VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody

For use with VENTANA MMR RxDx Panel

For use with VENTANA MMR IHC Panel

REF 760-5092

08033676001

IVD Σ 50

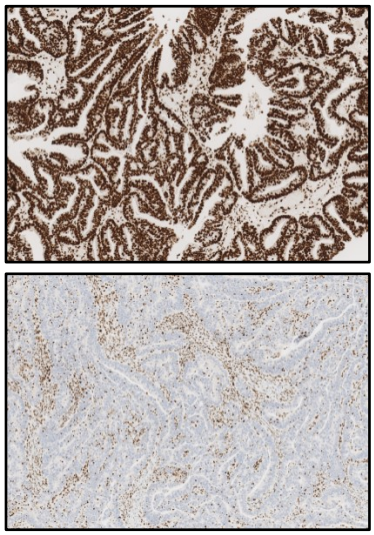

Figure 1. VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody staining with Intact (top) or Loss (bottom) of expression in endometrial carcinoma tissue.

INTENDED USE

VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody (VENTANA anti-MSH6 (SP93) antibody) is intended for laboratory use in the qualitative immunohistochemical detection of MSH6 protein in formalin-fixed, paraffin-embedded (FFPE) sections by light microscopy. VENTANA anti-MSH6 (SP93) antibody is ready to use on a BenchMark IHC/ISH instrument with the OptiView DAB IHC Detection Kit and ancillary reagents.

VENTANA anti-MSH6 (SP93) antibody is part of the VENTANA MMR RxDx Panel and the VENTANA MMR IHC Panel listed in Table 1.

Table 1. Diagnostic Indications for VENTANA MMR Panels.

Panel Name	Panel Antibodies[a]	Clinical Application
VENTANA MMR RxDx Panel	MLH1, PMS2, MSH2 and MSH6	The VENTANA MMR RxDx Panel is indicated as an aid in identifying deficient MMR (dMMR) endometrial carcinoma patients eligible for treatment with JEMPERLI (dostarlimab) in accordance with the approved therapeutic product labeling
VENTANA MMR IHC Panel	MLH1, PMS2, MSH2, MSH6 and BRAF V600E	The VENTANA MMR IHC Panel is indicated in detection of mismatch repair protein deficiency as a test for the identification of individuals at risk for Lynch syndrome in patients diagnosed with colorectal cancer (CRC), and, with BRAF V600E status, as an aid to differentiate between sporadic and probable Lynch syndrome CRC in the absence of MLH1 protein expression.

[a] MLH1- VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody
PMS2- VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody
MSH2- VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody
MSH6- VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody
BRAF- VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody

Test results of this panel should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

VENTANA anti-MSH6 (SP93) antibody is a rabbit monoclonal antibody produced against a synthetic peptide corresponding to an internal region of human MSH6 protein. VENTANA anti-MSH6 (SP93) antibody recognizes MSH6, which is one of several clinically important DNA mismatch repair (MMR) proteins.[1,2] VENTANA anti-MSH6 (SP93) antibody is part of VENTANA MMR RxDx Panel and VENTANA MMR IHC Panel, both of which are immunohistochemistry (IHC)- based systems for identifying tumors with deficient expression of any of the 4 MMR proteins (e.g. MLH1, PMS2, MSH2, MSH6) that are ordinarily ubiquitously expressed in proliferating normal and malignant cells.[3] These tumors are considered MMR-Deficient (dMMR).

MMR is a conserved molecular mechanism that functions to correct the improper base substitutions that spontaneously occur during DNA replication.[4] 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.[5,6,7] 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.[7,8] 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.[9]

CLINICAL SIGNIFICANCE

Endometrial Carcinoma (EC)

EC is the most common gynecological malignant disease, and the fourth most common cancer in North American women.[10,11] It is one of the leading causes of cancer-related death in the world.[12] EC is frequently noted to have many genetic alterations including MSI.[10] Approximately 20-40% of EC tumors are dMMR or exhibit MSI.[13,14,15] While the treatment of endometrial carcinoma varies depending on the grade, histology and stage of disease, evaluation of the MMR status of EC tumors is helpful for prognosis and guiding treatment.[16]

Emerging immunotherapies, particularly those that modify cellular pathways involving the programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1) proteins, are reshaping clinicians' therapeutic strategies. PD-1 is an inhibitory receptor expressed on T-cells after T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer.[17] PD-L1 expression has been observed in immune cells and malignant cells, and aberrant expression of PD-L1 on tumor cells has been reported to impede anti-tumor immunity, resulting in immune evasion.[17,18] Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity. Multiple studies have demonstrated that MMR deficiency correlates with higher expression of PD-1 or PD-L1, possibly due to increased neoantigen expression associated with the tumor mutation burden that results from replication errors.[10,19] Thus, MMR proteins may be useful as predictive biomarkers for PD-1 targeted therapy; specifically, a loss of expression of one or more MMR proteins might predict an increased likelihood of response to such therapy.[20,21,22] PD-1 inhibitors can be beneficial in cancers with a high frequency of MMR deficiency and /or MSI-H such as endometrial carcinoma.[19,20,22] Hence, patients with EC who are considering PD-1-targeted therapy will benefit from a companion diagnostic (CDx) assay to determine if they may be eligible for treatment with PD-1 or PD-L1 checkpoint inhibition therapy.

A loss of expression of any of the essential MMR proteins, including MLH1, PMS2, MSH2, or MSH6, causes MMR deficiency. As part of VENTANA MMR RxDx Panel, VENTANA anti-MLH1 (M1) antibody aids in determining the MMR IHC status of tumors by classifying them as Intact or Loss for MMR protein expression. The presence of staining for all four MMR protein markers in the tumor using VENTANA MMR RxDx Panel indicates that the case is MMR-Proficient (pMMR). The absence of staining for any of the MMR protein makers using VENTANA MMR RxDx Panel indicates that the case is dMMR.

Colorectal Carcinoma (CRC) and Lynch Syndrome

Colorectal cancer is the third most common cancer and the fourth most prevalent cause of cancer death in the world.[23] The majority of colorectal cancers show chromosomal instability, however approximately 15% of cancers develop through an alternative pathway characterized by defective function of the DNA mismatch repair (MMR) system. The dMMR colorectal cancers are often poorly differentiated and frequently show proximal colon predominance, mucinous, medullary, or signet ring histologic features and increased numbers of tumor-infiltrating lymphocytes.[24,25] In general, MMR deficiency may be caused either by germline mutations in one of the MMR genes with subsequent loss of the corresponding normal allele through genetic or epigenetic mechanisms, somatic mutations in the alleles, or by epigenetic inactivation of the MLH1 gene through methylation.[26]

Lynch syndrome was described in the 1960s and identified a link between the loss of MMR function and cancer.[27] Loss of MMR proteins (MLH1, PMS2, MSH2, or MSH6) may lead to MSI and a higher lifetime risk of not only colorectal cancer, but also cancers of the stomach, brain, pancreas, skin, endometrium and ovaries. Patients with Lynch syndrome have a 50-80% lifetime risk for colorectal cancer.[7,28,29] Lynch syndrome is unique from other hereditary cancer syndromes as direct testing on tumor tissue aids in the identification of patients at risk for Lynch syndrome and helps inform subsequent germline genetic testing. Families with Lynch syndrome benefit from advanced cancer screening protocols.

Various guidelines, including National Comprehensive Cancer Network (NCCN) guidelines, recommend that all colorectal cancers should be screened for potential Lynch syndrome to identify patients and families that will benefit from further genetic testing and counseling.[27,30-33] Using the VENTANA MMR IHC Panel will aid in determining the MMR status of colorectal cancers by classifying them as Intact or Loss for MMR protein expression. Loss of MLH1 or MSH2 expression is almost invariably accompanied with the loss of its heterodimer partner, PMS2 or MSH6, respectively. However, loss of PMS2 or MSH6 does not lead to loss of MLH1 or MSH2. Loss of PMS2, MSH2, and/or MSH6 is consistent with probable Lynch syndrome, and patients should be referred for additional testing and counseling consistent with clinical practice.

Loss of MLH1 protein may indicate a sporadic occurrence or potential Lynch syndrome. In 15% or more of sporadic colorectal cancer, loss of MLH1 protein is due to hypermethylation of the MLH1 promoter.[7,34,35] Importantly, the BRAF V600E mutation is observed in about two thirds of tumors with loss of MLH1 expression from MLH1 promoter hypermethylation. In contrast, the BRAF V600E mutation is very rarely observed in Lynch syndrome tumors.[34] Therefore, if the result of the VENTANA anti-MLH1 (M1) antibody indicates loss of MLH1 protein, VENTANA anti-BRAF V600E (VE1) antibody may stratify the tumor as sporadic or probable Lynch syndrome.[7,36] In colorectal cancer, loss of MLH1 protein with a BRAF V600E status of positive strongly indicates that the tumor is the result of a sporadic occurrence, virtually eliminating Lynch syndrome as the underlying cause of malignancy.[37,38] When loss of MLH1 protein is accompanied with a BRAF V600E status of negative, the MLH1 loss is consistent with a high probability of Lynch syndrome.[39]

PRINCIPLE OF THE PROCEDURE

The VENTANA anti-MSH6 (SP93) antibody is a rabbit monoclonal antibody produced against a synthetic peptide corresponding to an internal region of human MSH6 protein. The VENTANA anti-MSH6 (SP93) antibody binds to MSH6 protein in FFPE tissue sections. The antibody can be localized using a haptenated secondary antibody followed by a multimer anti-hapten-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, the BenchMark IHC/ISH 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.

In addition to staining with VENTANA anti-MSH6 (SP93) antibody, a second slide should be stained with the rabbit monoclonal negative reagent, Rabbit Monoclonal Negative Control Ig. The negative reagent control is used to assess background staining.

MATERIAL PROVIDED

VENTANA anti-MSH6 (SP93) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-MSH6 (SP93) antibody contains approximately 5 µg of a rabbit monoclonal antibody.

The antibody is diluted in Tris-HCl with carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 1 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-MSH6 (SP93) antibody is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate interpretation guide for detailed instructions for interpretation of VENTANA MMR RxDx Panel or VENTANA MMR IHC Panel staining in specific indications:

- VENTANA MMR RxDx Panel Interpretation Guide for EC indication (P/N 1020315EN)
- Interpretation Guide for Staining Colorectal Tissue for VENTANA MMR IHC Panel (P/N 1016703EN)

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody (Cat. No. 760-5091 / 08033668001)
3. VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody (Cat. No. 760-5094 / 08033692001)
4. VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody (Cat. No. 760-5093 / 08033684001)
5. For VENTANA MMR IHC Panel only, VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody (Cat. No. 760-5095 / 08033706001)
6. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
7. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
8. Microscope slides, positively charged
9. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
10. For VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody only, OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 or Cat. No. 860-099 / 06718663001)
11. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
12. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
13. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
14. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
15. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
16. Cell Conditioning Solution (CC1) Cat. No. 950-124 / 05279801001)
17. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
18. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
19. Permanent mounting medium
20. Cover glass
21. Automated coverslipper
22. General purpose laboratory equipment
23. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. Tissue should

be fixed immediately following excision for use with the VENTANA MMR RxDx Panel or VENTANA MMR IHC Panel antibodies. A delay to fixation of more than 6 hours has been shown to have an adverse effect on stain intensity of the tissue. Tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours is recommended. Fixation times of less than 6 hours and more than 72 hours may result in a loss of staining for MSH6. The amount of NBF used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).[40,41]

Fixatives such as zinc formalin, Z-5, 95% alcohol, alcohol-formalin-acetic acid (AFA) and PREFER fixative have demonstrated weak or variable staining; they are not recommended for use with this assay. Users who deviate from the specified specimen preparation must accept responsibility for interpretation of patient results.

Sections should be cut at 4 µm thick and mounted on positively-charged glass slides. No other thicknesses have been validated. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time and may be compromised 45 days after cutting from the FFPE tissue block. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[42,43]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No 1272/2008:

Table 2. Hazard information.

Hazard	Code	Statement
WARNING 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, a reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA anti-MSH6 (SP93) antibody has been developed for use on a BenchMark IHC/ISH instrument in combination with OptiView DAB IHC Detection Kit and ancillary reagents. Refer to Table 3 for the recommended staining protocol for VENTANA MMR RxDx Panel or Table 4 for the recommended staining protocol for VENTANA MMR IHC Panel.

This antibody has been optimized for specific incubation times, but the user must validate results obtained with this reagent. The effect of varying time and temperature of the antigen retrieval (cell conditioning) and antibody incubation from the recommended staining protocol in Table 3 or Table 4 may result in sub-optimal staining and false deficient and false proficient results. It is strongly recommended not to deviate from the recommended staining protocol in Table 3 or Table 4. Appropriate controls should be employed and documented. Users who deviate from the listed protocol must accept responsibility for interpretation of patient results.

The parameters for the automated procedures can be displayed, printed, and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding IHC staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 760-5092.

Table 3. Recommended staining protocol for VENTANA anti-MSH6 (SP93) antibody and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments for VENTANA MMR RxDx Panel.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Staining Procedure	GX MMR Panel	XT MMR Panel	ULTRA MMR Panel
Antibody (Primary)	anti-MSH6 Rabbit Mono Ab Selected Or Negative Control Selected	anti-MSH6 Rabbit Mono Ab Selected Or Negative Control Selected	anti-MSH6 Rabbit Mono Ab Selected Or Negative Control Selected
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes, 100°C	CC1, 64 minutes, 100°C	ULTRA CC1, 64 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	12 minutes, 37°C	12 minutes, 37°C	12 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Note: Any deviation from recommended test procedures may invalidate results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patients' results

Table 4. Recommended staining protocol for VENTANA anti-MSH6 (SP93) antibody and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments for VENTANA MMR IHC Panel.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Staining Procedure	BMK OptiView DAB IHC Par[b]	XT OptiView DAB IHC[c]	U OptiView DAB IHC[d]
Antibody (Primary)	anti-MSH6 Rabbit Mono Ab Selected Or Negative Control Selected	anti-MSH6 Rabbit Mono Ab Selected Or Negative Control Selected	anti-MSH6 Rabbit Mono Ab Selected Or Negative Control Selected
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes, 100°C	CC1, 64 minutes, 100°C	ULTRA CC1, 64 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	12 minutes, 37°C	12 minutes, 37°C	12 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

[b] Users have the option to use GX MMR Panel staining procedure.

[c] Users have the option to use XT MMR Panel staining procedure.

[d] Users have the option to use ULTRA MMR Panel staining procedure.

Note: Any deviation from recommended test procedures may invalidate results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patients' results

INTERNAL POSITIVE CONTROLS

Normal tissue elements (e.g. lymphocytes, fibroblasts, or normal epithelium) in the immediate vicinity of the tumor will serve as internal positive controls. Unequivocal nuclear staining in these cells validates the staining run. If the internal positive controls fail to demonstrate appropriate staining, results with the test specimen should be considered invalid.

NEGATIVE REAGENT CONTROL

A negative reagent control should be used to stain an adjacent section of the patient specimen tissue on a separate slide from the VENTANA anti-MSH6 (SP93) antibody stained slide. A negative reagent control rabbit monoclonal antibody (Rabbit Monoclonal Negative Control Ig), is recommended for use in place of the primary antibody to evaluate nonspecific staining. The staining parameters for the negative reagent control antibody should be the same as those for the primary antibody.

POSITIVE TISSUE CONTROL

A positive tissue control must be run with every staining procedure performed. Optimal laboratory practice is to include a positive control section on the same slide as the patient tissue. This helps identify any failures applying reagents to the slide. Tissue with weak

positive staining is best suited for quality control. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to the test sections. Such tissues may monitor all steps of the procedure from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

CRC or EC tissue with an MSH6 Clinical Status of Intact, or normal colon or normal endometrium tissue pre-qualified with VENTANA anti-MSH6 (SP93) antibody, may be used as a positive tissue control. Normal colon or normal endometrium will stain intact for MSH6 using VENTANA anti-MSH6 (SP93) antibody. The positive tissue control should exhibit unequivocal nuclear staining in viable tumor cells, in the presence of internal positive controls (nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor).

NEGATIVE TISSUE CONTROL

Since the MLH1, PMS2, MSH2, and MSH6 proteins are expressed in all tissues, a normal negative tissue control does not exist for these biomarkers. However, CRC or EC tissue with an MSH6 Clinical Status of Loss pre-qualified with VENTANA anti-MSH6 (SP93) antibody may be used as a negative tissue control. The negative tissue control should be used only to monitor the correct performance of processed tissues, test reagents, and instruments and not as an aid in formulating a specific diagnosis of patient samples.

ASSAY VERIFICATION

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing on a series of tissues with known IHC performance characteristics representing tissues Intact for MSH6 protein Clinical Status. (Refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist,[44] or the CLSI Approved Guideline.[45])

STAINING INTERPRETATION / EXPECTED RESULTS

VENTANA anti-MSH6 (SP93) antibody has a nuclear staining pattern in actively proliferating cells. Tumor tissue stained with VENTANA anti-MSH6 (SP93) antibody is assigned a Clinical Status by a trained pathologist based on their evaluation of the presence or absence of specific nuclear staining in the tumor. A Clinical Status of Intact is assigned to cases with unequivocal nuclear staining in viable tumor cells, in the presence of acceptable internal positive controls (nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor). A Clinical Status of Loss is assigned to cases with unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls as shown in Table 5.

If unequivocal nuclear staining is absent in internal positive controls and/or background staining interferes with interpretation, then the assay results should be considered unacceptable and repeated. Punctate nuclear staining of tumor cells should be considered negative (Loss). In cases with focal tumor cell staining, some specimens may exhibit focal staining in the tumor cells and staining intensity may vary from weak to strong. Based on the VENTANA MMR RxDx Panel scoring algorithm, focal weak equivocal nuclear staining in viable tumor cells in the presence of internal positive controls should be given a Clinical Status of Loss. On the other hand, focal strong unequivocal nuclear staining in viable tumor cells in the presence of internal positive controls should be given a Clinical Status of Intact.

Table 5. Staining interpretation for VENTANA anti-MSH6 (SP93) antibody.

Clinical Status	Description
Intact MSH6 Expression	Unequivocal nuclear staining in viable tumor cells in the presence of acceptable internal positive controls (e.g. nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor)
Loss of MSH6 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.

VENTANA anti-MSH6 (SP93) antibody-stained cases are categorized as Intact or Loss according to the presence or absence of specific staining in the tumor.

The interpretation for overall panel-level MMR Status is provided below in Table 6.

Table 6. Staining interpretation for VENTANA MMR RxDx Panel and VENTANA MMR IHC Panel.

Proficient (Negative)	Deficient (Positive)
All 4 markers (MLH1, PMS2, MSH2, and MSH6) in the panel exhibit Intact protein expression	At least 1 marker (MLH1, PMS2, MSH2, and MSH6) in the panel exhibits Loss of protein expression

SPECIFIC LIMITATIONS

Deviation from the recommended conditions for antigen retrieval provided in the listed protocol may invalidate expected results. Appropriate controls should be used and documented. Users who deviate from the listed protocol must accept responsibility for interpretation of patient results.

VENTANA anti-MSH6 (SP93) antibody has been solely cleared for use on BenchMark IHC/ISH instruments with OptiView DAB IHC Detection Kit and is not cleared for use with any other detection methods or automated staining instruments.

Some cases may be particularly challenging due to the following issues:

- Nonspecific background: Some specimens may exhibit nonspecific background staining for reasons that are not well understood. For this reason, evaluation of a VENTANA anti-MSH6 (SP93) antibody slide must include a comparison of the slide to the negative reagent control slide to determine the level of nonspecific background staining. Cytoplasmic staining, if present, should be disregarded in VENTANA anti-MSH6 (SP93) antibody IHC interpretation.
- Focal Staining: Some specimens may exhibit focal staining in the tumor cells; the staining intensity may vary from weak to strong. As specified by the VENTANA anti-MSH6 (SP93) antibody IHC scoring algorithm, focal weak equivocal nuclear staining in viable tumor cells in the presence of acceptable internal positive control staining should be categorized as Loss.
- Punctate Staining: Some specimens may exhibit discrete punctate staining within a few nuclei of the tumor; the staining intensity may vary from weak to strong. This staining pattern should be ignored. If only this type of staining pattern is present, the Clinical Status is Loss.
- Speckling: In contrast to punctate staining, speckling has a finer, more granular appearance and can be focal or occur across many tumor cells. This staining pattern, if seen in the tumor cell nuclei, should be ignored and the slide given a clinical status of Loss.
- Tissue or Staining Artifact: Histologic artifacts originating from the sample processing and microtomy processes can also complicate the determination of VENTANA anti-MSH6 (SP93) antibody IHC Clinical Status. These artifacts may include, but are not limited to, fixation gradients and edge effects, DAB trapping, nuclear bubbling, lack of staining in some regions of the tissue, tearing or folding of the tissue, and loss of the tissue section. In some instances, repeat staining of new sections or acquisition of a new specimen may be required.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS FOR VENTANA MMR RXDX PANEL

ANALYTICAL PERFORMANCE

Staining tests for staining sensitivity, specificity, repeatability, and intermediate precision, as well as tests for reader precision, Inter-Laboratory Reproducibility, and clinical outcome were conducted and the results are listed in the following section.

Sensitivity and Specificity

Analytical sensitivity was evaluated by characterizing dMMR prevalence in the intended use endometrial carcinoma tissue samples. The overall prevalence for MMR deficiency within endometrial carcinoma tissue samples was 22.5%, which correlates with the dMMR prevalence found in the literature for endometrial carcinoma.[46]

Analytical specificity was determined by staining multiple cases of normal and neoplastic human tissues with VENTANA anti-MSH6 (SP93) antibody. The results are listed in Table 7 and Table 8. Positive staining is nuclear unless otherwise specified. No unexpected staining was observed with VENTANA anti-MSH6 (SP93) antibody on the normal and neoplastic tissues. As expected, since MMR is present in all actively proliferating cells, all normal and neoplastic tissues demonstrated positive staining.

Table 7. Specificity of VENTANA anti-MSH6 (SP93) antibody staining on FFPE normal tissues.

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Adrenal Gland	3/3	Lung	4/4
Bladder	3/3	Lymph node	3/3
Bone Marrow	3/3	Mesothelium	3/3
Ovary	5/5	Pancreas	3/3
Breast	3/3	Parathyroid Gland	3/3
Cerebellum	3/3	Peripheral Nerve	5/5
Cerebrum	3/3	Prostate	3/3
Cervix	3/3	Skeletal Muscle	3/3
Colon	3/3	Skin	3/3
Endometrium	2/3	Spleen	3/3
Esophagus	3/3	Stomach	3/3
Heart	3/3	Testis	3/3
Pituitary gland	3/3	Thymus	3/3
Intestine	3/3	Thyroid	4/4
Kidney	3/3	Tongue / Salivary Gland	3/3
Liver	3/3	Tonsil	3/3

Note: MMR proteins such as MSH6 are present in all actively proliferating cells. For all tissues, positive/negative staining was determined for tissue specific elements in the presence of positive staining in normal control cells (lymphocytes, fibroblasts, and epithelial cells).

Table 8. Specificity of VENTANA anti-MSH6 (SP93) antibody staining on a variety of FFPE neoplastic tissues.

Pathology	# Positive / Total Cases
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebrum)	1/1
Serous adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Pancreas)	1/1
Pancreatic neuroendocrine neoplasm (Pancreas)	1/1
Seminoma (Testis)	2/2
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	1/1
Microinvasive ductal carcinoma (Breast)	1/1
Invasive ductal carcinoma (Breast)	1/1
B-cell lymphoma; NOS (Spleen)	1/1
Small cell carcinoma (Lung)	1/1
Squamous cell carcinoma (Lung)	1/1
Adenocarcinoma (Lung)	1/1
Neuroendocrine carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	1/1
Signet ring carcinoma (Stomach)	1/1
Adenocarcinoma (Small intestine)	1/1
Stromal sarcoma (small intestine)	1/1
Adenocarcinoma (Colon)	1/1
Adenocarcinoma (Rectum)	1/1
Gastrointestinal stromal tumor (GIST) (Rectum)	1/1
Hepatocellular carcinoma (Liver)	1/1
Hepatoblastoma (Liver)	1/1
Clear cell carcinoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Adenocarcinoma (Uterus)	1/1
Clear cell carcinoma (Endometrium)	1/1
Squamous cell carcinoma (Cervix)	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	1/1
Squamous cell carcinoma (Skin)	1/1
Neuroblastoma (Retroperitoneum)	1/1
Mesothelioma (Peritoneum)	1/1
B-cell lymphoma; NOS (Lymph node)	2/2
Hodgkin lymphoma (Lymph node)	1/1
Diffuse anaplastic large cell lymphoma	1/1
Leiomyosarcoma (Bladder)	1/1
Osteosarcoma	1/1
Spindle cell rhabdomyosarcoma (Peritoneum)	1/1
Leiomyosarcoma (Smooth muscle)	1/1

Note: MMR proteins such as MSH6 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).

Precision – VENTANA MMR RxDx Panel

Repeatability and Intermediate Precision - Marker-Level Study

Twenty-eight (15 Intact and 13 Loss) FFPE cases from a variety of solid tumor tissues including 6 (3 intact and 3 loss) endometrial carcinoma cases were evaluated in this study. The study designs verified staining precision on tumor tissues stained with VENTANA anti-MSH6 (SP93) antibody.

- Three lots of anti-MSH6 (SP93) (between-antibody lots)
- Three lots of OptiView DAB IHC Detection Kit (between-detection kits)
- Three BenchMark ULTRA instruments (between-instruments)
- Across 3 days (between-day)
- Across all intermediate precision conditions (within-run)

Each case was assigned one mode based on the samples aggregated per test condition for between-antibody lots, between-detection kit lots, between-instruments and between-days. For the within-run condition, each case was compared within its duplicate samples per test run. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti-MSH6 (SP93) antibody (Table 5). Results are summarized in Table 9 for endometrial carcinoma tissues and Table 10 for variety of solid tumor tissues including endometrial carcinoma.

Table 9. Repeatability and intermediate precision of VENTANA anti-MSH6 (SP93) antibody on endometrial carcinoma tissues as measured by MSH6 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	PPA	18/18	100.0	(82.4, 100.0)
	NPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)
Between-Detection Kits	PPA	18/18	100.0	(82.4, 100.0)
	NPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)
Between-Instruments (BenchMark ULTRA)	PPA	18/18	100.0	(82.4, 100.0)
	NPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)
Between-Day	PPA	18/18	100.0	(82.4, 100.0)
	NPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)
Within-Run	PPA	27/27	100.0	(87.5, 100.0)
	NPA	27/27	100.0	(87.5, 100.0)
	OPA	54/54	100.0	(93.4, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method with 2000 bootstrap samples. CIs for 100% PPA, NPA, and OPA were calculated using the Wilson score method.

Note: For the purpose of this analysis, a marker status of Intact was considered negative and a marker status of Loss was considered positive.

Table 10. Repeatability and intermediate precision of VENTANA anti-MSH6 (SP93) antibody on a variety of solid tumor tissues including endometrial carcinoma as measured by MSH6 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	PPA	78/78	100.0	(95.3, 100.0)
	NPA	90/90	100.0	(95.9, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Between-Detection Kits	PPA	78/78	100.0	(95.3, 100.0)
	NPA	90/90	100.0	(95.9, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Between-Instruments (BenchMark ULTRA)	PPA	78/78	100.0	(95.3, 100.0)
	NPA	90/90	100.0	(95.9, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Between-Day	PPA	78/78	100.0	(95.3, 100.0)
	NPA	90/90	100.0	(95.9, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Within-Run	PPA	117/117	100.0	(96.8, 100.0)
	NPA	135/135	100.0	(97.2, 100.0)
	OPA	252/252	100.0	(98.5, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson score method.

Note: For the purposes of this analysis, a marker status of Intact was considered negative and a marker status of Loss was considered positive.

Between-Day Intermediate Precision - Marker-Level Study

Twenty-four (12 Intact and 12 Loss) FFPE cases from a variety of solid tumor tissues including 8 (4 Intact and 4 Loss) endometrial carcinoma cases were included in this study. The study design verified staining precision on tumor tissues stained with the VENTANA anti-MSH6 (SP93) antibody across 5 non-consecutive days.

For each sample, the mode of the staining result was determined as the most frequently observed staining result among the 10 replicates stained on the 5 non-consecutive days using a single lot of antibody and single lot of detection on one instrument. The result from each test sample was then compared to the respective mode and deemed concordant or discordant. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti-MSH6 (SP93) antibody (Table 5). Results are summarized Table 11 for endometrial carcinoma tissues and Table 12 for variety of solid tumor tissues including endometrial carcinoma.

Table 11. Between-Day intermediate precision of VENTANA anti-MSH6 (SP93) antibody on endometrial carcinoma tissues as measured by MSH6 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Day	PPA	40/40	100.0	(91.2, 100.0)
	NPA	40/40	100.0	(91.2, 100.0)
	OPA	80/80	100.0	(95.4, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson score method.

Note: For the purposes of this analysis, a marker status of Intact was considered negative and a marker status of Loss was considered positive.

Table 12. Between-Day intermediate precision of VENTANA anti-MSH6 (SP93) antibody on a variety of solid tumor tissues including endometrial carcinoma as measured by MSH6 Clinical Status (Intact/ Loss)

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Day	PPA	120/120	100.0	(96.9, 100.0)
	NPA	120/120	100.0	(96.9, 100.0)
	OPA	240/240	100.0	(98.4, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson score method.

Note: For the purposes of this analysis, a marker status of Intact was considered negative and a marker status of Loss was considered positive.

Between-Platform and Between-Instrument Intermediate Precision - Marker-Level Study in EC

Ten (5 Intact and 5 Loss) FFPE EC cases were included in this study. The study design verified intermediate precision on EC tissues stained with VENTANA anti-MSH6 (SP93) antibody across BenchMark ULTRA, BenchMark XT and BenchMark GX platforms and between 3 BenchMark ULTRA, 3 BenchMark XT and 3 BenchMark GX instruments. For each case, the mode of the staining result was determined as the most frequently observed staining result. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti-MSH6 (SP93) antibody (Table 5). The result of each test sample was compared to the respective mode and deemed concordant or discordant. Results for intermediate precision between platform and between BenchMark ULTRA, BenchMark XT and BenchMark GX instruments are summarized in Table 13 for EC cases.

Table 13. Between-platform and between-instrument intermediate precision of VENTANA anti-MSH6 (SP93) antibody on EC tissues as measured by MSH6 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Platform	PPA	90/90	100.0	(95.9, 100.0)
	NPA	90/90	100.0	(95.9, 100.0)
	OPA	180/180	100.0	(97.9, 100.0)
Between-Instrument (BenchMark XT)	PPA	30/30	100.0	(88.6, 100.0)
	NPA	30/30	100.0	(88.6, 100.0)
	OPA	60/60	100.0	(94.0, 100.0)
Between-Instrument (BenchMark ULTRA)	PPA	30/30	100.0	(88.6, 100.0)
	NPA	30/30	100.0	(88.6, 100.0)
	OPA	60/60	100.0	(94.0, 100.0)
Between-Instrument (BenchMark GX)	PPA	30/30	100.0	(88.6, 100.0)
	NPA	30/30	100.0	(88.6, 100.0)
	OPA	60/60	100.0	(94.0, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method with 2000 bootstrap samples. CIs for 100% PPA, NPA and OPA were calculated using the Wilson score method.

Note: For the purposes of this analysis, a marker status of Intact was considered negative and a marker status of Loss was considered positive.

BenchMark IHC/ISH Instrument Concordance - Marker-Level Study in EC

Forty-four (23 intact and 21 loss) FFPE EC cases were included in this study. The study design verified concordant Clinical Status and stain intensity on endometrial carcinoma tissues stained with VENTANA anti-MSH6 (SP93) antibody on BenchMark ULTRA, BenchMark XT and BenchMark GX platforms. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti-MSH6 (SP93) antibody (Table 5). The result of each test sample was compared to the Clinical Status derived from a slide stained on a BenchMark ULTRA instrument and deemed concordant or discordant. Results for assay migration are summarized in Table 14 for EC cases.

Table 14. Between-platform concordance of VENTANA anti-MSH6 (SP93) antibody on EC tissues as measured by MSH6 Clinical Status (Intact/Loss).

Concordance	Agreement			
	Type	n/N	%	95% CI
BenchMark ULTRA: BenchMark XT	PPA	21/21	100.0	(84.5, 100.0)
	NPA	23/23	100.0	(85.7, 100.0)
	OPA	44/44	100.0	(92.0, 100.0)
BenchMark ULTRA: Benchmark GX	PPA	21/21	100.0	(84.5, 100.0)
	NPA	22/23	95.7	(79.0, 99.2)
	OPA	43/44	97.7	(88.2, 99.6)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method with 2000 bootstrap samples. CIs for 100% PPA, NPA and OPA were calculated using the Wilson score method.

Note: For the purposes of the concordance analysis, a marker status of Intact was considered negative and a marker status of Loss was considered positive.

Reader Precision - Panel-Level Study

Between-Reader and Within-Reader precision was assessed by evaluating the concordance of MMR RxDx status between three readers and within individual readers using 162 (100 proficient and 62 deficient) cases from a variety of tumor types including 34 (17 proficient and 17 deficient) endometrial carcinoma cases. FFPE cases from each of the following organ systems were evaluated in this study: urinary, reproductive, gastrointestinal, endocrine, hepato-pancreatobiliary, soft tissue/skin, thoracic and other (head and neck). Specimens were blinded and randomized prior to evaluation for MSH6 status (Intact or Loss) and panel-level status (Proficient or Deficient) using the VENTANA MMR RxDx Panel scoring algorithm (Table 6). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers and within-reader are summarized in Table 15 for endometrial carcinoma tissues and in Table 16 for variety of solid tumor tissues including endometrial carcinoma.

Table 15. Within-Reader and Between-Reader Precision of VENTANA MMR RxDx Panel on endometrial carcinoma tissues as measured by MMR Clinical Status (Proficient/ Deficient).

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	100/101	99.0	(97.0 ,100.0)
	ANA	102/103	99.0	(97.1 ,100.0)
	OPA	101/102	99.0	(97.1 ,100.0)
Between-Reader	APA	98/100	98.0	(93.8 ,100.0)
	ANA	102/104	98.1	(94.4 ,100.0)
	OPA	100/102	98.0	(94.1 ,100.0)

Note: Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method with 2000 bootstrap samples.

Note: For the purposes of this analysis, an MMR Panel status of Deficient (dMMR) was considered positive and an MMR Panel status of Proficient (pMMR) was considered negative.

Table 16. Within-Reader and Between-Reader Precision of VENTANA MMR RxDx Panel on a variety of solid tumor tissues including endometrial carcinoma as measured by MMR Clinical Status (Proficient/ Deficient).

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	(364/366)	99.5	(98.6 ,100.0)
	ANA	(598/600)	99.7	(99.2 ,100.0)
	OPA	(481/483)	99.6	(99.0 ,100.0)
Between-Reader	APA	(364/366)	99.5	(98.3 ,100.0)
	ANA	(596/598)	99.7	(99.0 ,100.0)
	OPA	(480/482)	99.6	(98.8 ,100.0)

Note: Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method with 2000 bootstrap samples.

Note: For the purposes of this analysis, an MMR Panel status of Deficient (dMMR) was considered positive and an MMR Panel status of Proficient (pMMR) was considered negative.

Inter-Laboratory Reproducibility Study: Panel-Level Study

An Inter-Laboratory Reproducibility study of VENTANA MMR RxDx Panel was completed to demonstrate the reproducibility of the assay in determining the MMR status of EC specimens. The study included 30 archival, de-identified FFPE specimens that were stained on a BenchMark ULTRA instrument at each of 3 external laboratories on each of 3 non-consecutive days (spanning at least 20 days in total). Each staining day at each site produced a 5-slide panel [4 biomarker antibody-stained slides and 1 slide stained with Negative Control (Monoclonal)] using the PMS2 staining protocol] that was independently evaluated for the status of each marker (Intact or Loss) and for MMR status (Deficient or Proficient) by 2 pathologists at the site.

The study included 540 total observations for 30 samples (including 4 challenging samples) stained over 3 days across 3 sites with 2 readers per site. The MMR status results for all readers, sites, and days for the cases were combined and analyzed versus the reader modes for the same cases to determine the overall reproducibility of MMR status. The summary of the agreement rates across all evaluable observations, using the case-level reader modes for MMR panel level status as the reference is shown in Table 17.

Table 17. Inter-Laboratory Reproducibility for agreement rates for VENTANA MMR RxDx Panel in EC.

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	263/268	98.1	(95.5, 100.0)
	NPA	269/269	100.0	(98.6, 100.0)
	OPA	532/537	99.1	(97.8, 100.0)
Site- Stratified	PPA	263/268	98.1	(95.5, 100.0)
	NPA	269/269	100.0	(98.6, 100.0)
	OPA	532/537	99.1	(97.8, 100.0)
Reader-Stratified	PPA	263/265	99.2	(98.1, 100.0)
	NPA	272/272	100.0	(98.6, 100.0)
	OPA	535/537	99.6	(99.1, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method with 2000 replicates. In the case of 100% agreement, the Wilson score method was used.

Note: For the purposes of this analysis, an MMR Panel status of Deficient (dMMR) was considered positive and an MMR Panel status of Proficient (pMMR) was considered negative.

In addition, pairwise comparisons of MMR status were made between-sites, between-readers, and between-days. As summarized in Table 18, the assay was reproducible across 3 days, 3 sites, and 6 readers.

Table 18. Inter-Laboratory Reproducibility pairwise agreement rates for VENTANA MMR RxDx Panel in EC.

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Inter-Site	APA	3072/3132	98.1	(95.3, 100.0)
	ANA	3216/3276	98.2	(95.7, 100.0)
	OPA	3144/3204	98.1	(95.5, 100.0)
Inter-Reader	APA	258/263	98.1	(95.3, 100.0)
	ANA	268/273	98.2	(95.7, 100.0)
	OPA	263/268	98.1	(95.5, 100.0)
Inter-Day	APA	518/522	99.2	(98.1, 100.0)
	ANA	542/546	99.3	(98.2, 100.0)
	OPA	530/534	99.3	(98.1, 100.0)

Note: Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method with 2000 replicates.

Note: For the purposes of this analysis, an MMR Panel status of Deficient (dMMR) was considered positive and an MMR Panel status of Proficient (pMMR) was considered negative.

CLINICAL PERFORMANCE

Clinical Performance of dostarlimab (JEMPERLI) in the GARNET Study

The efficacy and safety of JEMPERLI were investigated in the GARNET study, a multicentre, uncontrolled, multiple parallel cohort, open-label study. The GARNET study included expansion cohorts in subjects with recurrent or advanced solid tumours who have limited available treatment options. Cohort A1 enrolled patients with mismatch repair deficient (dMMR)/microsatellite instability-high (MSI-H) EC who have progressed on or after a platinum-containing regimen.

Patients received 500 mg dostarlimab every 3 weeks for 4 cycles followed by 1000 mg dostarlimab every 6 weeks. Treatment continued until unacceptable toxicity or disease progression for up to two years.

The major efficacy outcome measures were objective response rate (ORR) and duration of response (DOR) as assessed by blinded independent central radiologists' (BICR) review according to response evaluation criteria in solid tumors (RECIST) v 1.1. The efficacy population was defined as patients who had measurable disease by BICR at baseline and had minimum of 24 weeks follow-up or had less than 24 weeks of follow-up and discontinued due to adverse events or disease progression.

The first planned interim analysis for GARNET included dMMR EC patients enrolled in GARNET before the clinical cutoff date (CCOD) of 08-Jul-2019. The efficacy population for the first interim analysis consisted of a cohort of 71 patients with dMMR EC. The efficacy population for the second planned interim analysis consisted of a cohort of 209 patients with dMMR solid tumors, including EC, who were enrolled in the study before the CCOD of 01-Mar-2020.

The performance of VENTANA MMR RxDx Panel was measured by evaluating its ability to identify patients with EC (first interim analysis), who were likely to respond to treatment with JEMPERLI (i.e., the efficacy results observed in GARNET). Specifically, the diagnostic performance evaluation sought to determine the efficacy of JEMPERLI among patients with recurrent or advanced dMMR EC who could have been enrolled in GARNET had VENTANA MMR RxDx Panel (CDx assay) been used for enrollment screening rather than the CTA. A bridging approach was required for these analyses because patients were not screened for GARNET study enrollment using the CDx assay. The diagnostic performance evaluation for VENTANA MMR RxDx Panel used only a subset of cases from Biologics License Application (BLA) safety population; patients had to meet the diagnostic study criteria at the interim analyses to be included in the diagnostic performance analyses.

GARNET Study Clinical Results - EC (dMMR)

A summary of efficacy results for subjects with dMMR EC in the primary BLA efficacy analysis set is presented in Table 19.

Table 19. Efficacy results in GARNET study for patients with dMMR/MSI-H EC

Endpoint	JEMPERLI N = 108
Objective Response Rate (ORR)	
ORR, n (%)	47 (43.5 %) [a]
(95% CI)	(34.0, 53.4)
Complete response rate n (%)	11 (10.2 %)
Partial response rate n (%)	36 (33.3 %)
Disease control rate (DCR) (95 % CI)	55.6% (45.7, 65.1)
Stable disease % (95 % CI)	12% (6.6, 19.7)
Duration of Response (DOR)	
Median in months (range)	Not reached [b] (2.6, 28.1+)
Probability of maintaining response at 6 months by K-M (95 % CI)	97.9 % (85.8, 99.7)
Probability of maintaining response at 12 months by K-M (95 % CI)	90.9 % (73.7, 97.1)

[a] At time of data cut-off (01 March 2020)

[b] At the time of data cut-off, the median DOR had not been reached.

K-M: Kaplan-Meier curve estimate.

CI: confidence interval.

MSI-H: microsatellite instability high.

VENTANA MMR RxDx Panel Clinical Performance for GARNET dMMR Endometrial Cancer Population

The population for the diagnostic performance evaluation of VENTANA MMR RxDx Panel efficacy in EC included patients enrolled in GARNET who received study treatment prior to the CCOD for the first planned interim analysis and whose eligibility for that study was confirmed using a CTA, defined as any locally or centrally performed MMR IHC test other than VENTANA MMR RxDx Panel.

As part of the bridging analysis, the agreement of MMR status between CTA and CDx results were calculated using the CTA results as the reference. For the purpose of the analyses, a Proficient MMR status was considered negative, and a Deficient MMR status was considered positive. Among all the clinical samples from the original GARNET study BLA safety population with both evaluable CDx result and an evaluable CTA result, the inter-assay concordance results are shown in Table 20.

Table 20. MMR Status concordance between the GARNET CTA and VENTANA MMR RxDx Panel for EC population.

Analysis Population [a]	Agreement		
	Measure [b]	n/N	% (95% CI)
IU Concordance	PPA	51/55	92.7 (82.7, 97.1)
	NPA	68/68	100.0 (94.7, 100.0)
	OPA	119/123	96.7 (91.9, 98.7)
ITD Concordance	PPA	70/76	92.1 (83.8, 96.3)
	NPA	90/91	98.9 (94.0, 99.8)
	OPA	160/167	95.8 (91.6, 98.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson score method.

[a] Analyses were performed in the Concordance population (all patients in the Safety Population with an evaluable VENTANA MMR RxDx Panel (CDx) staining result). The Intent-to-Diagnose (ITD) and Concordance populations were equivalent in this study. The Intended Use (IU) Concordance population includes only the subset of patients who are also in the IU population (ie, for whom VENTANA MMR RxDx Panel testing attempt was performed according to the requirements of the diagnostic protocol).

[b] For the purpose of the analyses, an MMR status of Deficient (dMMR) was considered positive, and an MMR status of Proficient (pMMR) was considered negative.

Additional analyses were conducted to estimate the drug efficacy in patients with recurrent or advanced EC whose tumors were or would have been dMMR upon testing using VENTANA MMR RxDx Panel, including a primary analysis using different multiple imputation (MI) approaches and sensitivity analyses using the lower bounds of the observed PPA and NPA 95% CIs as the assumed PPA and NPA, respectively, and using an adjusted assumption for the prevalence of cases that would be dMMR if tested using the CTA. These analyses utilizing an imputed CDx status to evaluate efficacy in the CDx-selected population yielded ORR and DOR results similar to those actually observed in the GARNET dMMR EC population selected using the CTA.

PERFORMANCE CHARACTERISTICS FOR VENTANA MMR IHC PANEL

Precision – VENTANA MMR IHC Panel

Within-Run Repeatability and Between-Day Intermediate Precision

The repeatability and precision of VENTANA anti-MSH6 (SP93) antibody were evaluated on the BenchMark ULTRA instrument in combination with the OptiView DAB IHC Detection Kit.

Within-run repeatability was evaluated using 10 colorectal cancer specimens (5 Intact and 5 Loss for MSH6 expression). Five replicate slides from each of the colorectal cancer specimens were stained with VENTANA anti-MSH6 (SP93) antibody on a single BenchMark ULTRA instrument within a single day. Each VENTANA anti-MSH6 (SP93) 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 10 colorectal cancer specimens (5 Intact and 5 Loss for MSH6 expression). Replicate slides from each of the colorectal cancer specimens were stained with VENTANA anti-MSH6 (SP93) antibody on a BenchMark ULTRA instrument on each of 5 non-consecutive days. Each VENTANA anti-MSH6 (SP93) 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% positive percent agreement (PPA), 100% negative percent agreement (NPA) and 100% overall percent agreement (OPA). A summary of the results is shown in Table 21.

Table 21. Within-run repeatability and between-day intermediate precision of the VENTANA anti-MSH6 (SP93) antibody as measured by Clinical Status (Intact or Loss).

Repeatability/ Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Run Repeatability	Intact	PPA	25/25	100.0	(86.7,100.0)
	Loss	NPA	25/25	100.0	(86.7,100.0)
	Total	OPA	50/50	100.0	(92.9,100.0)
Between-Day Precision	Intact	PPA	50/50	100.0	(92.9,100.0)
	Loss	NPA	50/50	100.0	(92.9,100.0)
	Total	OPA	100/100	100.0	(96.3,100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson score method.

Between-Instrument Intermediate Precision

BenchMark ULTRA instrument between-instrument intermediate precision of the VENTANA anti-MSH6 (SP93) antibody was determined by staining replicate slides of 10 colorectal cancer specimens (5 Intact and 5 Loss for MSH6 expression) across 3 BenchMark ULTRA instruments with VENTANA anti-MSH6 (SP93) antibody using the OptiView DAB IHC Detection Kit.

Each VENTANA anti-MSH6 (SP93) 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% PPA, NPA, and OPA. A summary of the results can be found in Table 22.

Table 22. BenchMark ULTRA instrument between-instrument intermediate precision of the VENTANA anti-MSH6 (SP93) antibody as measured by Clinical Status (Intact or Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Between-Instrument Intermediate Precision	Intact	PPA	30/30	100.0	(88.6,100.0)
	Loss	NPA	30/30	100.0	(88.6, 100.0)
	Total	OPA	60/60	100.0	(94.0,100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson sore method.

In addition, Between-instrument intermediate precision of the VENTANA anti-MSH6 (SP93) antibody was determined by staining replicate slides of 6 colorectal cancer specimens (4 Intact and 2 Loss for MSH6 expression) across 3 BenchMark XT and 3 BenchMark GX instruments with VENTANA anti-MSH6 (SP93) antibody using the OptiView DAB IHC Detection Kit.

There were 15 observations per case when pooling the 3 instruments together; the median for each case was determined from these 15 observations. Individual observations of that same case were deemed to be concordant with the median case signal intensity if they were within 0.5 signal intensity. For BenchMark XT and BenchMark GX instrument Between-Instrument Intermediate Precision, pairwise comparisons of stain intensity scores of tumor for each specimen were made and demonstrated 100% OPA between 3 BenchMark XT instruments and 100% OPA between 3 BenchMark GX instruments. For all slides background staining was acceptable (≤ 0.5) on both the BenchMark XT and BenchMark GX instruments.

BenchMark IHC/ISH Instrument Concordance

Concordance across the BenchMark IHC/ISH instruments for the VENTANA anti-MSH6 (SP93) antibody was determined by staining colorectal carcinoma specimens with VENTANA anti-MSH6 (SP93) antibody using the OptiView DAB IHC Detection Kit. All slides were evaluated for Clinical Status (Intact/Loss) by a single pathologist.

Pairwise comparisons of Clinical Status for the colorectal cancer specimens were made between platforms including BenchMark GX to BenchMark ULTRA instruments (134 Intact and 20 Loss cases), BenchMark GX to BenchMark XT instruments (133 Intact and 21 Loss cases) and BenchMark ULTRA to BenchMark XT instruments (136 Intact and 20 Loss cases). All pairwise comparisons made between platforms demonstrated 100% average positive agreement (APA), average negative agreement (ANA), and OPA.

Reader Precision Studies

Within- and between-reader precision was evaluated on 20 colorectal cancer specimens (11 Intact and 9 Loss cases) stained with VENTANA anti-MSH6 (SP93) antibody and the OptiView DAB IHC Detection Kit. Each VENTANA anti-MSH6 (SP93) antibody-stained slide was paired with an hematoxylin and eosin (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 MSH6 Clinical Status. Pathologists were blinded to the case diagnosis. Following a two-week washout period, the VENTANA anti-MSH6 (SP93) antibody-stained slides were re-randomized for a second evaluation of the MSH6 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 20 colorectal cancer slide comparisons per pathologist. Comparisons from the 3 pathologists were pooled and demonstrated 98.5% APA, 98.1% ANA, and 98.3% OPA for within-reader precision. A summary of the results can be found in Table 23.

Between-reader precision compared all slide evaluations (20 colorectal cancer x 2 evaluations/case x 3 pathologists = 120 slide evaluations) to a modal case status for each colorectal cancer case. The results demonstrate 100% PPA, 98.1% NPA, and 99.2% OPA for between-reader precision. A summary of the results can be found in Table 23.

Table 23. Within-reader and between-reader precision of the VENTANA anti-MSH6 (SP93) antibody on colorectal cancer cases as measured by MSH6 Clinical Status (Intact/Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Reader	Intact	APA	66/67	98.5	(88.0,100.0)
	Loss	ANA	52/53	98.1	(83.3,100.0)
	Total	OPA	59/60	98.3	(85.0,100.0)
Between Reader	Intact	PPA	66/66	100.0	(94.5,100.0)
	Loss	NPA	53/54	98.1	(90.2,99.7)
	Total	OPA	119/120	99.2	(95.4,99.9)

Note: Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

Note: For within-reader, the APA and ANA 95% Confidence Intervals (CI) were calculated using the Clopper-Pearson based method; the OPA 95% CI was calculated using the percentile bootstrap method. For Between-Reader, 95% CIs were calculated using the Wilson Score method.

Lot-to-Lot Precision

Lot-to-lot precision of VENTANA anti-MSH6 (SP93) antibody was determined by testing 3 production lots of the VENTANA anti-MSH6 (SP93) antibody each on triplicate slides of 10 colorectal cancer specimens (5 Intact and 5 Loss for MSH6 expression) on a BenchMark ULTRA instrument using the OptiView DAB IHC Detection Kit.

Each VENTANA anti-MSH6 (SP93) 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-MSH6 (SP93) 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-MSH6 (SP93) antibody Lot-to-lot precision, all slide evaluations were compared to a modal case status for each colorectal cancer case. The OPA between the VENTANA anti-MSH6 (SP93) antibody lots was 100%; demonstrating that VENTANA anti-MSH6 (SP93) antibody staining is reproducible across antibody lots.

A summary of the results for Lot-to-lot precision of the VENTANA anti-MSH6 (SP93) antibody is shown in Table 24.

Table 24. Lot-to-lot precision of the VENTANA anti-MSH6 (SP93) antibody as measured by Clinical Status (Intact or Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Lot-to-Lot	Intact	PPA	45/45	100.0	(92.1,100.0)
	Loss	NPA	45/45	100.0	(92.1,100.0)
	Total	OPA	90/90	100.0	(95.9,100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson sore method.

Inter-Laboratory Reproducibility Study

An Inter-Laboratory Reproducibility study of the VENTANA MMR IHC Panel was completed to demonstrate reproducibility of each VENTANA MMR IHC Panel assay to determine Clinical Status. The study included 6 colorectal cancer tissue specimens (3 Intact and 3 Loss) for each MMR protein and 16 colorectal cancer tissue specimens (8 Positive and 8 Negative) for BRAF V600E run across 3 BenchMark ULTRA instruments on each of 5 non-consecutive days over 21 days at three external laboratories. Each

antibody-stained slide was paired with an hematoxylin and eosin (H&E) and negative reagent control stained slide from the same case. All slide sets were randomized and evaluated by a total of 6 readers (2 readers/site) who were blinded to the MMR Clinical Status of the study set. Each of the 40 cases in the study had 30 observations across all days, sites, and readers. The modal case reference status was derived for each case based on the most often observed status of the 30 observations. The study included a total of 1200 observations for all five proteins. For all evaluable cases, the acceptability rate for morphology and background in this study was 100%. A summary of the pooled (all five proteins) agreement statistics between the modal case reference status and individual observations can be found in Table 25.

Table 25. Agreement between the VENTANA MMR IHC Panel and Modal Case Reference Status.

Inter-Laboratory Reproducibility	Clinical Status	Agreement			
		Type	n/N	%	95% CI
All Proteins	Intact/Positive	PPA	598/600	99.8	(98.7,100.0)
	Loss/Negative	NPA	593/600	98.9	(97.4, 99.5)
	Total	OPA	1191/1200	99.4	(98.6, 99.7)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Clinical Status is defined as Intact or Loss for protein expression for MMR protein and Positive or Negative for BRAF V600E protein.

Note: 95% Confidence Intervals (CI) were calculated using a generalized linear mixed model (GLMM) approach.

In addition, pairwise comparisons were made between-site, between-day and between-reader for the VENTANA anti-MSH6 (SP93) antibody. For MSH6, this study set included a total of 180 observations. A summary of the results can be found in Table 26. The data indicate assay reproducibility across 5 days, 3 sites, and 6 readers.

Table 26. Inter-Laboratory Reproducibility pairwise agreement rates for the VENTANA anti-MSH6 (SP93) antibody as measured by Clinical Status (Intact or Loss).

Inter-Laboratory Reproducibility		Agreement			
		Type	n/N	%	95% CI
Between-Site (3 sites)		APA	360/364	98.9	(96.8, 100.0)
		ANA	352/356	98.9	(96.6, 100.0)
		OPA	356/360	98.9	(96.7, 100.0)
Between-Day (5 non-consecutive days)	Site A	APA	120/120	100.0	(96.9, 100.0)
		ANA	120/120	100.0	(96.9, 100.0)
		OPA	120/120	100.0	(96.9, 100.0)
	Site B	APA	120/120	100.0	(96.9, 100.0)
		ANA	120/120	100.0	(96.9, 100.0)
		OPA	120/120	100.0	(96.9, 100.0)
	Site C	APA	120/124	96.8	(90.9, 100.0)
		ANA	112/116	96.6	(88.9, 100.0)
		OPA	116/120	96.7	(90.0, 100.0)
Between-Reader (2 pathologists per site)		APA	90/91	98.9	(96.8, 100.0)
		ANA	88/89	98.9	(96.6, 100.0)
		OPA	89/90	98.9	(96.7, 100.0)

Note: Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method; in instances where the point estimate was 100%, Wilson score method was used.

Accuracy Study: Method Comparison of VENTANA MMR IHC Panel Results to Molecular Testing (DNA sequencing and MLH1 promoter hypermethylation)

A study was conducted to compare the performance of the VENTANA MMR IHC Panel to molecular testing including a comprehensive DNA sequencing colon panel for the identification of colorectal cancers that (i) are MMR deficient (dMMR), and (ii) contain the BRAF V600E mutation. 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, sequential colorectal cancer 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 molecular testing. Following review, 105 sequential cases meeting these criteria were enrolled into the study. In addition, 13 colorectal cancer cases showing a Clinical status of Loss by IHC were included to ensure that Loss of each marker was represented in the study. Sections of all cases in the study were stained by IHC with the VENTANA MMR IHC Panel and appropriate negative reagent controls. Additional sections were subjected to the DNA sequencing colon panel. MLH1 promoter hypermethylation is one of the mechanisms which may lead to loss of MLH1 protein expression, and it is linked to sporadic colorectal cancer rather than potential Lynch syndrome diagnosis. Therefore, all MLH1 loss cases identified by IHC in the study were tested for hypermethylation of the MLH1 promoter.

In the final study set of 118 cases, the analysis included PPA and NPA for all markers pooled (i.e. all observations pooled) where molecular testing acted as the reference status for IHC comparison. The analysis included a comparison of MMR protein status (Intact / Loss) to molecular status defined as Normal (no pathogenic mutation(s), negative for

MLH1 promoter hypermethylation, and wild-type BRAF (no V600E mutation)) or Abnormal (presence of pathogenic mutation(s), positive for MLH1 promoter hypermethylation, and/or positive for the BRAF V600E mutation). 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 were 99.4% PPA, 93.5% NPA and 98.8% OPA as shown in Table 27.

A pooled analysis comparing the four MMR IHC markers (without the VENTANA anti-BRAF V600E (VE1) antibody) to molecular testing results was also performed. Point estimates were 99.3% PPA, 89.7% NPA and 98.5% OPA as summarized in Table 28.

An additional analysis compared the four MMR IHC marker results to the molecular testing results for the MMR genes at the case level to include the status of all markers and create a dMMR/pMMR outcome for the two methods. This analysis is shown in Table 29, and exhibits an OPA of 97.4% between the two methods.

IHC MMR status and molecular testing MMR status were also compared for individual MMR markers within the study. The OPA of each MMR marker, when compared to the combined results of the DNA sequencing colon panel and MLH1 promoter hypermethylation testing, was 100.0% for VENTANA anti-MLH1 (M1) antibody, 99.1% 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 anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody.

BRAF V600E Clinical status in colorectal cancers obtained by IHC using the VENTANA anti-BRAF V600E (VE1) antibody was also compared to BRAF mutational status results determined by DNA sequencing. The PPA, NPA, and OPA of IHC testing using the VENTANA anti-BRAF V600E (VE1) antibody using DNA sequencing as the reference all were 100% (Table 30). Additional testing was performed to verify the ability of the VENTANA anti-BRAF V600E (VE1) antibody to further stratify CRC cases showing a loss of MLH1 protein expression. Of the 23 positive BRAF V600E cases, 20 cases had loss of MLH1 protein by IHC and were positive for MLH1 promoter hypermethylation. These data are consistent with the close association of BRAF V600E positive status with MLH1 promoter hypermethylation status. The remaining three cases were pMMR (intact for all MMR proteins). All BRAF V600E positive specimens were identified as sporadic colorectal cancer. The results verified that the VENTANA anti-BRAF V600E (VE1) antibody correctly identifies colorectal cancers having the BRAF V600E mutation. The data also supported the use of VENTANA anti-BRAF V600E (VE1) antibody to differentiate between sporadic and probable Lynch syndrome colorectal cancer in the absence of MLH1 expression.

Table 27. Pooled analysis for VENTANA MMR IHC Panel agreement between IHC and molecular testing.

Status (Molecular/IHC)	Agreement			
	Type	n/N	%	95% CI
Normal/Intact	PPA	523/526	99.4	(98.7,100.0)
Abnormal/Loss	NPA	58/62	93.5	(87.1, 98.6)
Total	OPA	581/588	98.8	(98.0, 99.7)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: For IHC, MMR Status is Intact or Loss for protein expression. For this analysis, BRAF V600E negative and positive cases were included in Intact or Loss categories, respectively. Molecular testing indicates absence (Normal) or presence (Abnormal) of potential pathogenic mutations or MLH1 promoter hypermethylation.

Note: 95% Confidence Intervals (CI) were calculated using the percentile bootstrap method.

Table 28. Pooled analysis for four MMR IHC markers (without VENTANA anti-BRAF V600E (VE1) antibody) agreement between IHC and molecular testing.

Status (Molecular/IHC)	Agreement			
	Type	n/N	%	95% CI
Normal/Intact	PPA	428/431	99.3	(98.4,100.0)
Abnormal/Loss	NPA	35/39	89.7	(79.4, 97.7)
Total	OPA	463/470	98.5	(97.3, 99.6)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: For IHC, Status is Intact or Loss for protein expression. Molecular testing indicates absence (Normal) or presence (Abnormal) of potential pathogenic mutations or MLH1 promoter hypermethylation.

Note: 95% Confidence Intervals (CI) were calculated using the percentile bootstrap method.

Table 29. Agreement between the four MMR IHC markers and molecular testing results for MMR status (dMMR/pMMR).

MMR Status	Agreement			
	Type	n/N	%	95% CI
pMMR	PPA	79/80	98.8	(93.3,99.8)
dMMR	NPA	35/37	94.6	(82.3, 98.5)
Total	OPA	114/117	97.4	(92.7, 99.1)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: For IHC, pMMR status for a case is represented by Intact status for all MMR proteins, while dMMR status is represented by Loss of one or more MMR proteins. For molecular testing, pMMR status is represented by the absence of pathogenic mutations or MLH1 promoter hypermethylation, while dMMR status is represented by the presence of pathogenic mutations or MLH1 promoter hypermethylation.

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson sore method.

Table 30. Agreement between IHC using VENTANA anti-BRAF V600E (VE1) antibody and molecular testing.

BRAF V600E Status (Molecular/IHC)	Agreement			
	Type	n/N	%	95% CI
Positive/Abnormal	PPA	23/23	100.0	(85.7, 100.0)
Negative/Normal	NPA	95/95	100.0	(96.1, 100.0)
Total	OPA	118/118	100.0	(96.8, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

Note: Status for BRAF V600E was defined as Positive or Negative IHC results and Abnormal (presence of the V600E mutation) or Normal (wild-type BRAF) results by molecular testing.

Note: Two-sided 95% confidence intervals (CIs) were calculated using the Wilson sore method.

Performance of VENTANA anti-MSH6 (SP93) Antibody on the BenchMark ULTRA PLUS Instrument

Concordance Between BenchMark ULTRA PLUS and BenchMark ULTRA Instruments for MSH6 (SP93) Antibody

Three laboratories, from separate institutions in the United States, participated in a concordance study between the BenchMark ULTRA PLUS instrument and the BenchMark ULTRA instrument. There were 120 unique colorectal carcinoma, endometrial carcinoma, and "other" solid tumor organ systems cases which represented the antibody status range of the VENTANA anti-MSH6 (SP93) Antibody, with equal distribution between MSH6 Loss and MSH6 Intact cases for each indication as determined by RTD consensus review. Tissue slides from all cases were stained with H&E, a negative reagent control, and VENTANA anti-MSH6 (SP93) Assay on a BenchMark ULTRA instrument using the recommended staining protocol. Unstained tissue slides from all cases were randomized and equally distributed (40 cases per site such that each site received a representative sample of study cases) for staining on a BenchMark ULTRA PLUS instrument using the recommended VENTANA MSH6 (SP93) staining protocol. Two pathologists per site, blinded to case status, evaluated the slides stained on the BenchMark ULTRA PLUS instrument and determined the MSH6 status. After a two week washout period, corresponding case slides previously stained at Roche on the BenchMark ULTRA instrument were distributed to the appropriate sites for clinical evaluation. Additionally, one RTD pathologist reviewed all study slides and was included as a third pathologist for each of the sites. The results were analyzed by Roche. The OPA, LPA and IPA rates were 96.1% (342/356), 96.0% (169/176), and 96.1% (173/180), respectively. The results are summarized in Table 31.

Table 31. Pooled Agreement of MSH6 status for cases stained with VENTANA anti-MSH6 (SP93) antibody on BenchMark ULTRA PLUS versus BenchMark ULTRA instrument.

BenchMark ULTRA PLUS MSH6 (SP93) Status	BenchMark ULTRA MSH6 (SP93) Status		
	Loss	Intact	Total
Loss	169	7	176
Intact	7	173	180
Total	176	180	356
	n/N	% (95% CI)	
LPA	169/176	96.0 (93.0, 98.9)	
IPA	173/180	96.1 (92.8, 98.9)	
OPA	342/356	96.1 (93.8, 98.0)	

Note: Two-sided 95% CI calculated using the percentile bootstrap method with 2000 replicates stratified by indication and biomarker status (Intact, Loss, Challenging), for a total of 9 bins.

Note: The pooled agreement pools all cases and readers for each marker.

Note: LPA = Loss Percent Agreement; IPA = Intact Percent Agreement; OPA = Overall Percent Agreement.

Inter-Laboratory Reproducibility Study- BenchMark ULTRA PLUS

An Inter-Laboratory Reproducibility study of the VENTANA MMR RxDx Panel was completed to demonstrate the reproducibility of the assay in determining the MMR status of solid pan tumor specimens. The study included 42 archival, de-identified FFPE specimens that were stained on a BenchMark ULTRA PLUS instrument at each of 3 external laboratories on each of 3 non-consecutive days (spanning at least 20 days in total). Each staining day at each site produced a 5-slide panel [4 biomarker antibody-stained slides and 1 slide stained with Negative Control (Monoclonal) using the PMS2 staining protocol] that was independently evaluated for the status of each marker (Intact or Loss) and for MMR status (Deficient or Proficient) by 2 pathologists at the site.

The study included 756 total observations for 42 samples (including 4 challenging samples) stained over 3 days across 3 sites with 2 readers per site. The MMR status results for all readers, sites, and days for the cases were combined and analyzed versus the reader modes for the same cases to determine the overall reproducibility of MMR status. The summary of the agreement rates across all evaluable observations, using the case-level reader modes for MMR panel level status as the reference is shown in Table 32.

Table 32. Inter-laboratory reproducibility for overall agreement rates for VENTANA MMR RxDx Panel in solid pan tumor.

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	dMPA	373/375	99.5	(98.7, 100.0)
	pMPA	378/378	100.0	(99.0, 100.0)
	OPA	751/753	99.7	(99.3, 100.0)
Site- Stratified	dMPA	373/375	99.5	(98.7, 100.0)
	pMPA	378/378	100.0	(99.0, 100.0)
	OPA	751/753	99.7	(99.3, 100.0)
Reader-Stratified	dMPA	373/375	99.5	(98.7, 100.0)
	pMPA	378/378	100.0	(99.0, 100.0)
	OPA	751/753	99.7	(99.3, 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 made between-sites, between-readers, and between-days. As summarized in Table 33, the assay was reproducible across 3 days, 3 sites, and 6 readers.

Table 33. Inter-laboratory reproducibility pairwise agreement rates for the VENTANA MMR RxDx Panel in endometrial carcinoma.

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Inter-Site	ADPA	4416/4440	99.5	(98.6, 100.0)
	APPA	4536/4560	99.5	(98.7, 100.0)
	OPA	4476/4500	99.5	(98.7, 100.0)
Inter-Reader	ADPA	370/372	99.5	(98.6, 100.0)
	APPA	378/380	99.5	(98.7, 100.0)
	OPA	374/376	99.5	(98.7, 100.0)
Inter-Day	ADPA	738/741	99.6	(99.2, 100.0)
	APPA	756/759	99.6	(99.2, 100.0)
	OPA	747/750	99.6	(99.2, 100.0)

Note: ADPA = Average dMMR Percent Agreement; APPA = Average pMMR Percent Agreement; OPA = Overall Percent Agreement.

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:
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Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number

Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
E	Inclusion of BenchMark Ultra PLUS instrument. Updates made to Staining Procedure, Performance Characteristics for Ventana MMR RxDx Panel, Analytical Performance and Performance Characteristics for Ventana MMR IHC Panel sections. Updated to the latest template.

INTELLECTUAL PROPERTY

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