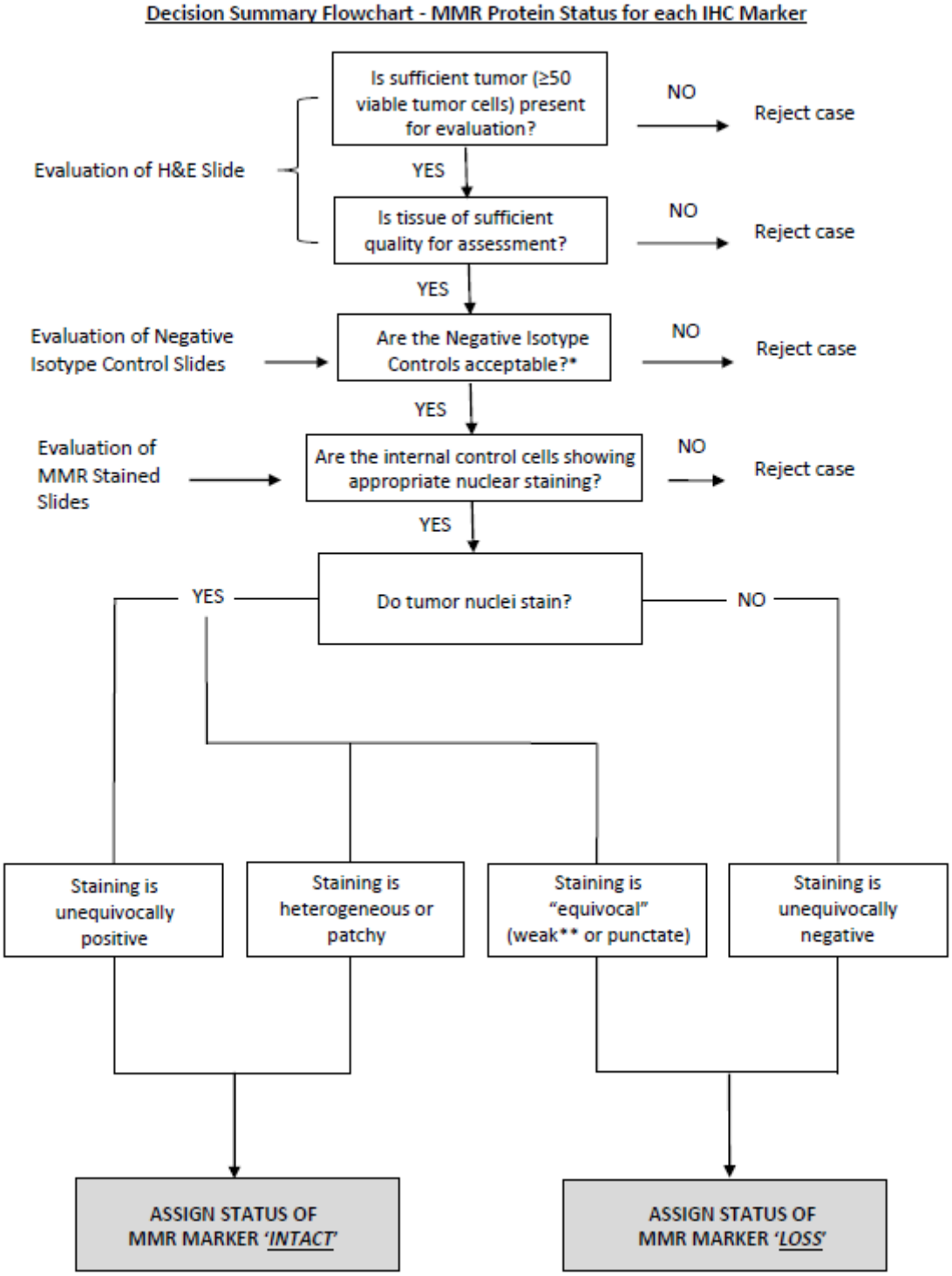
1. The tissue of the patient specimen has been subjected to suitable processing and fixation to allow the epitope of the target protein to be detected via IHC.
2. The staining procedure has been performed correctly on the slide mounted with the patient specimen.
3. The primary antibody is performing as intended and staining the corresponding protein.

4.5.2 Negative Reagent Control

For each patient specimen, a negative reagent control must be used to stain an additional slide-mounted tissue section to that stained with the primary antibody. This will determine whether the primary antibody is reacting with the patient tissue in a non-specific manner. BOND Ready-to-Use Negative Control Negative (Mouse) is recommended as the negative reagent control for use in conjunction with MLH1 (Mismatch Repair Protein) (ES05) and MSH2 (Mismatch Repair Protein) (79H11). BOND Ready-to-Use Negative Control Negative (Rabbit) is recommended as the negative reagent control for use in conjunction with MSH6 (Mismatch Repair Protein) (EP49) and PMS2 (Mismatch Repair Protein) (EP51).

4.6 Interpretation of Results

Hematoxylin and Eosin stained slides are used to assess the overall quality of the sample for each case. An assessment should be made of the quality and quantity of the tumor content of the CRC sample. Cases should be voided if there is insufficient tumor (less than 50 viable tumor cells) available to score or if the sample is poorly fixed or otherwise of a poor enough quality that renders interpretation of the case impossible. The MMR protein status for each IHC marker shall be assigned following the decision summary illustrated in the flowchart below.



*Any staining seen in the Negative Isotype Controls should be taken into account when interpreting the MMR antibody stained test sections. If any staining is judged to be of sufficient intensity to adversely affect the interpretation of the test sections then this should be classed as unacceptable and the case voided.

** Weaker than internal positive control cells

To obtain the BOND MMR Antibody Panel results, each individual marker (MLH1, MSH2, MSH6 and PMS2) will be scored as protein loss or protein intact according to the device's scoring criteria. The loss of one or more of these proteins within one specimen will be considered an MMR loss. To obtain an intact panel result, all four proteins must be scored as intact.

5. Intended Use/Indications for Use

The BOND MMR Antibody Panel is intended to be used for the qualitative identification by light microscopy of human mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 in formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue sections by immunohistochemical staining. The BOND MMR Antibody Panel includes BOND Ready-to-Use Primary Antibody MLH1 (Mismatch Repair Protein) (ES05), BOND Ready-to-Use Primary Antibody MSH2 (Mismatch Repair Protein) (79H11), BOND Ready-to-Use Primary Antibody MSH6 (Mismatch Repair Protein) (EP49) and BOND Ready-to-Use Primary Antibody PMS2 (Mismatch Repair Protein) (EP51). The BOND MMR Antibody Panel is intended for use on the BOND-III or BOND-MAX fully automated systems with BOND Polymer Refine Detection.

The BOND MMR Antibody Panel is indicated for the detection of MMR protein deficiency as an aid in the identification of potential hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch Syndrome in patients diagnosed with CRC. Patients with "MMR Loss" results should receive additional diagnostic testing consistent with clinical practice guidelines for diagnosis of Lynch syndrome. The BOND MMR Antibody Panel is not intended for use in indications other than CRC. This test should not be used for diagnosis of CRC.

The clinical interpretation of any staining or its absence when using the BOND MMR Antibody Panel should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

The clinical performance of this device to guide treatment of MMR deficient patients has not been established.

Limitations

- MLH1 (Mismatch Repair Protein) (ES05), MSH2 (Mismatch Repair Protein) (79H11), MSH6 (Mismatch Repair Protein) (EP49), and PMS2 (Mismatch Repair Protein) (EP51) have been solely cleared for use on the BOND-III and BOND-Max instrument with the BOND Polymer Refine Detection Kit and is not cleared with any other automated staining instruments or detection methods.
- MLH1 (Mismatch Repair Protein) (ES05), MSH2 (Mismatch Repair Protein) (79H11), MSH6 (Mismatch Repair Protein) (EP49), and PMS2 (Mismatch Repair Protein) (EP51) have been optimized at Leica Biosystems for use with BOND Polymer Refine Detection and BOND ancillary reagents.
- The effect of retrieval, incubation times or temperatures other than those specified is unknown and may give erroneous results. Users who deviate from the listed protocol must accept responsibility for interpretation of patient results.
- The protocol times may vary, due to variation in tissue fixation and the effectiveness of antigen enhancement, and must be determined empirically.

Negative reagent controls should be used when optimizing retrieval conditions and protocol times.

- For staining interpretation, there must be ≥50 viable tumor cells present for evaluation.
- The stratification of MMR loss cases via BRAF is not recommended as the presence of BRAFV600E does not definitively exclude Lynch Syndrome as a possible aetiologyetiology. Use of this test with a BRAF test may lead to increased false negative results.
- Test Results obtained using the product must be interpreted by healthcare professionals in conjunction with other clinical findings, family history and other laboratory data.
- The clinical performance of this device to guide treatment of MMR deficient patients has not been established.

6. Comparison of technological characteristics with the predicate device

Comparison Table of the BOND MMR Antibody Panel [subject device] and the Ventana MMR IHC Panel (DEN170030) [predicate device]		
Characteristics	**Leica Biosystems BOND MMR Antibody Panel [Subject Device]**	**VENTANA MMR IHC Panel (DEN170030) [Predicate Device]**
	Similarities	
Intended Use/Indications for Use	**For *In Vitro* Diagnostic Use** The BOND MMR Antibody Panel is intended to be used for the qualitative identification by light microscopy of human mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 in formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue sections by immunohistochemical staining. The BOND MMR Antibody Panel includes BOND Ready-to-Use Primary Antibody MLH1 (Mismatch Repair Protein) (ES05), BOND Ready-to-Use Primary Antibody MSH2 (Mismatch Repair Protein) (79H11), BOND Ready-to-Use Primary Antibody MSH6 (Mismatch Repair Protein) (EP49) and BOND Ready-to-Use Primary Antibody PMS2 (Mismatch Repair Protein) (EP51). The BOND MMR Antibody Panel is intended for use on the BOND-III or BOND-MAX fully automated systems with BOND Polymer Refine Detection. The BOND MMR Antibody Panel is indicated for the detection of MMR protein deficiency as an aid in the identification of potential hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch Syndrome in patients diagnosed with CRC. Patients with "MMR Loss" results should receive additional diagnostic testing consistent with clinical practice guidelines for diagnosis of Lynch syndrome. The BOND MMR Antibody Panel is not intended for use in indications other than CRC. This test should not be used for diagnosis of CRC. The clinical interpretation of any staining or its absence when using the BOND MMR Antibody Panel should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. The clinical performance of this device to guide treatment of MMR deficient patients has not been established.	**For *In Vitro* Diagnostic Use** The VENTANA MMR IHC Panel is a qualitative immunohistochemistry (IHC) test intended for use in the light microscopic assessment of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, and MSH6) and BRAF V600E proteins in formalin-fixed, paraffin-embedded colorectal cancer (CRC) tissue sections. The opt iView DAB IHC Detection Kit is used with MLH1, MSH2, MSH6 and BRAF V600E, and the OptiView DAB IHC Detection Kit with OptiView Amplification Kit is used for PMS2 detection. The VENTANA MMR IHC Panel is for use on the VENTANA BenchMark ULTRA instrument. The VENTANA MMR IHC Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody, and VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody. The VENTANA MMR IHC Panel is indicated in patients diagnosed with colorectal cancer (CRC) to detect mismatch repair (MMR) proteins deficiency as an aid in the identification of probable Lynch syndrome and to detect BRAFV600E protein as an aid to differentiate between sporadic CRC and probable Lynch syndrome. Results from the Ventana MMR IHC Panel should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls. The clinical performance of this device to guide treatment of MMR deficient patients has not been established.
Technology	Immunohistochemistry based protein expression	Same

Tissue Type	Formalin-Fixed Paraffin-Embedded Colorectal Cancer Tissue Sections	Same
Assay Type	Qualitative	Same
Staining Pattern	Nuclear	Same
Read/Review Equipment	Light Microscopy	Same
Target Population	Patients diagnosed with CRC	Same
Controls	Internal positive tissue controls and negative reagent controls	External and internal positive tissue controls and negative reagent controls
Result Interpretation	Pathologist assessment	Same
Differences		
Antibody Type	• Anti-MLH1 (ES05): Mouse Monoclonal • Anti-MSH2 (79H11): Mouse Monoclonal • Anti-MSH6 (EP49): Rabbit Monoclonal • Anti-PMS2 (EP51): Rabbit Monoclonal	• Anti-MLH1 (M1): Mouse Monoclonal • Anti-MSH2 (G219-1129): Mouse Monoclonal • Anti-MSH6 (SP93): Rabbit Monoclonal • Anti-PMS2 (A16-4): Mouse Monoclonal • Anti-BRAF V600E (VE1): Mouse Monoclonal
Mismatch Repair (MMR) Protein IHC Panel	• Anti-MLH1 • Anti-MSH2 • Anti-MSH6 • Anti-PMS2	• Anti-MLH1 • Anti-MSH2 • Anti-MSH6 • Anti-PMS2 • Anti-BRAF V600E
Assay Target	4 -Mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2	4 -Mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2, and BRAF6 V600E
Detection Kit	BOND Polymer Refine Detection (DS9800): Biotin-free, polymeric HRP-linker antibody conjugate, for MLH1, MSH2, MSH6 and PMS2.	OptiView DAB IHC Detection Kit: Biotin-free, Multimeric HRP-linker antibody conjugate for MLH1, MSH2, MSH6 and BRAF V600E. OptiView DAB IHC Detection Kit with OptiView Amplification Kit: Biotin-free, Multimeric HRP-linker antibody conjugate, with tyramide- based amplification for PMS2.
Staining Instruments	LBS BOND -MAX and BOND-III Instruments	VENTANA BenchMark ULTRA Instrument

6.1 Comparison Between the Device and Predicate

The BOND MMR Antibody Panel [subject device] and the VENTANA MMR IHC Panel (DEN170030) [predicate device] share the same technological and Immunohistochemistry principles for a Lynch Syndrome Test System.

Based on the data generated from the results of the Non-Clinical Performance Testing and the Clinical Performance Testing conducted on the subject device, it may be concluded that the BOND MMR Antibody Panel [subject device] is as safe and effective, and performs as well as, the legally marketed predicate device, the VENTANA MMR IHC Panel (DEN170030) [predicate device]. The similar Indications for Use / Intended Use, and substantially equivalent usability, functionality, and performance characteristics for the proposed the subject device have been assessed to be substantially equivalent (SE) to the predicate device, and any differences do not raise new or different issues of safety and effectiveness when compared to the predicate device.

6.2 Special Controls

The device meets all General Controls and Special Controls listed for regulation 21CFR 864.1866. Documentation and data supporting the Special Controls specific to this product classification were documented in the submission and summarized in this document and the product label.

7. Performance Characteristics

7.1 Analytical performance

7.1.1 *Precision*

Three studies were performed to evaluate the precision of the BOND MMR Antibody Panel.

i. Precision across Lots and Days on the BOND-III and BOND-MAX Instruments

This study was conducted at 1 site using 3 lots of BOND Ready To Use MMR Primary Antibody for each of the 4 MMR proteins. Slides from 40 FFPE CRC tissue cases, 10 cases per MMR protein, with 5 cases being protein deficient and 5 cases expressing intact protein were tested. Each case was stained 18 times [3 Slide Staining Assembly (SSAs) x 6 days] on 1 BOND-III and 1 BOND-MAX instrument. Testing was performed over 6 nonconsecutive days following a randomized testing order. Therefore, a total of 1440 slides (4 antibodies x 10 samples x 3 SSAs x 2 instruments x 6 days) were evaluated. A single pathologist read and scored all stained slides.

Positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) were calculated, comparing the antibody status of each processed slide determined by the pathologist to the majority score. Point estimates along with 2-sided 95% confidence intervals were calculated separately by instrument and by antibody. All slides from one case for MSH2 staining were excluded from BOND-III data analysis (n = 18) and from BOND-MAX data analysis (n = 18) because the case had insufficient tumor.

Intra-run Repeatability:

Intra-run repeatability was evaluated using 40 FFPE CRC tissue cases, 10 cases per MMR protein, with 5 cases being protein deficient (protein loss) and 5 cases

expressing intact protein (intact). Six (6) slides were stained using each panel antibody. Data were obtained using 1 lot of each panel antibody from 60 total observations (10 cases x 3 replicates x 2 days x 1 instrument) for each panel antibody on BOND-III and from 60 total observations (10 cases x 3 replicates x 2 days x 1 instrument) for each panel antibody on BOND-MAX. Intra-run repeatability OPA was 100% for each panel antibody on both instruments except MSH2 on BOND-III (OPA 98.1%, PPA 100%, NPA 95.8%) and PMS2 on BOND-MAX (OPA 98.3%, PPA 100%, NPA 96.7%). Results for individual panel antibodies are shown in Table 1 below.

Table 1 Summary of Intra-run Precision

Antibody	Agreement on BOND-III				Agreement on BOND-MAX			
	Type	n/N*	%	95% CI	Type	n/N*	%	95% CI
Anti-MLH1 (ES05)	PPA	30/30	100	[88.6% - 100%]	PPA	30/30	100	[88.6% - 100%]
	NPA	30/30	100	[88.6% - 100%]	NPA	30/30	100	[88.6% - 100%]
	OPA	60/60	100	[94.0% - 100%]	OPA	60/60	100	[94.0% - 100%]
Anti-MSH2 (79H11)	PPA	30/30	100	[88.6% - 100%]	PPA	30/30	100	[88.6% - 100%]
	NPA	23/24	95.8	[79.8% - 99.3%]	NPA	24/24	100	[86.2% - 100%]
	OPA	53/54	98.1	[90.2% - 99.7%]	OPA	54/54	100	[93.4% - 100%]
Anti-MSH6 (EP49)	PPA	30/30	100	[88.6% - 100%]	PPA	30/30	100	[88.6% - 100%]
	NPA	30/30	100	[88.6% - 100%]	NPA	30/30	100	[88.6% - 100%]
	OPA	60/60	100	[94.0% - 100%]	OPA	60/60	100	[94.0% - 100%]
Anti-PMS2 (EP51)	PPA	30/30	100	[88.6% - 100%]	PPA	30/30	100	[88.6% - 100%]
	NPA	30/30	100	[88.6% - 100%]	NPA	29/30	96.7	[83.3% - 99.4%]
	OPA	60/60	100	[94.0% - 100%]	OPA	59/60	98.3	[91.1% - 99.7%]

*Number of agreement/Number of pairs

Between-day Precision:
Between-day repeatability was evaluated using the same CRC cases used for intra-run testing. Three (3) slides were stained using each of the 4 panel antibodies across 6 non-consecutive days on BOND-III and BOND-MAX. For Day 1 and 2, the first six slides from each case from Intra-run precision study were used. Data were obtained from 180 total observations (10 cases x 3 replicates x 6 days x 1 instrument) for each panel antibody on BOND-III and from 180 total observations (10 cases x 3 replicates x 6 days x 1 instrument) for each panel antibody on BOND-MAX [Note: MSH2 evaluation was conducted with 4 intact and 5 loss status cases (9 total cases) for a total of 162 observations per instrument since 1 case was excluded due to insufficient tumor]. Between-day repeatability OPA was 100% for each panel antibody on both instruments except MSH2 on BOND-III (OPA 98.8%, PPA 98.9%, NPA 98.6%), MSH6 on BOND-III (OPA 99.4%, PPA 98.9%, NPA 100%) and BOND-MAX (OPA 99.4%, PPA 100%, NPA 98.9%), PMS2 on BOND-III (OPA 98.9%, PPA 98.9%, NPA 98.9%) and BOND-MAX (OPA 99.4%, PPA 100%, NPA 98.9%). The study met pre-specified acceptance criteria of ≥85% lower bound confidence interval. Results for individual panel antibodies are shown in Table 2 below.

Table 2 Summary of Between-day Precision

Antibody	Agreement on BOND-III				Agreement on BOND-MAX			
	Type	n/N*	%	95% CI	Type	n/N*	%	95% CI
Anti-MLH1 (ES05)	PPA	90/90	100	[95.9% - 100%]	PPA	90/90	100	[95.9% - 100%]
	NPA	90/90	100	[95.9% - 100%]	NPA	90/90	100	[95.9% - 100%]
	OPA	180/180	100	[97.9% - 100%]	OPA	180/180	100	[97.9% - 100%]
Anti-MSH2 (79H11)	PPA	89/90	98.9	[94.0% - 99.8%]	PPA	90/90	100	[95.9% - 100%]
	NPA	71/72	98.6	[92.5% - 99.8%]	NPA	72/72	100	[94.9% - 100%]
	OPA	160/162	98.8	[95.6% - 99.7%]	OPA	162/162	100	[97.7% - 100%]
Anti-MSH6 (EP49)	PPA	89/90	98.9	[94.0% - 99.8%]	PPA	90/90	100	[95.9% - 100%]
	NPA	90/90	100	[95.9% - 100%]	NPA	89/90	98.9	[94.0% - 99.8%]
	OPA	179/180	99.4	[96.9% - 99.9%]	OPA	179/180	99.4	[96.9% - 99.9%]
Anti-PMS2 (EP51)	PPA	89/90	98.9	[94.0% - 99.8%]	PPA	90/90	100	[95.9% - 100%]
	NPA	89/90	98.9	[94.0% - 99.8%]	NPA	89/90	98.9	[94.0% - 99.8%]
	OPA	178/180	98.9	[96.0% - 99.7%]	OPA	179/180	99.4	[96.9% - 99.9%]

*Number of agreement/Number of pairs

Between-lot Precision:

The study evaluated lot to lot repeatability with 3 final lots of each of the 4 panel antibodies using the same CRC cases as intra-run testing. Three (3) slides per case were stained using each panel antibody across 6 non-consecutive days (2 days per lot) on BOND-III and BOND-MAX. Data were obtained from 180 total observations (10 cases x 3 replicates x 2 days x 3 lots) for each panel antibody on BOND-III and from 180 total observations (10 cases x 3 replicates x 2 days x 3 lots) for each panel antibody on BOND-MAX. [Note: MSH2 evaluation was conducted with 4 intact and 5 loss status cases (9 cases total) for a total of 162 observations per instrument since 1 case was excluded due to insufficient tumor]. Between-lot repeatability OPA was 100% for each panel antibody on both instruments with the exception of MSH2 on BOND-III (OPA 98.8%, PPA 98.9%, NPA 98.6%), MSH6 on BOND-III (OPA 99.4%, PPA 98.9%) and BOND-MAX (OPA 99.4%, NPA 98.9%), PMS2 on BOND-III (OPA 98.9%, PPA 98.9%, NPA 98.9%) and BOND-MAX (OPA 99.4%, NPA 98.9%). The study met pre-specified acceptance criteria of ≥85% lower bound confidence interval. Results for individual panel antibodies are shown in Table 3 below.

Table 3 Summary of Between-lot Precision

Antibody	Agreement on BOND-III				Agreement on BOND-MAX			
	Type	n/N*	%	95% CI	Type	n/N*	%	95% CI
Anti-MLH1 (ES05)	PPA	90/90	100	[95.9% - 100%]	PPA	90/90	100	[95.9% - 100%]
	NPA	90/90	100	[95.9% - 100%]	NPA	90/90	100	[95.9% - 100%]
	OPA	180/180	100	[97.9% - 100%]	OPA	180/180	100	[97.9% - 100%]
Anti-MSH2 (79H11)	PPA	89/90	98.9	[94.0% - 99.8%]	PPA	90/90	100	[95.9% - 100%]
	NPA	71/72	98.6	[92.5% - 99.8%]	NPA	72/72	100	[94.9% - 100%]
	OPA	160/162	98.8	[95.6% - 99.7%]	OPA	162/162	100	[97.7% - 100%]
Anti-MSH6 (EP49)	PPA	89/90	98.9	[94.0% - 99.8%]	PPA	90/90	100	[95.9% - 100%]
	NPA	90/90	100	[95.9% - 100%]	NPA	89/90	98.9	[94.0% - 99.8%]
	OPA	179/180	99.4	[96.9% - 99.9%]	OPA	179/180	99.4	[96.9% - 99.9%]
Anti-PMS2 (EP51)	PPA	89/90	98.9	[94.0% - 99.8%]	PPA	90/90	100	[95.9% - 100%]
	NPA	89/90	98.9	[94.0% - 99.8%]	NPA	89/90	98.9	[94.0% - 99.8%]
	OPA	178/180	98.9	[96.0% - 99.7%]	OPA	179/180	99.4	[96.9% - 99.9%]

*Number of agreement/Number of pairs

The results support that the BOND MMR Antibody Panel on BOND-III and on BOND-MAX have acceptable levels of precision (repeatability) across lots and days.

ii. Reproducibility across Laboratories and Pathologists on the BOND-III and BOND-MAX Instruments

This study was conducted at 3 sites using 1 lot of the BOND MMR antibody for each of the 4 MMR proteins. Slides from 30 FFPE CRC tissue cases were tested, including cases being protein deficient and cases expressing intact proteins. Each case was stained on 1 BOND-III and 1 BOND MAX instrument per site, for a total of 6 times (3 sites x 2 instruments). Testing was performed on 6 non-consecutive days over a minimum 20-day testing period, following randomized testing orders. A single pathologist per site read and scored all stained slides from that site. In addition, 3 pathologists read and scored a set of 30 cases stained at 1 site in 3 scoring sessions separated by a washout period of at least 14 days.

Table 4 MMR antibody Case Status

Marker/Antibody	Positive/Intact	Deficient/Loss	Total
MLH1	25	5	30
MSH2	27	3	
MSH6	26	4	
PMS2	24	6	

PPA, NPA and OPA were calculated for point estimates along with 2-sided 95% confidence intervals to evaluate intra-pathologist, inter-pathologist, inter-laboratory and inter-instrument precision.

Results of the study are summarized in Table 5 to Table 9. The results support that the BOND MMR Antibody Panel on BOND-III and on BOND-MAX have

acceptable levels of precision (reproducibility).

Intra- and Inter-Pathologist Reproducibility:
Intra-pathologist and inter-pathologist reproducibility were assessed by evaluating concordance of marker status across 3 pathologists and within individual pathologists using 30 cases of CRC stained at 1 site on BOND-III and BOND-MAX. These 30 CRC specimens consisted of varying number of cases with loss or intact MMR protein status as shown in Table 4. Each reader scored all 30 cases in three rounds that were separated by a two week wash out period. Data was obtained from 270 total observations (30 cases x 3 replicate x 3 pathologists) for each of the 4 panel antibodies for BOND-III, and from 270 total observations (30 cases x 3 replicate x 3 pathologists) for each panel antibody for BOND-MAX. Intra-pathologist reproducibility OPA ranged from 99.3% to 100%, PPA ranged from 99.2% to 100%. The results are shown below in Table 5. Inter-pathologist reproducibility OPA ranged from 99.3% to 100%, PPA ranged from 99.2% to 100%. The results are shown below in Table 6. The studies met the pre-specified acceptance criteria.

Table 5 Summary of Intra-Pathologist Reproducibility

Antibody	**Agreement on BOND-III**				**Agreement on BOND-MAX**			
	Type	**n/N***	**%**	**95% CI**	**Type**	**n/N***	**%**	**95% CI**
Overall call	PPA	180/180	100	[97.9% - 100%]	PPA	180/180	100	[97.9% - 100%]
	NPA	90/90	100	[95.9% - 100%]	NPA	90/90	100	[95.9% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]
Anti-MLH1 (ES05)	PPA	225/225	100	[98.3% - 100%]	PPA	225/225	100	[98.3% - 100%]
	NPA	45/45	100	[92.1% - 100%]	NPA	45/45	100	[92.1% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]
Anti-MSH2 (79H11)	PPA	252/252	100	[98.5% - 100%]	PPA	250/252	99.2	[97.2% - 99.8%]
	NPA	18/18	100	[82.4% - 100%]	NPA	18/18	100	[82.4% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	268/270	99.3	[97.3% - 99.8%]
Anti-MSH6 (EP49)	PPA	234/234	100	[98.4% - 100%]	PPA	234/234	100	[98.4% - 100%]
	NPA	36/36	100	[90.4% - 100%]	NPA	36/36	100	[90.4% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]
Anti-PMS2 (EP51)	PPA	216/216	100	[98.3% - 100%]	PPA	216/216	100	[98.3% - 100%]
	NPA	54/54	100	[93.4% - 100%]	NPA	54/54	100	[93.4% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]

*Number of agreement/Number of pairs

Table 6 Summary of Inter-Pathologist Reproducibility

Antibody	Agreement on BOND-III				Agreement on BOND-MAX			
	Type	n/N*	%	95% CI	Type	n/N*	%	95% CI
Overall call	PPA	180/180	100	[97.9% - 100%]	PPA	180/180	100	[97.9% - 100%]
	NPA	90/90	100	[95.9% - 100%]	NPA	90/90	100	[95.9% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]
Anti-MLH1 (ES05)	PPA	225/225	100	[98.3% - 100%]	PPA	225/225	100	[98.3% - 100%]
	NPA	45/45	100	[92.1% - 100%]	NPA	45/45	100	[92.1% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]
Anti-MSH2 (79H11)	PPA	252/252	100	[98.5% - 100%]	PPA	250/252	99.2	[97.2% - 99.8%]
	NPA	18/18	100	[82.4% - 100%]	NPA	18/18	100	[82.4% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	268/270	99.3	[97.3% - 99.8%]
Anti-MSH6 (EP49)	PPA	234/234	100	[98.4% - 100%]	PPA	234/234	100	[98.4% - 100%]
	NPA	36/36	100	[90.4% - 100%]	NPA	36/36	100	[90.4% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]
Anti-PMS2 (EP51)	PPA	216/216	100	[98.3% - 100%]	PPA	216/216	100	[98.3% - 100%]
	NPA	54/54	100	[93.4% - 100%]	NPA	54/54	100	[93.4% - 100%]
	OPA	270/270	100	[98.6% - 100%]	OPA	270/270	100	[98.6% - 100%]

*Number of agreement/Number of pairs

Inter-Instrument Reproducibility:

Instrument to instrument reproducibility was evaluated by one pathologist using the same cases as Inter-pathologist and Intra-pathologist reproducibility. One slide was stained using each of the 4 panel antibodies, one slide was stained with Negative Control (Mouse), and one slide was stained with Negative Control (Rabbit) for each case across 3 different BOND-III and 3 different BOND-MAX instruments. Data was obtained from 90 total observations (30 cases x 1 replicate x 3 instruments) for each panel antibody for BOND-III, and from 90 total observations (30 cases x 1 replicate x 3 instruments) for each panel antibody for BOND-MAX. Inter-instrument reproducibility OPA ranged from 98.9% to 100%, PPA ranged from 98.8% to 100%, NPA ranged from 93.3% to 100%. The study met pre-specified acceptance criteria. Results for the overall panel and each panel antibody on BOND-III and BOND-MAX are shown in Table 7 below.

Table 7 Summary of Inter-Instrument Reproducibility

Antibody	Agreement on BOND-III				Agreement on BOND-MAX			
	Type	n/N*	%	95% CI	Type	n/N*	%	95% CI
Overall call	PPA	60/60	100	[94.0% - 100%]	PPA	60/60	100	[94.0% - 100%]
	NPA	30/30	100	[88.6% - 100%]	NPA	29/30	96.7	[83.3% - 99.4%]
	OPA	90/90	100	[95.9% - 100%]	OPA	89/90	98.9	[94.0% - 99.8%]
Anti-MLH1 (ES05)	PPA	75/75	100	[95.1% - 100%]	PPA	75/75	100	[95.1% - 100%]
	NPA	15/15	100	[79.6% - 100%]	NPA	14/15	93.3	[70.2% - 98.8%]
	OPA	90/90	100	[95.9% - 100%]	OPA	89/90	98.9	[94.0% - 99.8%]
Anti-MSH2 (79H11)	PPA	84/84	100	[95.6% - 100%]	PPA	83/84	98.8	[93.6% - 99.8%]
	NPA	6/6	100	[61.0% - 100%]	NPA	6/6	100	[61.0% - 100%]
	OPA	90/90	100	[95.9% - 100%]	OPA	89/90	98.9	[94.0% - 99.8%]
Anti-MSH6 (EP49)	PPA	78/78	100	[95.3% - 100%]	PPA	78/78	100	[95.3% - 100%]
	NPA	12/12	100	[75.8% - 100%]	NPA	12/12	100	[75.8% - 100%]
	OPA	90/90	100	[95.9% - 100%]	OPA	90/90	100	[95.9% - 100%]
Anti-PMS2 (EP51)	PPA	72/72	100	[94.9% - 100%]	PPA	72/72	100	[94.9% - 100%]
	NPA	18/18	100	[82.4% - 100%]	NPA	17/18	94.4	[74.2% - 99.0%]
	OPA	90/90	100	[95.9% - 100%]	OPA	89/90	98.9	[94.0% - 99.8%]

*Number of agreement/Number of pairs

Inter-laboratory Reproducibility:

The reproducibility of the BOND MMR Antibody Panel was assessed at 3 sites using the same cases as Inter-pathologist and Intra-pathologist reproducibility. Multiple tissue sections were cut from each case. Three (3) sites (2 external and 1 internal) stained all cases with the designated antibody and the appropriate negative reagent control (NRC) antibody spanning a period of at least 20 days. One pathologist at each site independently evaluated each case to determine the status (intact or loss).

Data was obtained from 90 total observations (30 cases x 1 replicate x 3 sites) for each of the 4 panel antibodies for BOND-III, and from 90 total observations (30 cases x 1 replicate x 3 sites) for each panel antibody for BOND-MAX. Reproducibility OPA ranged from 98.9% to 100%, PPA ranged from 98.8% to 100%. The study met pre-specified acceptance criteria. Results for the overall panel and each panel antibody on BOND-III and BOND-MAX are shown in Table 8 below.

Table 8 Summary of Inter-Laboratory Reproducibility

Antibody	Agreement on BOND-III				Agreement on BOND-MAX			
	Type	n/N*	%	95% CI	Type	n/N*	%	95% CI
Overall call	PPA	59/59	100	[93.9% - 100%]	PPA	60/60	100	[94.0% - 100%]
	NPA	30/30	100	[88.6% - 100%]	NPA	30/30	100	[88.6% - 100%]
	OPA	89/89	100	[95.9% - 100%]	OPA	90/90	100	[95.9% - 100%]
Anti-MLH1 (ES05)	PPA	74/74	100	[95.1% - 100%]	PPA	75/75	100	[95.1% - 100%]
	NPA	15/15	100	[79.6% - 100%]	NPA	15/15	100	[79.6% - 100%]
	OPA	89/89	100	[95.9% - 100%]	OPA	90/90	100	[95.9% - 100%]
Anti-MSH2 (79H11)	PPA	83/83	100	[95.6% - 100%]	PPA	83/ 84	98.8	[93.6% - 99.8%]
	NPA	6/6	100	[61.0% - 100%]	NPA	6/6	100	[61.0% - 100%]
	OPA	89/89	100	[95.9% - 100%]	OPA	89/90	98.9	[94.0% - 99.8%]
Anti-MSH6 (EP49)	PPA	77/77	100	[95.2% - 100%]	PPA	78/78	100	[95.3% - 100%]
	NPA	12/12	100	[75.8% - 100%]	NPA	12/12	100	[75.8% - 100%]
	OPA	89/89	100	[95.9% - 100%]	OPA	90/90	100	[95.9% - 100%]
Anti-PMS2 (EP51)	PPA	71/71	100	[94.9% - 100%]	PPA	72/72	100	[94.9% - 100%]
	NPA	18/18	100	[82.4% - 100%]	NPA	18/18	100	[82.4% - 100%]
	OPA	89/89	100	[95.9% - 100%]	OPA	90/90	100	[95.9% - 100%]

*Number of agreement/Number of pairs

iii. Reproducibility across Days and Laboratories on BOND-III

To evaluate the inter-day and inter-site reproducibility of the BOND MMR Antibody Panel on the BOND-III Instrument, 24 FFPE CRC tissue cases were tested, including 3 intact and 3 loss cases for each of the 4 MMR proteins, at 3 testing sites. At each site, slides were stained with 1 reagent lot of the antibodies using 1 BOND-III instrument. Two staining runs were performed each day to test slides from the 24 cases on each of 5 nonconsecutive test days at each site. A total of 360 slides (6 cases × 4 antibodies × 3 sites × 5 days) were evaluated. At each site, one pathologist read and scored stained slides stained at their site in accordance with the scoring guidance. The slides stained on the same day were scored together in the same scoring session. Different scoring sessions occurred on nonconsecutive days.

PPA, NPA and OPA were calculated for point estimates along with 2-sided 95% confidence intervals by comparing the antibody status of each processed slide determined by the pathologist to the majority score. The majority score was determined by combining runs across all sites. At one site, 12 slides failed due to temperature error. New unstained slides were repeat stained and read and scored successfully.

Results of the study are summarized in Table 9 to Table 13. The overall reproducibility OPA ranged from 94.4% to 100%, PPA ranged from 91.7% to 100%, NPA ranged from 97.8% to 100%. The lower bound of the two-sided 95% Confidence Interval for OPA, PPA and NPA for each antibody are ≥ 85% with 1 exception. For MSH6, PPA was 91.7% (95% CI: 81.9% - 96.4%). All 5 discordant results were from one case and 4 of the 5 slides were stained at a single site. This

case was investigated and the study pathologists agreed that it was challenging to interpret due to the very focal staining impacting scoring.

The results support that the BOND MMR Antibody Panel on BOND-III have acceptable levels of precision (reproducibility) across days and testing sites.

Table 9: Summary of Inter-Laboratory Reproducibility

Antibody	Agreement on BOND-III			
	Type	n/N*	%	95% CI
Overall call	PPA	190/195	97.4%	[94.1% - 98.9%]
	NPA	164/165	99.4%	[96.6% - 99.9%]
	OPA	354/360	98.3%	[96.4% - 99.2%]
Anti-MLH1 (ES05)	PPA	45/45	100.0%	[92.1% - 100.0%]
	NPA	45/45	100.0%	[92.1% - 100.0%]
	OPA	90/90	100.0%	[95.9% - 100.0%]
Anti-MSH2 (79H11)	PPA	45/45	100.0%	[92.1% - 100.0%]
	NPA	45/45	100.0%	[92.1% - 100.0%]
	OPA	90/90	100.0%	[95.9% - 100.0%]
Anti-MSH6 (EP49)	PPA	55/60	91.7%	[81.9% - 96.4%]
	NPA	30/30	100.0%	[88.6% - 100.0%]
	OPA	85/90	94.4%	[87.6% - 97.6%]
Anti-PMS2 (EP51)	PPA	45/45	100.0%	[92.1% - 100.0%]
	NPA	44/45	97.8%	[88.4% - 99.6%]
	OPA	89/90	98.9%	[94.0% - 99.8%]

*Number of agreement/Number of pairs

Table 10: Percent Agreements for Overall and by Site--MLH1

Anti-MLH1 (ES05)		Agreement on BOND-III			
		Type	n/N*	%	95% CI
Overall call		PPA	45/45	100%	[92.1% - 100.0%]
		NPA	45/45	100%	[92.1% - 100.0%]
		OPA	90/90	100%	[95.9% - 100.0%]
Between-Day (5 non-consecutive days)	Site 1	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]
	Site 2	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]
	Site 3	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]

*Number of agreement/Number of pairs

Table 11: Percent Agreements for Overall and by Site--MSH2

Anti-MSH2 (79H11)		Agreement on BOND-III			
		Type	n/N*	%	95% CI
Overall call		PPA	45/45	100%	[92.1% - 100.0%]
		NPA	45/45	100%	[92.1% - 100.0%]
		OPA	90/90	100%	[95.9% - 100.0%]
Between-Day (5 non-consecutive days)	Site 1	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]
	Site 2	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]
	Site 3	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]

*Number of agreement/Number of pairs

Table 12: Percent Agreements for Overall and by Site--MSH6

Anti-MSH6 (EP49)		Agreement on BOND-III			
		Type	n/N*	%	95% CI
Overall call		PPA	55/60	91.7%	[81.9% - 96.4%]
		NPA	30/30	100.0%	[88.6% - 100.0%]
		OPA	85/90	94.4%	[87.6% - 97.6%]
Between-Day (5 non-consecutive days)	Site 1	PPA	20/20	100%	[83.9% - 100.0%]
		NPA	10/10	100.0%	[72.2% - 100.0%]
		OPA	30/30	100.0%	[88.6% - 100.0%]
	Site 2	PPA	19/20	95.0%	[76.4% - 99.1%]
		NPA	10/10	100.0%	[72.2% - 100.0%]
		OPA	29/30	96.7%	[83.3% - 99.4%]
	Site 3	PPA	16/20	80.0%	[58.4% - 91.9%]
		NPA	10/10	100.0%	[72.2% - 100.0%]
		OPA	26/30	86.7%	[70.3% - 94.7%]

*Number of agreement/Number of pairs

Table 13: Percent Agreements for Overall and by Site--PMS2

Anti-PMS2 (EP51)		Agreement on BOND-III			
		Type	n/N*	%	95% CI
Overall call		PPA	45/45	100.0%	[92.1% - 100.0%]
		NPA	44/45	97.8%	[88.4% - 99.6%]
		OPA	89/90	98.9%	[94.0% - 99.8%]
Between-Day (5 non-consecutive days)	Site 1	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]
	Site 2	PPA	15/15	100%	[79.6% - 100.0%]
		NPA	15/15	100%	[79.6% - 100.0%]
		OPA	30/30	100%	[88.6% - 100.0%]
	Site 3	PPA	15/15	100.0%	[79.6% - 100.0%]
		NPA	14/15	93.3%	[70.2% - 98.8%]
		OPA	29/30	96.7%	[83.3% - 99.4%]

*Number of agreement/Number of pairs

7.1.2 Stability:

The below stability studies were conducted for the BOND MMR Antibody Panel :

- Reagent Stability Studies
  - Real-Time Stability Tests
  - Accelerated Stability Tests
  - Freeze Thaw Stability Tests
- Cut Slide Stability Studies

i. Reagent Stability Studies:

Stability testing of the BOND MMR antibodies was evaluated by performing Accelerated stability testing and Real Time shelf-life testing. The Accelerated stability was evaluated using accelerated shelf-life tests and was followed up by long term stability with Real Time shelf-life tests.

The stability of the BOND MMR antibodies was evaluated using three batches of each of the four antibodies.

Prior to the initiation of both accelerated and real-time stability testing, one batch of each antibody underwent transport simulation as preconditioning. The Transport Simulation Batches were stored for a minimum of 24 hours at 2-8°C, the required aliquots were removed from recommended storage conditions and transferred to 27-33°C for 2 days, then returned to recommended storage conditions for at least 1 day, then transferred to 37-43°C for 2 days.

A Day 0 test was performed where a minimum of 3 cases were stained and a 0-4 scale used as the reference. Day 0 slides were required to have a staining intensity of at least 2+ in the most intensely-staining tumor region to be included in the study.

Accelerated Stability

Following transport cycling, the lot of the antibody was stored at 34-40 ºC and at 24-30°C. Timepoints for testing at 34-40 ºC were 7 and 10 weeks; timepoints for testing at 24-30°C were 14 and 20 weeks. If a testing failure occurred at either timepoint for 34-40 ºC then the study reverted to testing units stored at 24-30°C. If units stored at 24-30°C failed then accelerated stability was discontinued and real time data used. At each time point, staining intensities of the slides were compared with the ones stained at Day 0. The intensity had to be at least 2+ for all slides to pass the acceptance criteria.

Real Time Shelf-Life Tests

Real time stability was evaluated for three conditions: long term stability (Real-time Shelf Life Test), in-use stability (In Use Test), transport simulation (Transport Test). The study was continued up to 545 Days (18-month timepoint including a safety margin)

1. The in-use stability test batch was stored at 2-8ºC until required for testing where an 'In Use' aliquot of the antibody for each time points was cycled for 35 cycles by storing at 34-40°C for 7 hours. At the end of each 7 hour period containers were returned to 2-8°C. The units were tested on or after 35 cycles.
2. The transport simulation batches were stored for a minimum of 24 hours at 2-8°C, the required aliquots were removed from recommended storage conditions and transferred to 27-33°C for 2 days, then returned to recommended storage conditions for at least 1 day, then transferred to 37-43°C for 2 days. Units were returned thereafter to their recommended storage conditions for a period of at least 24 hours before testing.
3. The real time stability test for the three batches were performed on Day 0 and then subsequent time points. Tissues stained at each time point were compared to a Day 0 control tissue and a -77 - -83°C control.

Freeze-Thaw Tests

The freeze-thaw batch underwent freeze-thaw preconditioning prior to day 0 testing. Three vials of the batch were stored at -77 - -83°C for a minimum of 24 hours. The aliquots were then placed at 2-8ºC to thaw overnight and were tested alongside and compared to an aliquot stored only at 2-8°C. The freeze-thaw batch was not subjected to transport simulation.

At each time point, staining intensities of the slides were compared with the ones stained at Day 0. The intensity was required to be at least 2+ for all slides to pass the acceptance criteria. If the test failed, then study either reverted to the lower temperatures or in the case of a real time failure the study would be aborted.

Results

All four antibodies passed the transport simulation testing and the freeze-thaw testing. The anti-MLH1, anti-MSH2 and anti-PMS2 antibodies passed real

time stability testing at 545 days. This supports a maximum shelf life for these antibodies of 12 months. The antibody of anti-MSH6 passed the accelerated stability testing at 7 weeks and the real time stability testing at 365 days. This supports a maximum shelf life for this antibody of 7 months.

ii. Cut slide Stability:

To evaluate the stability of the cut slides, 60 x 3µm sections were cut from each of three FFPE CRC tissue cases for a total of 180 slides. Six slides of each FFPE CRC tissue case were stained on Day 0 on the BOND-III system with one lot of each of the four antibodies along with the negative reagent controls, scored on a 0-4+ scale and used as the reference. All three cases are intact for all four MMR proteins with the staining intensity being at least 2+ in the most intensely-staining tumor region. The remaining slides of each FFPE CRC tissue case were stored at ambient/room temperature for 7, 14, 28, 42, 56, 70 and 77 days. At each timepoint, six slides of each FFPE CRC tissue case were stained the same way as above and compared with the ones stained at baseline. In addition, at each of these timepoints, freshly cut sections of the same three tissue cases were stained with each of the four antibodies as a quality control. The intensity was required to be at least 2+ to pass the acceptance criteria. Results of the study showed that the epitopes recognized by each of the four antibodies in FFPE CRC tissue cases remain stable for a period of time up to and including 11 weeks post sectioning when the tissue sections are mounted onto slides and stored at ambient/room temperature, therefore supporting epitope stability claims in cut sections of up to 10 weeks.

7.1.3 *Analytical specificity:*

Analytical specificity was addressed in two separate studies for each of the MMR panel antibodies. The first addressed antibody specificity and the second addressed immunoreactivity in normal and neoplastic tumor specimen.

i. Western Blot and IHC:

Western blot analyses were conducted to demonstrate that the antibodies specifically detect the proteins of predicted molecular weight for each of the 4 BOND MMR antibodies using cell lines with known MMR loss or intact status. Cell lines used in the study were generated using lysates from the PANC-1 (human epithelial pancreas/duct carcinoma) for MLH1 and MSH2 and lysates from the A431 (human epidermoid carcinoma) for MSH6 and PMS2. Western Blots confirmed presence of reactive bands at expected molecular weighs for each of the 4 MMR proteins.

IHC tests were conducted using formalin-fixed, paraffin-embedded cell lines described in the table below to assess nonspecific binding in the context of use. The results of the IHC with engineered cell lines was consistent with expected reactivity.

Table 14 Western Blot Analysis

Antibody	Formalin-Fixed, Paraffin-Embedded Cell Lines for IHC Testing
MMR INTACT	Breast adenocarcinoma cell line that carries no specific MMR mutations
MLH1 & PMS2 LOSS	Prostate carcinoma cell line with a splice site mutation between exon 1 and exon 2 of the MLH1 gene which causes the deletion of 5 coding nucleotides
MSH2 LOSS	Colon adenocarcinoma cell line that carries a homozygous deletion of exons 3 to 8 in the MSH2 gene
MSH6 LOSS	A colon adenocarcinoma cell line that is known to harbour a 1 base pair deletion mutation in one allele of the MSH6 gene and a sequence deletion/insertion involving 5 base pairs in the other allele

The combined results from western blots and cell line IHC demonstrated specific antibody reactivity for each of the 4 MMR proteins included in the BOND MMR antibody panel.

ii. Immunoreactivity:

Immunoreactivity of the BOND MMR antibodies was tested across multiple cases of normal and tumor tissue types. The summary of staining results with the panel antibodies is shown in Table 15 to Table 17. Staining of these ubiquitously expressed nuclear proteins was observed in normal and neoplastic tissues as expected.

Table 15 Immunoreactivity in FFPE normal tissues

Tissue	No. of IHC positive cases / total cases			
	MLH1	MSH2	MSH6	PMS2
Cerebrum, grey matter tissue	3/3	3/3	3/3	3/3
Cerebrum, white matter tissue	3/3	3/3	3/3	3/3
Cerebellum	3/3	3/3	3/3	3/3
Adrenal gland	3/3	3/3	2/3	3/3
Ovary	3/3	3/3	3/3	3/3
Pancreas	3/3	3/3	3/3	3/3

Tissue	No. of IHC positive cases / total cases			
	MLH1	MSH2	MSH6	PMS2
Parathyroid	3/3	3/3	3/3	3/3
Pituitary	3/3	3/3	3/3	3/3
Testis	3/3	3/3	3/3	3/3
Thyroid gland	3/3	3/3	3/3	3/3
Breast	3/3	3/3	3/3	3/3
Spleen	3/3	3/3	3/3	3/3
Tonsil	3/3	3/3	3/3	3/3
Thymus gland	3/3	3/3	3/3	3/3
Bone marrow	3/3	3/3	3/3	3/3
Lung	3/3	3/3	3/3	3/3
Heart, cardiac muscle	2/3	3/3	1/3	1/3
Esophagus	3/3	3/3	3/3	3/3
Stomach	3/3	3/3	3/3	3/3
Small intestine, ileum	3/3	3/3	3/3	3/3
Colon	3/3	3/3	3/3	3/3
Liver	3/3	3/3	3/3	3/3
Salivary gland	3/3	3/3	3/3	3/3
Kidney	3/3	3/3	3/3	3/3
Prostate	3/3	3/3	3/3	3/3
Uterus, endometrium	3/3	3/3	3/3	3/3
Cervix	3/3	3/3	3/3	3/3
Skeletal muscle	2/3	3/3	2/3	1/3
Skin	3/3	3/3	3/3	3/3
Peripheral nerve	2/3	2/3	1/3	1/3
Mesothelium, umbilical cord	3/3	3/3	3/3	3/3
Larynx	3/3	3/3	3/3	3/3
Bladder	3/3	3/3	3/3	3/3

Table 16 Immunoreactivity in a variety of FFPE neoplastic tissues

Tissue	Pathology	No. of IHC positive cases / total cases			
		MLH1	MSH2	MSH6	PMS2
Bladder, urinary	Transitional cell carcinoma	2/2	2/2	2/2	2/2
Breast	Fibroadenoma	2/2	2/2	2/2	2/2
Breast	Invasive ductal carcinoma	2/2	2/2	2/2	2/2
Bone, tibia	Osteosarcoma	1/1	1/1	1/1	1/1
Bone, scapula	Chondrosarcoma	1/1	1/1	1/1	1/1
Brain	Meningioma, fibroblastic	1/1	1/1	1/1	1/1
Brain	Astrocytoma	1/1	1/1	1/1	1/1
Esophagus	Squamous cell carcinoma	2/2	2/2	2/2	2/2
Stomach	Adenocarcinoma	3/3	3/3	3/3	3/3
Intestine, small intestine	Adenoma	1/1	1/1	1/1	1/1
Intestine, small intestine	Adenocarcinoma	1/1	1/1	1/1	1/1
Intestine, colon	Adenoma	1/1	1/1	1/1	1/1
Intestine, colon	Adenocarcinoma	3/3	3/3	3/3	3/3
Intestine, rectum	Adenocarcinoma	2/3	3/3	3/3	2/3
Kidney	Clear cell carcinoma	1/1	1/1	1/1	1/1
Liver	Hepatocellular carcinoma	1/1	1/1	1/1	1/1
Lung	Adenocarcinoma	1/1	1/1	1/1	1/1
Lung	Small cell carcinoma	1/1	1/1	1/1	1/1

Tissue	Pathology	No. of IHC positive cases / total cases			
		MLH1	MSH2	MSH6	PMS2
Lymph node, neck	Lymphoma, Hodgkin lymphoma	1/1	1/1	1/1	1/1
Lymph node, neck	Lymphoma, anaplastic large cell lymphoma	1/1	1/1	1/1	1/1
Head and neck, oral cavity, hard palate	Adenocarcinoma	1/1	1/1	1/1	1/1
Tongue	Squamous cell carcinoma	1/1	1/1	1/1	1/1
Head and neck, nasopharynx	Nasopharyngeal carcinoma, NPC	1/1	1/1	1/1	1/1
Ovary	Granulosa cell tumor	1/1	1/1	1/1	1/1
Ovary	Adenocarcinoma	1/1	1/1	1/1	1/1
Ovary	Endometrioid adenocarcinoma	1/1	1/1	1/1	1/1
Pancreas	Adenocarcinoma	1/1	1/1	1/1	1/1
Prostate	Adenocarcinoma	2/2	2/2	2/2	2/2
Skin, trunk	Squamous cell carcinoma	1/1	1/1	1/1	1/1
Head and neck, nasal cavity	Melanoma	1/1	1/1	1/1	1/1
Testis	Seminoma	1/1	1/1	1/1	1/1
Thyroid	Adenoma	2/2	2/2	2/2	2/2
Thyroid	Follicular carcinoma	1/1	1/1	1/1	1/1
Thyroid	Follicular papillary adenocarcinoma	1/1	1/1	1/1	1/1

Tissue	Pathology	No. of IHC positive cases / total cases			
		MLH1	MSH2	MSH6	PMS2
Uterus, cervix	Squamous cell carcinoma	2/2	2/2	2/2	2/2
Uterus, endometrium	Adenocarcinoma	2/2	2/2	2/2	2/2
Liver	Metastatic colon adenocarcinoma	1/1	1/1	1/1	1/1
Lung	Metastatic cancers from gastrointestinal site	1/1	1/1	1/1	1/1
Ovary	Metastatic colon signet ring cell carcinoma	1/1	1/1	1/1	1/1
Lymph node	Metastatic esophagus squamous cell carcinoma	1/1	1/1	1/1	1/1

Table 17 Immunoreactivity in FFPE colorectal cancer tissues

Tissue	Pathology	No. of IHC positive cases / total cases			
		MLH1	MSH2	MSH6	PMS2
Colon	Adenocarcinoma	63/68	67/68	66/68	63/68
	Partly mucinous adenocarcinoma	12/12	11/12	11/12	12/12
	Mucinous adenocarcinoma	8/9	9/9	8/9	8/9
	Squamous carcinoma	1/1	1/1	1/1	1/1
Rectum	Adenocarcinoma	7/8	8/8	8/8	7/8
	Partly mucinous adenocarcinoma	3/3	3/3	3/3	3/3
	Mucinous adenocarcinoma	0/1	1/1	1/1	0/1

7.2 Clinical Performance:

The clinical validity of the BOND MMR Antibody Panel was determined in a study that assessed agreement between test results obtained from the BOND MMR Antibody Panel and a molecular test based on a DNA sequencing panel validated for detecting presence of pathogenic mutations likely to affect MMR protein expression in CRC. The purpose of the study was to evaluate the ability of the panel to correctly aid in the identification of patients needing additional Lynch syndrome genetic testing.

Eligible remnant FFPE CRC tissues ("cases") were procured, with at least 100 sequential cases with unknown mismatch repair (MMR) status (also referred to as the Sequential cohort) and at least 48 cases with known MMR protein deficiencies (also referred to as the Enrichment cohort). Specimens from the two cohorts were combined, randomized and processed to create slides evaluation with BOND MMR Antibody Panel testing and the DNA sequencing testing. Staining with the BOND MMR Antibody Panel was performed on BOND-III system in accordance with the instructions for use (IFUs). One pathologist at the testing site read and scored BOND MMR Antibody Panel stained slides in accordance with the scoring guidance.

Inclusion Criteria:

- FFPE CRC tissue block with matched normal blood, saliva, or FFPE tissue and
- (Sequential cohort) With unknown MMR protein status, sequentially obtained from a single US site or
- (Enrichment cohort) With known immunohistochemistry (IHC) MMR protein status obtained from multiple sites, including:
    - MLH1 and PMS2 loss
    - MSH2 and MSH6 loss
    - isolated PMS2 loss
    - isolated MSH6 loss

Exclusion Criteria:

- Tissue requirements for comparator DNA sequencing panel were not met
- Lack of sufficient tumor.

There were 155 cases procured for the study. One case was excluded due to not meeting the Enrichment cohort inclusion criteria. Of the remaining 154 cases eligible for the study, One case had insufficient tumor for both DNA sequencing and BOND MMR Antibody Panel testing, three cases had insufficient tumor for DNA sequencing, and seven cases did not have MMR Stained Slides interpreted due to the H&E Slide showing less than 50 viable tumor cells present for evaluation, not meeting the tissue requirements required by the BOND MMR Antibody Panel. Therefore, of the 154 eligible cases, 143 were tested by both methods (94 sequential cases and 49 enriched cases). Assessment of the demographic data associated with the study specimens determined that it was consistent with the intended use population.

Table 18 MMR Status of the Enrichment Cohort

	MLH1 and PMS2 loss	**MSH2 and MSH6 loss**	**PMS2 loss**	**MSH6 loss**
# Cases	25	14	6	4

BOND MMR Antibody Panel results (MMR loss or MMR intact) were compared to the

DNA sequencing panel results for pathogenic mutation(s) in the combined cohort (Sequential and Enrichment cohorts), and in the Sequential cohort and the Enrichment cohort, respectively. PPA, NPA, and OPA along with 2-sided 95% Wilson score confidence intervals (CIs) were calculated.

Of the 143 specimens tested by the BOND MMR Antibody Panel and the DNA sequencing panel, ten (10) cases had invalid DNA sequencing panel results due to testing not meeting quality control standards and were excluded from the agreement analysis. The remaining 133 of 143 cases had valid results by both methods and were evaluable. Results of the study are summarized in Table 19 to Table 21. The point estimates of agreement between the BOND MMR Antibody Panel and the DNA sequencing panel in the combined cohort were PPA 93.3%, NPA 95.9% and OPA 94.7%.

Table 19 Agreement between BOND MMR Antibody Panel Results and DNA Sequencing Panel Results: Combined Cohort

Combined Cohort		**DNA Sequencing Panel Results**			
		Pathogenic Mutations(s)	**No Pathogenic Mutations(s)**	**Invalid**	**Total**
BOND MMR Antibody Panel Results	**MMR Protein Loss**	56	3	4	63
	MMR Protein Intact	4	70	6	80
	Total	60	73	10	143

Agreement				
	Number of Agreements	**Number of Pairs**	**% Agreement**	**95% Confidence Interval**
PPA	56	60	93.3%	[84.1% - 97.4%]
NPA	70	73	95.9%	[88.6% - 98.6%]
OPA	126	133	94.7%	[89.5% - 97.4%]

Table 20 Agreement between BOND MMR Antibody Panel Results and DNA Sequencing Panel Results: Sequential Cohort

Sequential Cohort		**DNA Sequencing Panel Results**			
		Pathogenic Mutations(s)	**No Pathogenic Mutations(s)**	**Invalid**	**Total**
BOND MMR Antibody Panel Results	**MMR Protein Loss**	15	1	3	19
	MMR Protein Intact	3	66	6	75

	Total	18	67	9	94
Agreement					
	Number of Agreements	**Number of Pairs**	**% Agreement**	**95% Confidence Interval**	
PPA	15	18	83.3%	[60.8% - 94.2%]	
NPA	66	67	98.5%	[92.0% - 99.7%]	
OPA	81	85	95.3%	[88.5% - 98.2%]	

Table 21 Agreement between BOND MMR Antibody Panel Results and DNA Sequencing Panel Results: Enrichment Cohort

Enrichment Cohort		**DNA Sequencing Panel Results**			
		Pathogenic Mutations(s)	**No Pathogenic Mutations(s)**	**Invalid**	**Total**
BOND MMR Antibody Panel Results	**MMR Protein Loss**	41	2	1	44
	MMR Protein Intact	1	4	0	5
	Total	42	6	1	49
Agreement					
	Number of Agreements	**Number of Pairs**	**% Agreement**	**95% Confidence Interval**	
PPA	41	42	97.6%	[87.7% - 99.6%]	
NPA	4	6	66.7%	[30.0% - 90.3%]	
OPA	45	48	93.8%	[83.2% - 97.9%]	

Accuracy by MMR proteins: The concordance for MMR gene mutation status by the DNA sequencing panel and MMR protein loss by the BOND MMR Antibody Panel was also compared individually for each antibody. When compared to the results to the DNA sequencing panel, the OPA of each MMR protein ranged from 94.0% to 98.5%, the PPA ranged from 65.0% to 97.5%, the NPA ranged from 95.7% to 99.2%. Results are summarized in Table 22 to Table 24.

MLH1: Of the 37 MLH1 mutation positive cases, 33 had MLH1 protein loss when assessed by the BOND MMR Antibody Panel. Of the 4 MLH1 mutation positive cases with discordant BOND MMR Antibody results (i.e., intact), the following was found:

- One case had MLH1 promoter hypermethylation
- One case had a single somatic MLH1 pathogenic mutation with Copy-neutral loss of heterozygosity (CN-LOH)

- One case had 2 MLH1 somatic mutations as well as MLH1 promoter hypermethylation
- One case had a single somatic MLH1 pathogenic mutation and microsatellite instability-high (MSI-H) status.

Three of the cases also showed PMS2 protein loss.

MSH2: Of the 13 MSH2 mutation positive cases, 12 had MSH2 protein loss when assessed by the BOND MMR Antibody Panel. A single MSH2 mutation positive case with discordant BOND MMR Antibody results (i.e., intact) was identified. This case was characterized by a single MSH2 pathogenic mutation combined with MSI-H status; an MSH2 Variant of Unknown Significance (VUS) was also identified. MSH6 protein was also intact.

MSH6: Of the 20 MSH6 mutation positive cases, 13 had MSH6 protein loss when assessed by the BOND MMR Antibody Panel. Of the 7 MSH6 mutation positive cases with discordant BOND MMR Antibody results (ie, MSH6 intact), the cases were characterized as follows:

- A single MSH2 pathogenic mutation combined with MSI-H status; an MSH2 VUS was also identified. MSH2 protein was preserved.
- One pathogenic variant of somatic origin in the MSH2 gene, MSI-H status and MSH2 protein loss.
- Germline pathogenic mutation in MSH2 with CN-LOH and MSH2 protein loss, as well as a single germline pathogenic mutation in PMS2 and a single somatic pathogenic mutation in MSH6.
- A germline and a somatic mutation in MSH6 as well as multiple VUS.
- Two somatic mutations in MSH6 consistent with biallelic somatic mutations as well as a single germline mutation in PMS2 with PMS2 protein loss.
- Two pathogenic variants of somatic origin in MSH6 gene, and one pathogenic mutation of somatic mutation in MSH2, MSI-High status, and a PMS2 VUS was also identified.
- One pathogenic variant of somatic origin in MSH6 gene with MLH1 and PMS2 protein loss, as well as MSI-High.

Of the 7 MSH6 mutation positive cases with discordant BOND MMR Antibody results (i.e. MSH6 intact), 4 demonstrated IHC loss when stained using the BOND MMR Antibody Panel and these cases should receive additional diagnostic testing consistent with clinical practice guidelines for diagnosis of Lynch syndrome.

PMS2: Of the 40 PMS2 mutation positive cases, 39 had PMS2 protein loss when assessed by the BOND MMR Antibody Panel. A single PMS2 mutation positive cases with discordant BOND MMR Antibody results (i.e., intact) was identified. This case was characterized by a MLH1 promoter hypermethylation combined with MSI-S status. MLH1 protein staining was also intact.

Table 22 Agreement for Each Protein between BOND MMR Antibody Panel Results and DNA Sequencing Panel Results: Combined Cohort

Combined Cohort		DNA Sequencing Panel Results			
		Pathogenic Mutations(s)	No Pathogenic Mutations(s)	Invalid	Total
BOND MMR anti-MLH1 Results	Protein Loss	33	1	3	37
	Protein Intact	4	95	7	106
	Total	37	96	10	143
BOND MMR anti-MSH2 Results	Protein Loss	12	1	1	14
	Protein Intact	1	120	8	129
	Total	13	121	9	143
BOND MMR anti-MSH6 Results	Protein Loss	13	1	1	15
	Protein Intact	7	113	8	128
	Total	20	114	9	143
BOND MMR anti-PMS2 Results	Protein Loss	39	4	3	46
	Protein Intact	1	89	7	97
	Total	40	93	10	143

Agreement					
		Number of Agreements	Number of Pairs	% Agreement	95% Confidence Interval
Anti-MLH1	PPA	33	37	89.2%	[75.3% - 95.7%]
	NPA	95	96	99.0%	[94.3% - 99.8%]
	OPA	128	133	96.2%	[91.5% - 98.4%]
Anti-MSH2	PPA	12	13	92.3%	[66.7% - 98.6%]
	NPA	120	121	99.2%	[95.5% - 99.9%]
	OPA	132	134	98.5%	[94.7% - 99.6%]
Anti-MSH6	PPA	13	20	65.0%	[43.3% - 81.9%]
	NPA	113	114	99.1%	[95.2% - 99.8%]
	OPA	126	134	94.0%	[88.7% - 96.9%]
Anti-PMS2	PPA	39	40	97.5%	[87.1% - 99.6%]
	NPA	89	93	95.7%	[89.5% - 98.3%]
	OPA	128	133	96.2%	[91.5% - 98.4%]

Table 23 Agreement for Each Protein between BOND MMR Antibody Panel Results and DNA Sequencing Panel Results: Sequential Cohort

Sequential Cohort		DNA Sequencing Panel Results			
		Pathogenic Mutations(s)	No Pathogenic Mutations(s)	Invalid	Total
BOND MMR anti-MLH1 Results	Protein Loss	10	1	3	14
	Protein Intact	2	72	6	80
	Total	12	73	9	94
BOND MMR anti-MSH2 Results	Protein Loss	3	0	0	3
	Protein Intact	0	83	8	91
	Total	3	83	8	94
BOND MMR anti-MSH6 Results	Protein Loss	2	0	0	2
	Protein Intact	4	80	8	92
	Total	6	80	8	94
BOND MMR anti-PMS2 Results	Protein Loss	11	2	3	16
	Protein Intact	1	71	6	78
	Total	12	73	9	94

Agreement					
		Number of Agreements	Number of Pairs	% Agreement	95% Confidence Interval
Anti-MLH1	PPA	10	12	83.3%	[55.2% - 95.3%]
	NPA	72	73	98.6%	[92.6% - 99.8%]
	OPA	82	85	96.5%	[90.1% - 98.8%]
Anti-MSH2	PPA	3	3	100.0%	[43.9% - 100.0%]
	NPA	83	83	100.0%	[95.6% - 100.0%]
	OPA	86	86	100.0%	[95.7% - 100.0%]
Anti-MSH6	PPA	2	6	33.3%	[9.7% - 70.0%]
	NPA	80	80	100.0%	[95.4% - 100.0%]
	OPA	82	86	95.3%	[88.6% - 98.2%]

Anti-PMS2	**PPA**	11	12	91.7%	[64.6% - 98.5%]
	NPA	71	73	97.3%	[90.5% - 99.2%]
	OPA	82	85	96.5%	[90.1% - 98.8%]

Table 24 Agreement for Each Protein between BOND MMR Antibody Panel Results and DNA Sequencing Panel Results: Enrichment Cohort

Enrichment Cohort		**DNA Sequencing Panel Results**			
		Pathogenic Mutations(s)	**No Pathogenic Mutations(s)**	**Invalid**	**Total**
BOND MMR anti-MLH1 Results	**Protein Loss**	23	0	0	23
	Protein Intact	2	23	1	26
	Total	25	23	1	49
BOND MMR anti-MSH2 Results	**Protein Loss**	9	1	1	11
	Protein Intact	1	37	0	38
	Total	10	38	1	49
BOND MMR anti-MSH6 Results	**Protein Loss**	11	1	1	13
	Protein Intact	3	33	0	36
	Total	14	34	1	49
BOND MMR anti-PMS2 Results	**Protein Loss**	28	2	0	30
	Protein Intact	0	18	1	19
	Total	28	20	1	49

Agreement					
		Number of Agreements	**Number of Pairs**	**% Agreement**	**95% Confidence Interval**
Anti-MLH1	**PPA**	23	25	92.0%	[75.0% - 97.8%]
	NPA	23	23	100.0%	[85.7% - 100.0%]
	OPA	46	48	95.8%	[86.0% - 98.8%]
Anti-MSH2	**PPA**	9	10	90.0%	[59.6% - 98.2%]
	NPA	37	38	97.4%	[86.5% - 99.5%]
	OPA	46	48	95.8%	[86.0% - 98.8%]
Anti-MSH6	**PPA**	11	14	78.6%	[52.4% - 92.4%]
	NPA	33	34	97.1%	[85.1% - 99.5%]
	OPA	44	48	91.7%	[80.4% - 96.7%]

Anti-PMS2	**PPA**	28	28	100.0%	[87.9% - 100.0%]
	NPA	18	20	90.0%	[69.9% - 97.2%]
	OPA	46	48	95.8%	[86.0% - 98.8%]

8. Conclusions

The results of performed testing supports a determination of Substantial Equivalence (SE) of the BOND MMR Antibody Panel [subject device] when compared to the VENTANA MMR IHC Panel (DEN170030) [predicate device].

9. References

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