October 23, 2026

Roche Tissue Diagnostics, Inc. (Ventana Medical Systems, Inc.)
Mrunalini Deokar
Regulatory Affairs Manager
1910 E. Innovation Park Dr.
Tucson, Arizona 85755

Re: K260776
Trade/Device Name: VENTANA MMR IHC Panel
Regulation Number: 21 CFR 864.1866
Regulation Name: Lynch syndrome test systems
Regulatory Class: Class II
Product Code: PZJ
Dated: March 9, 2026
Received: March 10, 2026

Dear Mrunalini Deokar:

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 (the 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 available 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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality Management System Regulation (QMSR) (21 CFR Part 820), which includes, but is not limited to, ISO 13485 clause 7.3 (Design controls), ISO 13485 clause 8.3 (Nonconforming product), ISO 13485 clause 8.5.2 (Corrective action), and ISO 13485 clause 8.5.3 (Preventative action). Please note that regardless of whether a change requires premarket review, the QMSR requires device manufacturers to review and approve changes to device design and production (ISO 13485 clause 7.3 and ISO 13485 clause 7.5) and document changes and approvals in the Medical Device File (ISO 13485 clause 4.2.3).

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 Part 803) for devices or postmarketing safety reporting (21 CFR Part 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 Management System Regulation (QMSR) (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 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

Živana Težak, Ph.D.
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)*
K260776

Device Name
VENTANA MMR IHC Panel

Indications for Use *(Describe)*
VENTANA MMR IHC Panel

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 OptiView DAB IHC Detection Kit is used with MLH1, MSH2, MSH6, PMS2 (SP487) and BRAF V600E, and the OptiView DAB IHC Detection Kit with OptiView Amplification Kit is used for PMS2 (A16-4) detection. The VENTANA MMR IHC Panel is for use on the BenchMark ULTRA and BenchMark ULTRA PLUS instruments. The VENTANA MMR IHC Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody; VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody or VENTANA anti-PMS2 (SP487) Rabbit 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 BRAF V600E 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.
Intended for in vitro diagnostic (IVD) use. Prescription Use Only.

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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VENTANA MMR IHC Panel

510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Ventana Medical Systems, Inc. (Roche Tissue Diagnostics)
Address	1910 E Innovation Park Drive Tucson, AZ 85755
Contact	Mrunalini Deokar Regulatory Affairs Manager Email - minty.deokar@roche.com
Date Prepared	March 09, 2026
Proprietary Name	VENTANA MMR IHC Panel
Common Name	VENTANA MMR IHC Panel
Classification Name	Class II
Product Codes	PZJ
Regulation Number	21 CFR 864.1866
Regulation Name	Lynch Syndrome Test System
Predicate Devices	VENTANA MMR IHC Panel (DEN170030)
Establishment Registration	2028492

1. PROPOSED INTENDED USE

VENTANA MMR IHC Panel

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 OptiView DAB IHC Detection Kit is used with MLH1, MSH2, MSH6, PMS2 (SP487) and BRAF V600E, and the OptiView DAB IHC Detection Kit with OptiView Amplification Kit is used for PMS2 (A16-4) detection. The VENTANA MMR IHC Panel is for use on the BenchMark ULTRA and BenchMark ULTRA PLUS instruments. The VENTANA MMR IHC Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody; VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody or VENTANA anti-PMS2 (SP487) Rabbit 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 BRAF V600E 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.
Intended for in vitro diagnostic (IVD) use. Prescription Use Only.

2. DEVICE DESCRIPTION

The VENTANA MMR IHC Panel is a complete immunohistochemical diagnostic test system comprising the following primary antibodies and detection reagents:
VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody
VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody
or VENTANA anti-PMS2 (SP487) Rabbit Monoclonal Primary Antibody
VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody
VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody
VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody
OptiView DAB IHC Detection Kit and OptiView Amplification Kit on BenchMark ULTRA and BenchMark ULTRA PLUS instruments.
VENTANA anti-PMS2 (SP487) antibody is a rabbit monoclonal antibody produced against a recombinant PMS2 protein.
VENTANA anti-PMS2 (SP487) antibody recognizes PMS2, which is one of several clinically important DNA mismatch repair (MMR) proteins. VENTANA anti-PMS2 (SP487) antibody demonstrates equivalent specificity and improved staining quality when compared to VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary antibody. VENTANA anti-PMS2 (SP487) antibody is part of VENTANA MMR IHC Panel, an immunohistochemical (IHC) assay system for identifying tumors with MMR deficiency. MMR proteins (MLH1, PMS2, MSH2, and MSH6) are ubiquitously expressed in proliferating normal and malignant cells.
MMR is a conserved molecular mechanism that functions to correct the improper base substitutions that spontaneously occur during DNA replication. Polymerase chain reaction (PCR)-based methods have shown that MMR failure frequently leads to microsatellite instability (MSI), a condition in which short, tandem nucleotide repeats are inserted into the DNA. When the number of repeats is equal to or greater than 30% of the examined microsatellite loci, MSI can be further characterized as MSI-High (MSI-H). Defects in the MMR machinery have been attributed to mutations in the MMR proteins, most commonly MLH1, PMS2, MSH2, and MSH6. The MLH1 and PMS2 proteins normally function together in a heterodimeric complex, as do the MSH2 and MSH6 proteins. When MMR is functioning normally, the MSH6/MSH2 heterodimer binds to mismatched DNA. This binding induces a conformational change that allows the MLH1/PMS2 heterodimer to bind the DNA-bound MSH6/MSH2 complex, resulting in excision repair of the affected DNA. Mutations or deficiencies in these proteins result in frequent MSI and somatic mutation due to replication error. MMR IHC testing can be useful in identifying tumors with alterations in MMR. VENTANA anti-PMS2 (SP487) antibody binds to PMS2 protein in FFPE tissue sections. The antibody can be localized using a haptenated secondary antibody followed by a multimer antihapten-HRP conjugate

(OptiView DAB IHC Detection Kit). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, each BenchMark ULTRA or BenchMark ULTRA PLUS instrument washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies ULTRA LCS (Predilute) or LCS (Predilute), which minimizes evaporation of the aqueous reagents from the specimen slide.

3. COMPARISON TO PREDICATE

Table 1: Similarities and Differences between the Candidate VENTANA MMR IHC Panel and the VENTANA MMR IHC Panel

Comparator	Candidate Device: VENTANA MMR IHC Panel	Predicate Device: VENTANA MMR IHC Panel DEN170030
Proprietary Name	VENTANA MMR IHC Panel	VENTANA MMR IHC Panel
Regulation Number	21 CFR 864.1866	Same
Regulation Name	Lynch syndrome test systems	Same
Regulatory Class	Class II	Same
Product Code	PZJ, Lynch Syndrome Test System	Same
Intended Use	VENTANA MMR IHC Panel 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 OptiView DAB IHC Detection Kit is used with MLH1, MSH2, MSH6, PMS2 (SP487) and BRAF V600E, and the OptiView DAB IHC Detection Kit with OptiView Amplification Kit is used for PMS2 (A16-4) detection. The VENTANA MMR IHC Panel is for use on the BenchMark ULTRA and BenchMark ULTRA PLUS instruments. The VENTANA MMR IHC Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody; VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody or VENTANA anti-PMS2 (SP487) Rabbit 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 BRAF V600E 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. Intended for in vitro diagnostic (IVD) use. Prescription Use Only.	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 OptiView 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.
Conditions for use	Intended for in vitro diagnostic (IVD) use. Prescription use only.	Same
Sample Types	Formalin-Fixed Paraffin Embedded (FFPE)	Same
Analyte Targets	MLH1, PMS2, MSH2, MSH6, and BRAF V600E proteins	Same
Sample Preparation Procedure	Automated	Same
Amplification Technology	OptiView Amplification Kit [for PMS2 (A16-4)]	Same
Detection Chemistry	OptiView DAB IHC Detection Kit	Same
Controls used	Internal positive control, Positive and negative tissue controls, Negative Reagent Control (NRC)	Same
Result Analysis	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.	Same

Assay Method	IHC	Same

Comparator	Candidate Device: VENTANA MMR IHC Panel	Predicate Device: VENTANA MMR IHC Panel DEN170030
Detection Method	Qualitative	Same
Applications/Test Time	Each antibody [VENTANA anti-MLH1 (M1), VENTANA anti-PMS2 (A16-4), VENTANA anti-PMS2 (SP487), VENTANA anti-MSH2 (G219-1129), VENTANA anti-MSH6 (SP93), and VENTANA anti-BRAF V600E (VE1)] contains sufficient reagent for 50 tests	Each antibody [VENTANA anti-MLH1 (M1), VENTANA anti-PMS2 (A16-4), VENTANA anti-MSH2 (G219-1129), VENTANA anti-MSH6 (SP93), and VENTANA anti-BRAF V600E (VE1)] contains sufficient reagent for 50 tests
Instrument Platform	BenchMark ULTRA and BenchMark ULTRA PLUS instruments	BenchMark ULTRA instrument
Sample Matrix	MLH1 (M1): The antibody is diluted in a TBS buffer containing 0.3% carrier protein. PMS2 (A16-4): The antibody is diluted in PBS with 3% carrier protein and 0.05% ProClin300, a preservative. MSH2 (G219-1129): The antibody is diluted in phosphate buffer with 0.3% carrier protein, Brij 35, and 0.05% ProClin300, a preservative. MSH6 (SP93): The antibody is diluted in Tris-HCl with 1% carrier protein, and 0.10% ProClin 300, a preservative. PMS2 (SP487): The antibody is diluted in PBS with 3% carrier protein and 0.05% ProClin 300, a preservative. BRAF V600E (VE1): The antibody is diluted in phosphate buffer with 0.3% carrier protein, Brij 35, and 0.05% ProClin300, a preservative.	MLH1 (M1): The antibody is diluted in a TBS buffer containing 0.3% carrier protein. PMS2 (A16-4): The antibody is diluted in PBS with 3% carrier protein and 0.05% ProClin300, a preservative. MSH2 (G219-1129): The antibody is diluted in phosphate buffer with 0.3% carrier protein, Brij 35, and 0.05% ProClin300, a preservative. MSH6 (SP93): The antibody is diluted in Tris-HCl with 1% carrier protein, and 0.10% ProClin 300, a preservative. BRAF V600E (VE1): The antibody is diluted in phosphate buffer with 0.3% carrier protein, Brij 35, and 0.05% ProClin300, a preservative.

4. ANALYTICAL PEFORMANCE

Analytical performance studies were conducted to support the addition of the VENTANA anti-PMS2 (SP487) clone and the extension of the VENTANA MMR IHC Panel to the BenchMark ULTRA PLUS platform.

4.1. Specificity

Positive staining is nuclear unless otherwise specified. No unexpected staining was observed with VENTANA anti-PMS2 (SP487) antibody on the normal and neoplastic tissues. As expected, since mismatch repair is present in all actively proliferating cells, most normal and neoplastic tissues demonstrated positive staining (Table 2 and Table 3).
The VENTANA anti-PMS2 (SP487) Rabbit Monoclonal Primary Antibody demonstrates off-target staining in less than 10% of assessable tumor tissues. The VENTANA anti-PMS2 (SP487) Rabbit Monoclonal Primary Antibody exhibits target-specific nuclear staining of tumor cells in at least 90% of indication tissues with specific staining. The background and off-target staining for normal cases from the Tour of Body (TOB) were acceptable in 100% of cases

Table 2: Specificity of VENTANA anti-PMS2 (SP487) Antibody was Determined by Testing FFPE Normal Tissues

Tissue	No. Positive / Total Cases	Tissue	No. Positive / Total Cases
Adrenal Gland	3/3	Pancreas	8/8
Bladder/Urethra	6/6	Parathyroid	6/6
Bladder, urinary	7/7	Peripheral Nerve	3/3
Bone, bone marrow[a]	8/9	Pituitary Gland[a]	4/5
Brain, cerebellum	3/3	Placenta	2/2
Brain cerebral cortex	3/3	Pleura (Mesothelium)	7/7
Breast	3/3	Prostate/Prostate gland	8/8
Cervix	5/5	Salivary Gland	0/1
Esophagus	3/3	Skeletal Muscle	7/7
Fallopian tube[a]	2/3	Skin	11/11
Heart[b]	4/7	Spinal cord[a]	1/2
Intestine, colon	6/6	Spleen	3/3
Intestine, rectum	1/1	Stomach	7/7

Intestine, small intestine	7/7	Testis	7/7
Kidney, cortex[a]	3/4	Thymus	3/3
Kidney, medulla[a]	0/1	Thyroid	17/17
Liver[a]	1/3	Tonsil	5/5
Liver and intrahepatic bile ducts[c]	2/3	Ureter	1/1
Lung	7/7	Uterus, cervix[a]	2/3
Ovary[b]	4/5	Uterus, endometrium	30/30

[a] One cases had no definitive internal control staining.
[b] Three cases had no definitive internal control staining.
[c] One case no identifiable staining in tissue.

Note: Mismatch repair proteins such as PMS2 are present in all actively proliferating cells. For all tissues, positive/negative staining was determined for tumor cells in the presence of positive staining in normal control cells (lymphocytes, fibroblasts, and epithelial cells).

Table 3: Specificity of VENTANA anti-PMS2 (SP487) Antibody was Determined by Testing a Variety of FFPE Neoplastic Tissues

Pathology	No. Positive / Total Cases
Transitional cell carcinoma (bladder)	3/3
Invasive ductal carcinoma (breast)[a]	2/4
Invasive lobular carcinoma (breast)	1/1
Squamous cell carcinoma (esophagus)	2/2
Adenocarcinoma (intestine, colon)[b]	3/3
Adenocarcinoma (intestine, rectum)	3/3
Adenocarcinoma (intestine, small intestine)	1/1
Clear cell carcinoma (kidney)	3/3
Hepatocellular carcinoma (liver)[c]	2/3
Adenocarcinoma (lung)	3/3
Small cell carcinoma (lung)	1/1
Squamous cell carcinoma (lung)	1/1
Metastatic melanoma (lymph node)	1/1
Adenocarcinoma (ovary)	1/1
Endometrioid adenocarcinoma (ovary)	1/1
Serous cystadenocarcinoma (ovary)	1/1
Adenocarcinoma (prostate)	1/1
Squamous cell carcinoma (skin)	1/1
Adenocarcinoma (stomach)	2/2
Squamous cell carcinoma (uterus, cervix)	3/3
Adenocarcinoma (uterus, endometrium)[c]	2/3

[a] Two cases no definitive internal control cell staining.
[b] One case PMS2 Loss, other cases PMS2 Intact.

[c] One case no definitive internal control cells staining.
Note: Mismatch repair proteins such as PMS2 are present in all actively proliferating cells. For all tissues, positive/negative staining was determined for tumor cells in the presence of positive staining in normal control cells (lymphocytes, fibroblasts, and epithelial cells).

4.2. Within-Run Repeatability and Between-Day Intermediate Precision

The repeatability and precision of VENTANA anti-PMS2 (SP487) antibody were evaluated on the BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit. Within-Run Repeatability was evaluated using was evaluated using 6 CRC specimens (3 Intact and 3 Loss).

Replicate slides from each of the CRC specimens were stained with VENTANA antiPMS2 (SP487) antibody on a single BenchMark ULTRA instrument within a single day. Each VENTANA anti-PMS2 (SP487) antibody-stained slide was paired with a negative reagent control stained slide from the same case. All slide sets were randomized and then evaluated as Intact or Loss by a single pathologist blinded to the case diagnosis.

Between-Day Intermediate Precision was also evaluated using 6 CRC specimens (3 Intact and 3 Loss). Replicate slides from each of the CRC specimens were stained with VENTANA anti-PMS2 (SP487) antibody on a BenchMark ULTRA instrument on each of 3 non-consecutive days. Each VENTANA anti-PMS2 (SP487) antibody-stained slide was paired with a negative reagent control stained slide from the same case. All slide sets were randomized, and then evaluated as Intact or Loss by a single pathologist blinded to the case diagnosis.

None of the slides stained with the negative reagent control showed specific staining and background staining was ≤ 0.5. Using pooled data of all possible pairings, both Within-Run Repeatability and Between-Day Intermediate Precision studies demonstrated 100% Loss percent agreement (LPA), 100% Intact percent agreement (IPA), and 100% overall percent agreement (OPA). A summary of the results can be found in Table 4.

Table 4: Within-Run Repeatability and Between-Day Intermediate Precision of VENTANA anti-PMS2 (SP487) antibody on CRC cases as measured by Clinical Status (Intact or Loss)

Precision	Clinical Status	Agreement			
		Type[a]	n/N	%	95% CI[b]
Within-Run	Loss	LPA	27/27	100.0	(87.5, 100.0)
	Intact	IPA	27/27	100.0	(87.5, 100.0)
	Total	OPA	54/54	100.0	(93.4, 100.0)
Between-Day Intermediate Precision	Loss	LPA	18/18	100.0	(82.4, 100.0)
	Intact	IPA	18/18	100.0	(82.4, 100.0)
	Total	OPA	36/36	100.0	(90.4, 100.0)

[a] LPA = Loss Percent Agreement; IPA = Intact Percent Agreement; OPA = Overall Percent Agreement.
[b] 95% CIs were calculated using the Wilson Score method.

4.2.1. BenchMark ULTRA Instrument-to-Instrument Precision

BenchMark ULTRA Instrument Between-Instrument Intermediate Precision of VENTANA anti-PMS2 (SP487) antibody was determined by staining replicate slides of 6 CRC specimens (3 Intact and 3 Loss) across 3 BenchMark ULTRA instruments with VENTANA anti-PMS2 (SP487) antibody using OptiView DAB IHC Detection Kit.

Each VENTANA anti-PMS2 (SP487) antibody-stained slide was paired with a negative reagent control stained slide from the same case. All slide sets were randomized, and then evaluated for Clinical Status (Intact or Loss) by a single pathologist blinded to the case diagnosis. None of the slides stained with the negative reagent control showed specific staining and background staining was ≤ 0.5.

For BenchMark ULTRA Instrument Between-Instrument Intermediate Precision, pairwise comparisons of the Clinical Status of slides for each specimen were made between instruments and demonstrated 100% LPA, IPA, and OPA. A summary of the results can be found in Table 5.

Table 5: BenchMark ULTRA Instrument Between-Instrument Intermediate Precision of VENTANA anti-PMS2 (SP487) Antibody in CRC as Measured by Clinical Status (Intact or Loss)

Precision	Clinical Status	Agreement			
		Type[a]	n/N	%	95% CI[b]
Between-Instrument Intermediate Precision	Loss	LPA	18/18	100.0	(82.4, 100.0)
	Intact	IPA	18/18	100.0	(82.4, 100.0)
	Total	OPA	36/36	100.0	(90.4, 100.0)

[a] LPA = Loss Percent Agreement; IPA = Intact Percent Agreement; OPA = Overall Percent Agreement.
[b] 95% CIs were calculated using the Wilson Score method.

4.2.2. Reader Precision Studies

Within- and Between-Reader precision was evaluated on 36 CRC specimens (18 Intact and 18 Loss) stained with VENTANA anti-PMS2 (SP487) antibody and OptiView DAB IHC Detection Kit. Each VENTANA anti-PMS2 (SP487) antibody-stained slide was paired with an H&E and a negative reagent control stained slide from the same case.

All slide sets were randomized, and evaluated by 3 pathologists for Intact or Loss PMS2 Clinical Status. Pathologists were blinded to the case diagnosis. Following a two-week washout period, the VENTANA anti-PMS2 (SP487) antibody-stained slides were re-randomized for a second evaluation of the PMS2 Clinical Status by each of the 3 pathologists. None of the slides stained with the negative reagent control showed specific staining and background staining was ≤ 0.5.

Within-Reader precision compared initial and final slide evaluations from a single pathologist providing 36 CRC tumor slide comparisons per pathologist. Comparisons from the 3 pathologists were pooled and demonstrated 99.0% average Loss agreement (ALA), 99.1% average Intact agreement (AIA), and 99.1% OPA for Within-Reader precision. A summary of the results can be found in Table 6.

Between-Reader precision compared all slide evaluations (36 CRC tumor cases x 2 evaluations/case x 3 pathologists = 216 slide evaluations) to a modal case status for each case. The results demonstrate 95.2% ALA, 95.5% AIA, and 95.4% OPA for Between-Reader precision. A summary of the results can be found in Table 6.

Table 6: Within-Reader and Between-Reader Precision of VENTANA anti-PMS2 (SP487) Antibody on CRC as Measured by PMS2 Clinical Status (Intact or Loss)

Precision	Clinical Status	Agreement			
		Type[a]	n/N	%	95% CI[b]
Within-Reader	Loss	ALA	104/105	99.0	(97.1, 100.0)
	Intact	AIA	110/111	99.1	(97.2, 100.0)
	Total	OPA	107/108	99.1	(97.2, 100.0)
Between-Reader	Loss	ALA	200/210	95.2	(89.3, 100.0)
	Intact	AIA	212/222	95.5	(90.6, 100.0)
	Total	OPA	206/216	95.4	(89.8, 100.0)

[a] ALA = Average Loss Agreement; AIA = Average Intact Agreement; OPA = Overall Percent Agreement.
[b] For Within-Reader, the ALA, AIA and OPA 95% CIs were calculated using the percentile bootstrap method. For Between-Reader, the ALA, AIA, and OPA 95% Cis were calculated using the percentile bootstrap method.

4.2.3. Lot-to-Lot Precision

Lot-to-Lot Precision of VENTANA anti-PMS2 (SP487) antibody was determined by testing 3 production lots of VENTANA anti-PMS2 (SP487) antibody each on replicate slides of 6 CRC cases (3 Intact and 3 Loss) on a BenchMark ULTRA instrument using OptiView DAB IHC Detection Kit.

Each VENTANA anti-PMS2 (SP487) antibody-stained slide was paired with a negative reagent control stained slide from the same case. Slide sets were randomized and evaluated by a single pathologist blinded to the case diagnosis and VENTANA anti-PMS2 (SP487) antibody lot number. None of the slides stained with the negative reagent control showed specific staining and background staining was ≤ 0.5.

For VENTANA anti-PMS2 (SP487) antibody Lot-to-Lot Precision, all slide evaluations were compared to a modal case status for each case. The OPA between VENTANA anti-PMS2 (SP487) antibody lots was 100%; demonstrating that VENTANA anti-PMS2 (SP487) antibody staining is reproducible across antibody lots.

A summary of the results for Lot-to-Lot Precision of VENTANA anti-PMS2 (SP487) antibody is shown in Table 7.

Table 7: Lot-to-lot Precision of VENTANA anti-PMS2 (SP487) antibody on CRC as measured by Clinical Status (Intact or Loss)

Precision	Clinical Status	Agreement			
		Type[a]	n/N	%	95% CI[b]
Lot-to-Lot	Loss	LPA	18/18	100.0	(82.4, 100.0)
	Intact	IPA	18/18	100.0	(82.4, 100.0)
	Total	OPA	36/36	100.0	(90.4, 100.0)

[a] LPA = Loss Percent Agreement; IPA = Intact Percent Agreement; OPA = Overall Percent Agreement.

[b] 95% CIs were calculated using the Wilson Score method.

4.3. Inter-Laboratory Reproducibility and Method Comparison Study

An Inter-laboratory Reproducibility (ILR) study for VENTANA anti-PMS2 (SP487) antibody staining was conducted to evaluate the reproducibility of the assay in determining PMS2 protein expression (Loss/Intact) in CRC specimens on the BenchMark ULTRA and BenchMark ULTRA PLUS instruments independently. Additionally, a method comparison study between the BenchMark ULTRA and BenchMark ULTRA PLUS was performed to determine if the performance of VENTANA anti-PMS2 (SP487) antibody is equivalent on both BenchMark instruments. Twenty unique CRC tumor specimens (10 Intact, 10 Loss) were stained across 3 BenchMark ULTRA and 3 BenchMark ULTRA PLUS instruments on 3 non-consecutive days at 3 external laboratories. For each BenchMark instrument, a set of 3 stained slides per sample per staining day was randomized and evaluated by 2 readers per site. Each case had 6 results per site per BenchMark instrument (18 results per instrument, 36 results in total). At each site, the stained slides were independently evaluated using the VENTANA anti-PMS2 (SP487) antibody Table 8. Results for BenchMark ULTRA are summarized in Table 9 and Table 10. Results for BenchMark ULTRA PLUS are summarized in Table 11 and Table 12.

Table 8: Staining Interpretation for VENTANA anti-PMS2 (SP487) Antibody

Clinical Status	Description
Intact PMS2 Expression	Unequivocal nuclear staining in viable tumor cells, in the presence of acceptable internal positive controls (eg nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor)
Loss of PMS2 Expression	Unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of acceptable internal positive controls. Punctate nuclear staining will be considered negative.

Table 9: Inter-Laboratory Reproducibility for Overall Agreement Rates for VENTANA anti-PMS2 (SP487) in CRC Specimens on BenchMark ULTRA Instruments

BenchMark ULTRA Inter-Laboratory Reproducibility	Agreement			
	Type[a]	n/N	%	95% CI[b]
Overall	LPA	178/180	98.9	(96.7, 100.0)
	IPA	179/180	99.4	(98.3, 100.0)
	OPA	357/360	99.2	(97.8, 100.0)
Site- Stratified	LPA	178/180	98.9	(96.7, 100.0)
	IPA	179/180	99.4	(98.3, 100.0)
	OPA	357/360	99.2	(97.8, 100.0)
Reader-Stratified	LPA	178/180	98.9	(96.7, 100.0)
	IPA	179/180	99.4	(98.3, 100.0)
	OPA	357/360	99.2	(97.8, 100.0)

[a] IPA = Intact Percent Agreement; LPA = Loss Percent Agreement; OPA = Overall Percent Agreement.
[b] For point estimates equal to 100%, 2-sided 95% CIs were calculated using the Wilson score method; for other point estimates, they were calculated using the percentile bootstrap method with 2000 replicates and stratification by case qualification bin (Intact, Loss, Challenging Intact, or Challenging Loss).

In addition, pairwise comparisons of PMS2 status were evaluated between-sites, between-readers, and between-days. Reproducibility of the assay on BenchMark ULTRA across 5 days, 3 sites, and 6 readers is summarized in Table 10.

Table 10: Inter-Laboratory Reproducibility Pairwise Agreement Rates for VENTANA anti-PMS2 (SP487) in CRC on BenchMark ULTRA Instruments

BenchMark ULTRA Inter-Laboratory Reproducibility	Agreement			
	Type[a]	n/N	%	95% CI[b]
Inter-Site	ALA	2114/2148	98.4	(95.7, 100.0)
	AIA	2138/2172	98.4	(95.7, 100.0)
	OPA	2126/2160	98.4	(95.8, 100.0)
Inter-Reader	ALA	176/179	98.3	(95.5, 100.)
	AIA	178/181	98.3	(95.7, 100.0)
	OPA	177/180	98.3	(95.6, 100.0)
Inter-Day	ALA	352/358	98.3	(95.5, 100.)
	AIA	356/362	98.3	(95.7, 100.0)
	OPA	354/360	98.3	(95.6, 100.0)

[a] AIA = Average Intact Agreement; ALA = Average Loss Agreement; OPA = Overall Percent Agreement.
[b] For point estimates equal to 100%, 2-sided 95% CIs were calculated using the Wilson score method; for other point estimates, they were calculated using the percentile bootstrap method with 2000 replicates and stratification by case qualification bin (Intact, Loss, Challenging Intact, or Challenging Loss).

Table 11: Inter-Laboratory Reproducibility for Overall Agreement Rates for VENTANA anti-PMS2 (SP487) in CRC Specimens on BenchMark ULTRA PLUS Instruments

BenchMark ULTRA PLUS Inter-Laboratory Reproducibility	Agreement			
	Type[a]	n/N	%	95% CI[b]
Overall	LPA	178/180	98.9	(97.8, 100.0)
	IPA	178/180	98.9	(97.2, 100.0)
	OPA	356/360	98.9	(97.8, 99.7)
Site- Stratified	LPA	178/180	98.9	(97.2, 100.0)
	IPA	178/180	98.9	(97.2, 100.0)
	OPA	356/360	98.9	(97.8, 99.7)
Reader-Stratified	LPA	178/180	98.9	(97.2, 100.0)
	IPA	178/180	98.9	(97.2, 100.0)
	OPA	356/360	98.9	(97.8, 99.7)

[a] IPA = Intact Percent Agreement; LPA = Loss Percent Agreement; OPA = Overall Percent Agreement.
[b] For point estimates equal to 100%, 2-sided 95% CIs were calculated using the Wilson score method; for other point estimates, they were calculated using the percentile bootstrap method with 2000 replicates and stratification by case qualification bin (Intact, Loss, Challenging Intact, or Challenging Loss).

In addition, pairwise comparisons of PMS2 status were evaluated between-sites, between-readers, and between-days. Reproducibility of the assay on BenchMark ULTRA across 5 days, 3 sites, and 6 readers is summarized in Table 12.

Table 12: Inter-Laboratory Reproducibility Pairwise Agreement Rates for VENTANA PMS2 (SP487) antibody in CRC on BenchMark ULTRA PLUS Instruments

BenchMark ULTRA PLUS InterLaboratory Reproducibility	Agreement			
	Type[a]	n/N	%	95% CI[b]
Inter-Site	ALA	2112/2160	97.8	(95.6, 99.4)
	AIA	2112/2160	97.8	(95.6, 99.4)
	OPA	2112/2160	97.8	(95.6, 99.4)
Inter-Reader	ALA	176/180	97.8	(95.6, 99.4)
	AIA	176/180	97.8	(95.6, 99.4)
	OPA	176/180	97.8	(95.6, 99.4)
Inter-Day	ALA	352/360	97.8	(95.6, 99.4)
	AIA	352/360	97.8	(95.6, 99.4)
	OPA	352/360	97.8	(95.6, 99.4)

[a] AIA = Average Intact Agreement; ALA = Average Loss Agreement; OPA = Overall Percent Agreement. [b] For point estimates equal to 100%, 2-sided 95% CIs were calculated using the Wilson score method; for other point estimates, they were calculated using the percentile bootstrap method with 2000 replicates and stratification by case qualification bin (Intact, Loss, Challenging Intact, or Challenging Loss).

4.3.1. Method Comparison - BenchMark ULTRA versus BenchMark ULTRA PLUS

The performance equivalence analysis (method comparison) of the VENTANA anti-PMS2 (SP487) antibody on the BenchMark ULTRA and BenchMark ULTRA PLUS was conducted twice using each instrument as the reference in turn. A total of 40 colorectal carcinoma cases were stained on both instruments. Of the 40 cases, 20 had already been stained in the course of the ILR study conduct already described. For each of those 20 cases, only 3 of the total 18 ILR observations per case were used in the MC study analysis (specifically, Day 1 Reader 1 results, giving one observation per site per reader per case). Readers also evaluated an additional 60 independent cases, with each of 3 MC study readers evaluating one slide per case stained on BenchMark ULTRA and a second slide from that case stained on BenchMark ULTRA PLUS. Results of the method comparison study are presented in Table 13 below.

Table 13: Pooled Agreement of PMS2 status for CRC Tissue Cases stained with VENTANA anti-PMS2 (SP487) Antibody on the BenchMark ULTRA PLUS versus BenchMark ULTRA Instrument

Rate	ULTRA as Reference		ULTRA PLUS as Reference	
	% (n/N)	95% CI	% (n/N)	95% CI
LPA	100.0 (60/60)	(94.0, 100.0)	100.0 (60/60)	(94.0, 100.0)
IPA	100.0 (60/60)	(94.0, 100.0)	100.0 (60/60)	(94.0, 100.0)
OPA	100.0 (120/120)	(96.9, 100.0)	-	-

Note: Loss Percent Agreement (LPA), Intact Percent Agreement (IPA), Overall Percent Agreement (OPA).

The 100.0% OPA concordance between VENTANA anti-PMS2 (SP487) antibody staining on the BenchMark ULTRA compared to on the BenchMark ULTRA PLUS demonstrates comparable performance between the two BenchMark platforms. This study demonstrates sufficient evidence for extending labeling, including the clinical claims from the current on-market MMR panels to new MMR panels using the VENTANA anti-PMS2 (SP487) antibody on both the BenchMark ULTRA and the BenchMark ULTRA PLUS platforms.

PMS2 Clone Method Comparison - VENTANA PMS2 (A16-4) antibody versus VENTANA PMS2 (SP487) antibody

A Method Comparison study was conducted to demonstrate staining equivalence between VENTANA anti-PMS2 (A16-4) antibody and VENTANA anti-PMS2 (SP487) antibody. Overall, 40 archival, de-identified FFPE CRC specimens (20 Intact, 20 Loss, and some challenging cases for PMS2 expression) were stained on a BenchMark ULTRA instrument. Three pathologists, blinded to case status, independently evaluated the stained slides and determined PMS2 status (Intact or Loss). The MC study included 120 observations for 40 CRC samples. The OPA, LPA, and IPA rates for all cases were 100.0%. The results for all cases are summarized in Table 14.

Table 14: Pooled analysis of Method Comparison between VENTANA anti-PMS2 (A16-4) antibody and VENTANA anti-PMS2 (SP487) antibody on BenchMark ULTRA in CRC

Reader	PMS2 (SP487) Status	PMS2 (A16-4) Status			Measure [a]	% (n/N)	95% CI [b]
		Loss	Intact	Total			
Overall	Loss	60	0	60	LPA	100.0 (60/60)	(94.0, 100.0)
	Intact	0	60	60	IPA	100.0 (60/60)	(94.0, 100.0)
	Total	60	60	120	OPA	100.0 (120/120)	(96.9, 100.0)

[a] LPA = Loss Percent Agreement; IPA = Intact Percent Agreement; OPA = Overall Percent Agreement
[b] Two-sided 95% confidence intervals were calculated using the percentile bootstrap method with 2000 replicates. CIs for 100% IPA were calculated using Wilson score method.

The 100.0% LPA and IPA concordance between VENTANA anti-PMS2 (A16-4) antibody and VENTANA anti-PMS2 (SP487) antibody, with other analytical performance data, demonstrates comparable performance between the two PMS2 clones.

4.3.2. Accuracy Study: Concordance of VENTANA MMR IHC Panel Results to DNA Sequencing Results

A study was conducted to compare the performance of VENTANA MMR IHC Panel to a comprehensive DNA sequencing colon panel for the identification of CRCs that result from potential Lynch syndrome. The DNA sequencing colon panel included genomic analysis of variants present in MMR genes (MLH1, PMS2, MSH2, MSH6, EPCAM), BRAF, and other genes important in carcinogenesis (e.g., PIK3CA, KRAS, NRAS, ERBB2, etc.). Sequencing included all exons, intronic and flanking sequences as well as large deletions, duplications, and mosaicism.

For the study, 150 sequential CRC cases were stained by H&E and evaluated for indications of proper fixation and morphology including the presence of cellular elements (tumor and internal control cells). Each case was evaluated to determine if the specimen contained a minimum of 50% tumor content to provide sufficient representation of tumor cells in the sample as recommended for DNA sequencing. Of the 150 sequential CRC cases, 7 cases were excluded from the study set due to insufficient viable tumor (inadequate cellularity or lack of tumor content), 3 cases due to misclassification as CRC, and 1 due to clerical error. The remaining sequential study set cases were sectioned and a second H&E evaluation of bracketing slides was completed to ensure tissue integrity and tumor were represented through all sections, until sufficient cases (minimum of 100 cases) were enrolled into the study. Two cases were removed from the study due to lack of sufficient viable tumor content throughout the block and 1 was not

evaluated due to clerical error. Following review, the sequential study set included 111 cases meeting the selection criteria and were enrolled into the study. The remaining 25 sequential CRC cases were not evaluated and not enrolled into the study. In addition, an enrichment study set of 15 CRC cases showing a Clinical status of Loss by IHC were included to ensure that loss of each protein was represented in the study. Tissue sections of all cases in the study were stained by IHC with VENTANA MMR IHC Panel and appropriate negative reagent controls. Additional tissue sections from each case were subjected to the DNA sequencing colon panel. Of the 126 enrolled cases, 7 cases were excluded from analyses due to failure of sequencing.

In the final study set of 119 cases (including one case that failed to produce IHC results due to tissue loss), the analysis compared the results of VENTANA MMR IHC Panel to those for DNA sequencing at the case level, where DNA sequencing acted as the reference status for IHC comparison as shown in Table 15. For IHC, the MMR status (Intact / Loss) was stratified by BRAF V600E status, and for DNA sequencing, results were characterized by the presence or absence of potential pathogenic mutations. For this study, a pathogenic mutation within the tumor is defined as a germline or somatic mutation predicted to result in the loss of MMR protein expression. Point estimates for this comparison were 77.8% PPA, 97.0% NPA and 94.1% OPA.

IHC MMR status and DNA sequencing status were compared separately for individual MMR proteins within the study in Table 16. For MLH1 and PMS2 Loss cases, results were stratified by BRAF V600E. The OPA of each MMR protein, when compared to the results of the DNA sequencing colon panel, was 95.8% for VENTANA anti-MLH1 (M1) antibody, 94.1% for VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, 98.3% for VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody and 96.6% for VENTANA antiMSH6 (SP93) Rabbit Monoclonal Primary antibody. Out of three cases containing a potential pathogenic mutation in the MLH1 gene, all were MLH1 Loss cases by IHC. One of these cases was also BRAF V600E positive suggesting sporadic CRC. It is likely given the variable allelic frequency that the pathogenic mutation identified in the tumor does not represent a germline mutation, but an acquired mutation in this tumor. Four of the eight cases that contained a potential pathogenic mutation affecting MSH6 expression demonstrated MSH6 Intact status by IHC. Of these, two contained POLE mutations which variably affect the expression of MMR proteins and do not represent Lynch syndrome mutations. One case demonstrated MSH6 IHC staining in a small portion of the tumor and was designated Intact, but DNA sequencing showed several mutations in the MSH6 gene which likely result from somatic mutations. An analysis of VENTANA MMR IHC Panel and DNA sequencing results was also

performed for the sequential Table 17 and enrichment Table 18 study sets at the case level. In addition, an evaluation of the IHC results for each MMR protein and DNA sequencing within the sequential and enrichment study sets are presented in Table 19 and Table 20, respectively. VENTANA anti-BRAF V600E (VE1) antibody is included in VENTANA MMR IHC panel for the stratification of CRC cases showing a loss of MLH1 protein expression to sporadic or likely Lynch syndrome cancers. Of the 24 BRAF V600E IHC positive cases in this study, 20 cases had loss of MLH1 protein by IHC. The remaining four cases were pMMR (Intact for all MMR proteins). All 24 BRAF V600E positive specimens were identified as sporadic CRC. Thus the data support the use of VENTANA anti-BRAF V600E (VE1) antibody to differentiate between sporadic and probable Lynch syndrome CRC in the absence of MLH1 expression. In addition, BRAF V600E Clinical status in CRC obtained by IHC using VENTANA anti-BRAF V600E (VE1) antibody was also compared to BRAF mutational status results determined by DNA sequencing. For 23 BRAF V600E IHC positive cases (one case failed to yield DNA sequencing results), the PPA, NPA, and OPA of VENTANA anti-BRAF V600E (VE1) antibody based IHC testing using DNA sequencing results as the reference all were 100%. These results verified that VENTANA anti-BRAF V600E (VE1) antibody correctly identifies CRC having the BRAF V600E mutation.

Table 15: Evaluation of VENTANA MMR IHC Panel and DNA Sequencing Results at the Case Level – ALL CASES

A) Comparison of VENTANA MMR IHC Panel and DNA Sequencing Results

VENTANA MMR IHC Panel Results		DNA Sequencing Results			
		Pathogenic Mutation	No Pathogenic Mutation	Invalid	Total
MMR Loss	BRAF V600E +	1	19	0	20
	BRAF V600E -	14	3	1	18
MMR Intact	BRAF V600E +	0	3	1	4
	BRAF V600E -	2	76	5	83
Invalid		1	0	0	1
Total		18	101	7	126

Note: Invalids are defined as failure to produce results by IHC and/or DNA sequencing.

B) Agreement between VENTANA MMR IHC Panel and DNA Sequencing Results

A reement			
Type	**n/N**	**%**	**95% CI**
PPA	14/18	77.8	(54.8, 91.0)
NPA	98/101	97.0	(91.6, 99.0)
OPA	112/119	94.1	(88.4, 97.1)

Note: Only Invalids resulting from a failure by IHC are included in the analysis. 95% CIs were calculated using the Wilson Score method.

Note: The association between the test results and the final diagnosis with respect to potential Lynch Syndrome is an estimate because the study was enriched with potential Lynch syndrome positive cases.

Table 16: Evaluation of VENTANA MMR IHC Panel and DNA Sequencing Results at the Individual MMR Protein Level – ALL CASES

A) Comparison of individual MMR Protein Status and DNA Sequencing Results

IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MLH1 Loss	BRAF V600E +	1[a]	19	20
	BRAF V600E -	2	4	6
MLH1 Intact		0	92	92
Total		3	115	118
PMS2 (A16-4) Loss	BRAF V600E +	0	20	20
	BRAF V600E -	3	7	10
PMS2 (A16-4) Intact		0	88	88
Total		3	115	118
MSH2 Loss		3	2	5
MSH2 Intact		0	113	113
Total		3	115	118
MSH6 Loss		4	0	4
MSH6 Intact		4	110	114
Total		8	110	118

[a] Variant allele frequency indicates this is a rare MLH1 (p.K196Nfs*6) somatic mutation event and not a germline mutation.

B) Agreement between Individual MMR Protein and DNA Sequencing Results

Agreement				
Protein	**Type**	**n/N**	**%**	**95% CI[a]**
MLH1	PPA	2/3	66.7	(20.8, 93.9)
	NPA	111/115	96.5	(91.4, 98.6)
	OPA	113/118	95.8	(90.5, 98.2)
PMS2 (A16-4)	PPA	3/3	100.0	(43.9, 100.0)
	NPA	108/115	93.9	(88.0, 97.0)
	OPA	111/118	94.1	(88.3, 97.1)
MSH2	PPA	3/3	100.0	(43.9, 100.0)
	NPA	113/115	98.3	(93.9,99.5)
	OPA	116/118	98.3	(94.0,99.5)
MSH6	PPA	4/8	50.0	(21.5,78.5)
	NPA	110/110	100.0	(96.6, 100.0)
	OPA	114/118	96.6	(91.6, 98.7)

[a] 95% CIs were calculated using the Wilson Score method.

Table 17: Evaluation of VENTANA MMR IHC Panel and DNA Sequencing Results at the Case Level – SEQUENTIAL STUDY SET

A) Comparison of VENTANA MMR IHC Panel and DNA Sequencing Results

VENTANA MMR IHC Panel Results		DNA Sequencing Results			
		Pathogenic Mutation	No Pathogenic Mutation	Invalid	Total
MMR Loss	BRAF V600E +	1	18	0	19
	BRAF V600E -	4	2	0	6
MMR Intact	BRAF V600E +	0	3	1	4
	BRAF V600E -	1	76	5	82
Invalid		0	0	0	0
Total		6	99	6	111

Note: Invalids are defined as failure to produce results by IHC and/or DNA sequencing.

B) Agreement of VENTANA MMR IHC Panel and DNA Sequencing Results

Agree ıent			
Type	n/N	%	95% CI[a]
PPA	4/6	66.7	(30.0, 90.3)
NPA	97/99	98.0	(92.9, 99.4)
OPA	101/105	96.2	(90.6, 98.5)

Note: Only Invalids resulting from a failure by IHC are included in the analysis.
[a] 95% CIs were calculated using the Wilson Score method.

Table 18: Evaluation of VENTANA MMR IHC Panel and DNA Sequencing Results at the Case Level – ENRICHMENT STUDY SET

A) Comparison of VENTANA MMR IHC Panel and DNA Sequencing Results

VENTANA MMR IHC Panel Results		DNA Sequencing Results			
		Pathogenic Mutation	No Pathogenic Mutation	Invalid	Total
MMR Loss	BRAF V600E +	0	1	0	1
	BRAF V600E -	10	1	1	12
MMR Intact	BRAF V600E +	0	0	0	0
	BRAF V600E -	1	0	0	1
Invalid		1	0	0	1
Total		12	2	1	15

Note: Invalids are defined as failure to produce results by IHC and/or DNA sequencing.

B) Agreement of VENTANA MMR IHC Panel and DNA Sequencing Results

Agreement			
Type	n/N	%	95% CI[a]
PPA	10/12	83.3	(55.2, 95.3)
NPA	1/2	50.0	(9.5, 90.5)
OPA	11/14	78.6	(52.4, 92.4)

Note: Only Invalids resulting from a failure by IHC are included in the analysis.
[a] 95% CIs were calculated using the Wilson Score method.

Table 19: Evaluation of MMR Protein and DNA Sequencing Results at the Individual MMR Protein Level – SEQUENTIAL STUDY SET

A) Comparison of Individual MMR Protein Status and DNA Sequencing Results

IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MLH1 Loss	BRAF V600E +	1	18	19
	BRAF V600E -	1	4	5
MLH1 Intact		0	81	81
Total		2	103	105
PMS2 (A16-4) Loss	BRAF V600E +	0	19	19
	BRAF V600E -	0	5	5
PMS (A16-4) Intact		0	81	81
Total		0	105	105
MSH2 Loss		1	0	1
MSH2 Intact		0	104	104
Total		1	104	105
MSH6 Loss		0	0	0
MSH6 Intact		3	102	105
Total		3	102	105

B) Agreement of Individual MMR Protein Status and DNA Sequencing Results

Agreement				
Protein	**Type**	**n/N**	**%**	**95% CI[a]**
MLH1	PPA	1/2	50.0	(9.5, 90.5)
	NPA	99/103	96.1	(90.4, 98.5)
	OPA	100/105	95.2	(89.3, 97.9)
PMS2 (A16-4)	PPA	n.e.[b]	n.e.[b]	n.e.[b]
	NPA	100/105	95.2	(89.3, 97.9)
	OPA	100/105	95.2	(89.3, 97.9)
MSH2	PPA	1/1	100.0	(20.7, 100.0)
	NPA	104/104	100.0	(96.4, 100.0)
	OPA	105/105	100.0	(96.5, 100.0)
MSH6	PPA	0/3	0.0	(0.0, 56.1)
	NPA	102/102	100.0	(96.4, 100.0)
	OPA	102/105	97.1	(91.9, 99.0)

[a] 95% CIs were calculated using the Wilson Score method.

[b] n.e.= not estimable.

Table 20: Evaluation of MMR protein and DNA Sequencing Results at the Individual MMR Protein Level – ENRICHMENT STUDY SET

A) Comparison of Individual MMR Protein Status and DNA Sequencing Results

IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MLH1 Loss	BRAF V600E +	0	1	1
	BRAF V600E -	1	0	1
MLH1 Intact		0	11	11
Total		1	12	13
PMS2 (A16-4) Loss	BRAF V600E +	0	1	1
	BRAF V600E -	3	2	5
PMS2 (A16-4) Intact		0	7	7
Total		3	10	13
MSH2 Loss		2	2	4
MSH2 Intact		0	9	9
Total		2	11	13
MSH6 Loss		4	0	4
MSH6 Intact		1	8	9
Total		5	8	13

B) Agreement of Individual MMR Protein Status and DNA Sequencing Results

Agreement				
Protein	**Type**	**n/N**	**%**	**95% CI[a]**
MLH1	PPA	1/1	100.0	(20.7, 100.0)
	NPA	12/12	100.0	(75.8, 100.0)
	OPA	13/13	100.0	(77.2, 100.0)
PMS2 (A16-4)	PPA	3/3	100.0	(43.9, 100.0)
	NPA	8/10	80.0	(49.0, 94.3)
	OPA	11/13	84.6	(57.8, 95.7)
MSH2	PPA	2/2	100.0	(34.2, 100.0)
	NPA	9/11	81.8	(52.3, 94.9)
	OPA	11/13	84.6	(57.8, 95.7)
MSH6	PPA	4/5	80.0	(37.6, 96.4)
	NPA	8/8	100.0	(67.6, 100.0)
	OPA	12/13	92.3	(66.7, 98.6)

[a] 95% CIs were calculated using the Wilson Score method.

4.4. Platform Migration Study for Additional MMR Antibodies (MLH1, PMS2 [A16-4], MSH2, MSH6) on BenchMark ULTRA PLUS

To support the migration of the constituent antibodies of the VENTANA MMR IHC Panel—VENTANA anti-MLH1 (M1), VENTANA anti-PMS2 (A16-4), VENTANA anti-MSH2 (G219-1129), and VENTANA anti-MSH6 (SP93)—from the BenchMark ULTRA to the BenchMark ULTRA PLUS instrument, analytical method comparison (MC) and interlaboratory reproducibility (ILR) studies were conducted.

4.4.1. Method Comparison Study (BenchMark ULTRA vs. BenchMark ULTRA PLUS)

A method comparison study evaluated the concordance of biomarker status (Intact vs. Loss) for 40 CRC stained on BenchMark ULTRA and BenchMark ULTRA PLUS instruments across 3 external sites and evaluated by 7 pathologists. The pooled agreement results across all evaluable observations for each MMR biomarker are summarized in Table 21.

Table 21: Pooled Agreement of MMR Biomarker Status Between BenchMark ULTRA PLUS and BenchMark ULTRA Instruments in CRC

MMR Biomarker	Agreement Type	% (n/N)	95% CI
MLH1 (M1)	Loss Percent Agreement (LPA)	97.1% (136/140)	(93.4, 100.0)
	Intact Percent Agreement (IPA)	97.8% (135/138)	(94.0, 100.0)
	Overall Percent Agreement (OPA)	97.5% (271/278)	(95.1, 99.4)
PMS2 (A16-4)	Loss Percent Agreement (LPA)	97.8% (135/138)	(95.2, 100.0)
	Intact Percent Agreement (IPA)	98.6% (139/141)	(96.1, 100.0)
	Overall Percent Agreement (OPA)	98.2% (274/279)	(96.7, 99.6)
MSH2 (G219-1129)	Loss Percent Agreement (LPA)	97.8% (131/134)	(95.2, 99.4)
	Intact Percent Agreement (IPA)	99.2% (129/130)	(97.2, 100.0)
	Overall Percent Agreement (OPA)	98.5% (260/264)	(96.7, 99.7)
MSH6 (SP93)	Loss Percent Agreement (LPA)	96.5% (137/142)	(92.0, 100.0)
	Intact Percent Agreement (IPA)	98.5% (135/137)	(96.1, 100.0)

	Overall Percent Agreement (OPA)	97.5% (272/279)	(95.0, 99.6)

Note: 95% CIs were calculated using the percentile bootstrap method with 2,000 replicates.

4.4.2. Interlaboratory Reproducibility (ILR) Study

An ILR study was conducted using 14 FFPE CRC tumor specimens (7 dMMR and 7 pMMR cases) stained across 3 external sites on 3 non-consecutive days and evaluated by 2 pathologists per site (6 readers total) on BenchMark ULTRA PLUS instruments.

The primary analysis demonstrated high reproducibility for overall MMR status relative to the case mode, yielding a dMMR Percent Agreement (dMPA) of 100.0% (125/125; 95% CI: 97.0, 100.0) and a pMMR Percent Agreement (PMPA) of 100.0% (126/126; 95% CI: 97.0, 100.0), with an Overall Percent Agreement (OPA) of 100.0% (251/251; 95% CI: 98.5, 100.0). Inter-site, inter-reader, and inter-day pairwise agreement rates for overall MMR status were all 100.0%. Results are summarized in Table 22.

Table 22: Inter-Laboratory Reproducibility for overall agreement rates for VENTANA MMR RxDx Panel in CRC

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	dMPA	125/125	100.0	(97.0, 100.0)
	pMPA	126/126	100.0	(97.0, 100.0)
	OPA	251/251	100.0	(98.5, 100.0)
Site- Stratified	dMPA	125/125	100.0	(97.0, 100.0)
	pMPA	126/126	100.0	(97.0, 100.0)
	OPA	251/251	100.0	(98.5, 100.0)
Reader-Stratified	dMPA	125/125	100.0	(97.0, 100.0)
	pMPA	126/126	100.0	(97.0, 100.0)
	OPA	251/251	100.0	(98.5, 100.0)

Note: dMPA = dMMR Percent Agreement; pMPA = pMMR Percent Agreement;
OPA = Overall Percent Agreement.
Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates. CIs for 100% dMPA, pMPA and OPA were calculated using Wilson score method.

In addition, pairwise comparisons of MMR status were evaluated between-sites, between-readers, and between-days. Reproducibility of the assay on ULTRA PLUS across 3 days, 3 sites, and 6 readers is summarized in Table 23.

Table 23: Inter-Laboratory Reproducibility pairwise agreement rates for VENTANA MMR RxDx Panel in CRC

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Inter-Site	ADPA	1488/1488	100.0	(99.7, 100.0)
	APPA	1512/1512	100.0	(99.7, 100.0)
	OPA	1500/1500	100.0	(99.7, 100.0)
Inter-Reader	ADPA	124/124	100.0	(97.0, 100.0)
	APPA	126/126	100.0	(97.0, 100.0)
	OPA	125/125	100.0	(98.5, 100.0)
Inter-Day	ADPA	248/248	100.0	(98.5, 100.0)
	APPA	252/252	100.0	(98.5, 100.0)
	OPA	250/250	100.0	(98.5, 100.0)

Note: ADPA = Average dMMR Percent Agreement; APPA = Average pMMR Percent Agreement; OPA = Overall Percent Agreement.
Note: Two-sided 95% CIs for 100% ADPA and APPA were calculated using the transformed Wilson score method. CIs for 100% OPA were calculated using Wilson score method.

4.5. Platform Migration Study for VENTANA anti-BRAF V600E (VE1) Monoclonal Primary Antibody on BenchMark ULTRA PLUS

4.5.1. Method Comparison Study (BenchMark ULTRA vs. BenchMark ULTRA PLUS)

A method comparison study evaluated 120 unique FFPE colorectal cancer (CRC) tissue cases stained with VENTANA anti-BRAF V600E (VE1) antibody and OptiView DAB IHC Detection Kit on both BenchMark ULTRA and BenchMark ULTRA PLUS instruments.
The pairwise concordance between BenchMark ULTRA and BenchMark ULTRA PLUS instruments for BRAF V600E status (positive/negative) demonstrated an OPA of 98.3%. Both non-specific background acceptability and tissue morphology acceptability rates were 100.0% (120/120) on the BenchMark ULTRA PLUS instrument. (Table 24)

Table 24: Agreement Between BenchMark ULTRA and BenchMark ULTRA PLUS for VENTANA anti-BRAF V600E (VE1) Antibody

Agreement Type	% (n/N)	95% CI
Positive Percent Agreement (PPA)	97.1% (34/35)	(85.5, 99.5)
Negative Percent Agreement (NPA)	98.8% (84/85)	(93.6, 99.8)
Overall Percent Agreement (OPA)	98.3% (118/120)	(94.1, 99.5)

Note: 95% CIs were calculated using the Wilson score method.

4.5.2. Within-Laboratory Precision Study

Staining repeatability and intermediate precision for VENTANA anti-BRAF V600E (VE1) antibody were evaluated on BenchMark ULTRA PLUS instruments using 10 FFPE CRC tissue cases (5 BRAF V600E positive and 5 BRAF V600E negative).

The study evaluated within-run repeatability, between-day intermediate precision across 5 non-consecutive days, and between-instrument intermediate precision across 3 BenchMark ULTRA PLUS instruments. All endpoints met the 90% acceptance criteria, as summarized in Table 25.

Table 25: Precision and Repeatability Summary for VENTANA anti-BRAF V600E (VE1) Antibody on BenchMark ULTRA PLUS

Study Component	PPA % (n/N) [95% CI]	NPA % (n/N) [95% CI]	OPA % (n/N) [95% CI]
Within-Run Repeatability	100.0% (25/25) [86.7, 100.0]	100.0% (25/25) [86.7, 100.0]	100.0% (50/50) [92.9, 100.0]
Between-Day Intermediate Precision	100.0% (50/50) [92.9, 100.0]	100.0% (48/48) [92.6, 100.0]	100.0% (98/98) [96.2, 100.0]
Between-Instrument Intermediate Precision	100.0% (30/30) [88.6, 100.0]	100.0% (30/30) [88.6, 100.0]	100.0% (60/60) [94.0, 100.0]

Note: 95% CIs were calculated using the Wilson score method or percentile bootstrap. Background and morphology acceptability rates were 100.0% (168/168) across all precision testing runs.

4.6 Stability

a. Legacy Antibodies

Real-Time Stability and Ship Stress Stability for the legacy antibodies (MLH1, PMS2 (A16-4), MSH2, MSH6, BRAF V600E (VE1)) were leveraged from the original De Novo application. Please refer to the publicly available DEN170030 Decision Summary.

b. New Clone (SP487)

Cut Section Stability: The cut slide stability of VENTANA anti-PMS2 (SP487) was evaluated by staining slides sectioned from one CRC case, stored at 5°C ± 3°C and 30°C ± 5°C. All results met the study acceptance criteria; the data support a cut slide stability of at least 45 days, when stored at 5°C ± 3°C or 30°C ± 5°C.

Real-Time Stability: Real-time stability testing evaluated three lots of VENTANA anti-PMS2 (SP487) stored at the intended storage condition (2-8°C). A total of 40 CRC cases were enrolled in this study. Three cases are tested at each time point (one case per lot under test). The study met the acceptance criteria at Day 0 through Month 15 for VENTANA anti-PMS2 (SP487) Rabbit Monoclonal Primary Antibody for pan-tumor indication. An interim report has been submitted and the study will continue throughout the Month 27 time point.

Ship Stress Stability: Ship stress stability evaluated three lots of VENTANA anti-PMS2 (SP487) subjected to hot and cold stress profiles followed by intended storage. A total of 40 CRC cases were enrolled in this study. Three cases are tested at each time point (one case per lot under test). The acceptance criteria were met for this study from Day 0 through Month 15 for VENTANA anti-PMS2 (SP487) Rabbit Monoclonal Primary Antibody for the stress model tested for Shipping Category I and/or F. The VENTANA anti-PMS2 (SP487) Rabbit Monoclonal Primary Antibody is stable if frozen/thawed three times. The results from the timepoints completed thus far support an interim product stability of 12 months, and the study will continue throughout the Month 27 time point.

4.7 Analytical Specificity & Sensitivity

Prevalence (Analytical Sensitivity): The overall prevalence of PMS2 loss status was 25.0% in a cohort of 100 CRC cases.

Western Blot: Western blot analysis demonstrated the specific binding of VENTANA anti-PMS2 (SP487) to the PMS2 protein. The antibody produced a single band at the expected molecular weight.

Peptide Inhibition: Peptide inhibition studies demonstrated that specific staining of VENTANA anti-PMS2 (SP487) was abolished in the presence of the specific peptide containing the target epitope.