VENTANA

Roche

VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody

For use with VENTANA MMR IHC Panel

REF 730-7160

09607137001

IVD Σ 50

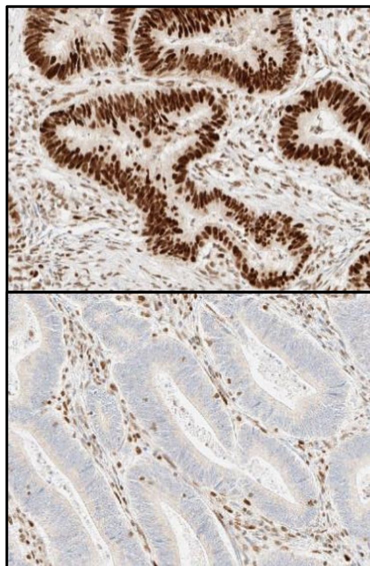


Figure 1. VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody staining with Intact (top) or Loss (bottom) of expression in colon cancer tissue.

INTENDED USE

VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody (VENTANA anti-MSH2 (G219-1129) antibody) is intended for the qualitative detection of MSH2 protein in formalin-fixed, paraffin-embedded tissue sections. VENTANA anti-MSH2 (G219-1129) antibody is ready to use on BenchMark IHC/ISH instruments with the OptiView DAB IHC Detection Kit and ancillary reagents.

VENTANA anti-MSH2 (G219-1129) antibody is part of the VENTANA MMR IHC Panel which includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-PMS2 (A16-4) 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 for the 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.

These products 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.

SUMMARY AND EXPLANATION

VENTANA anti-MSH2 (G219-1129) antibody is a mouse monoclonal antibody produced against a recombinant human MSH2 protein.

Colorectal cancer is the third most common cancer and the fourth most prevalent cause of cancer death in the world.[1] The majority of CRCs show chromosomal instability, however approximately 15% of cancers develop through an alternative pathway characterized by defective function of the DNA mismatch repair (MMR) system. As a consequence of the MMR deficiency, tumors exhibit microsatellite instability (MSI) resulting from the inability of MMR proteins to repair DNA replication errors.

CRCs with MMR defects are denoted as deficient MMR (dMMR) tumors. In contrast, CRCs with no MMR defects are denoted as proficient MMR (pMMR) tumors. 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.[2,3] 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.[4]

The four most commonly mutated MMR genes are *MLH1, PMS2, MSH2*, and *MSH6*. In normal cells, the MLH1 protein forms a complex (heterodimer) with the PMS2 protein, while the MSH2 protein forms a complex with the MSH6 protein.[5,6] When DNA mismatches occur, the MSH2/MSH6 heterodimer binds to the mismatched DNA, inducing a conformational change. The MLH1/PMS2 heterodimer binds the DNA-bound MSH2/MSH6 complex resulting in excision repair of the affected DNA.

The MLH1, PMS2, MSH2, and MSH6 proteins are clinically important MMR proteins encoded by genes that may be mutated in families with Lynch syndrome.[7,8] Carriers of these mutations have a high lifetime risk of developing colorectal and other cancers due to accumulation of DNA replication errors in proliferating cells. Lynch syndrome represents 1-6% of all CRCs. These tumors result from the inheritance of a germline autosomal dominant mutation in one of the four MMR genes, with MLH1 loss occurring in the majority of these Lynch syndrome associated CRCs.[5,9,10] More than 300 different mutations in the MMR family of proteins have been identified in patients with Lynch syndrome. The Lynch syndrome-associated tumor phenotype is generally characterized by immunohistochemical loss of expression in MMR proteins, particularly MLH1, PMS2, MSH2, and MSH6.[10-13] MMR IHC testing has been shown to be useful in the identification of the specific MMR gene in which either a germline or a somatic alteration is most likely to be found.[14]

As part of VENTANA MMR IHC Panel, VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody (VENTANA anti-BRAF V600E (VE1) antibody) aids to differentiate sporadic and probable Lynch syndrome CRC in the absence of MLH1 protein expression.[15,16] In CRC, loss of MLH1 protein is frequently the result of hypermethylation of the *MLH1* promoter and indicates a sporadic occurrence.[17] The presence of the BRAF V600E protein is tightly linked with hypermethylation of the *MLH1* promoter. As a result, a positive staining result with VENTANA anti-BRAF V600E (VE1) antibody indicates sporadic CRC.

CLINICAL SIGNIFICANCE

Lynch syndrome was described in the 1960s and identified a link between the loss of MMR function and cancer.[18] Loss of MMR proteins (MLH1, PMS2, MSH2, or MSH6) may lead to MSI and a higher lifetime risk of not only CRC, but also cancers of the stomach, brain, pancreas, skin, endometrium and ovaries. Patients with Lynch syndrome have a 50-80% lifetime risk for CRC.[5,19,20] 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 CRCs should be screened for potential Lynch syndrome to identify patients and families that will benefit from further genetic testing and counseling.[18,21-24] Using VENTANA MMR IHC Panel will aid in determining the MMR status of CRCs by classifying them as intact or loss for MMR protein expression. Detection of all four MMR proteins in the tumor indicates normal or intact MMR. 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 CRC, loss of MLH1 protein is due to hypermethylation of the MLH1 promoter.[5,25,26] 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.[25] Therefore, if the result of VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody (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.[5,27] In CRC, 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.[17,28] 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.[29]

PRINCIPLE OF THE PROCEDURE

VENTANA anti-MSH2 (G219-1129) antibody binds to MSH2 protein in formalin-fixed, paraffin-embedded (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, each 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.

MATERIAL PROVIDED

VENTANA anti-MSH2 (G219-1129) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-MSH2 (G219-1129) antibody contains approximately 100 µg of a mouse monoclonal antibody.

The antibody is diluted in a phosphate buffer containing carrier protein, Brij 35, and 0.05% ProClin300, a preservative.

Specific antibody concentration is approximately 20 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-MSH2 (G219-1129) antibody is a mouse monoclonal antibody produced as purified cell culture supernatant.

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. Microscope slides, positively charged
3. VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody (Cat. No. 730-7159 / 09605584001)
4. VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody (Cat. No. 730-7158 / 09607161001)
5. VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody (Cat. No. 730-7161 / 09606769001)
6. VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody (Cat. No. 760-5095 / 08033706001)
7. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
8. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
9. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
10. OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or Cat. No. 860-099 / 06718663001 (250 test))
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. The recommended tissue fixative is 10% neutral buffered formalin.[30] Sections should be cut at approximately 4 µm in thickness and mounted on positively-charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

For VENTANA MMR IHC Panel, it is recommended tissue be fixed within 6 hours of excision in 10% neutral buffered formalin (NBF) for 6-24 hours on the basis of staining in tonsil. Acceptable staining was also achieved with fixation in Zinc formalin and Z-5 fixatives for 6-24 hours. Alcohol formalin (AFA), 95% Ethanol, and PREFER fixatives are not recommended for use with VENTANA MMR IHC Panel as tissues demonstrate no or variable staining.

The amount of fixative 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).[30,31]

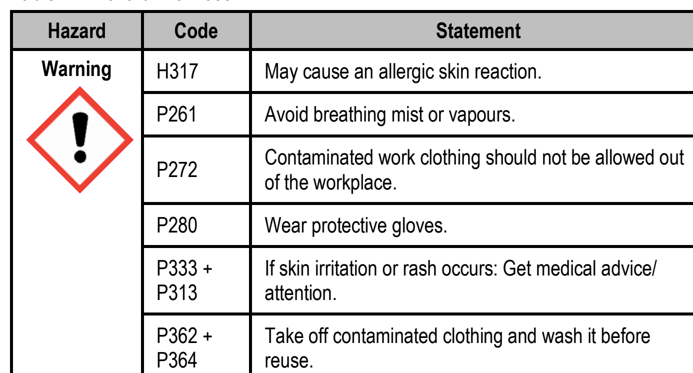
It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. 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.
6. 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.
7. 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.[32,33]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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 1. Hazard information.



Hazard	Code	Statement
Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing mist or vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	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.

VENTANA

Roche

Hazard	Code	Statement
	P501	Dispose of contents/ container to an approved waste disposal plant.

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

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer Table 2 below for recommended staining protocols.

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 on assay robustness is unknown. Thus, deviation from the recommended conditions for antigen retrieval provided in the listed protocol may invalidate expected results. 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 OptiView DAB IHC Detection Kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 730-7160.

Table 2. Recommended staining protocol for VENTANA anti-MSH2 (G219-1129) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1 40 minutes,	CC1, 40 minutes	ULTRA CC1, 40 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		

Deviation from the recommended conditions, especially for antigen retrieval, provided in the listed protocol may invalidate expected results. Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to “Immunohistochemistry: Principles and Advances.”[34]

QUALITY CONTROL PROCEDURES

Negative Reagent Control

In addition to staining with VENTANA anti-MSH2 (G219-1129) antibody, a second slide should be stained with the appropriate negative control reagent, Negative Control (Monoclonal). The negative reagent control is used to assess non-specific staining. The staining parameters for the negative reagent control antibody should be the same as that for the primary antibody.

Positive Tissue Control

A tissue control must be run with each staining run. Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue 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 reagents and instruments, not as an aid in determining a specific diagnosis of test samples. If the positive tissue control fails to demonstrate positive staining, results with the test specimen should be considered invalid.

Examples of positive control tissues for this antibody are CRC tissue with an MSH2 Clinical Status of Intact or normal colon tissue pre-qualified with VENTANA anti-MSH2 (G219-1129) antibody. Normal colon will stain intact for MSH2 using VENTANA anti-MSH2 (G219-1129) antibody. The positive tissue control should exhibit unequivocal nuclear staining in viable tumor cells and/or normal colon tissue elements. . For all tissues, 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 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 tissue with an MSH2 Clinical Status of Loss pre-qualified with VENTANA anti-MSH2 (G219-1129) 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 MSH2 Clinical Status. Refer to the Quality Control Procedures previously outlined in this method sheet and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[35] or the CLSI Approved Guideline.[31]

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for VENTANA anti-MSH2 (G219-1129) antibody is nuclear in actively proliferating cells. CRC specimens stained with VENTANA anti-MSH2 (G219-1129) antibody are 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 3.

If unequivocal nuclear stain is absent in internal positive controls and/or background staining interferes with interpretation, the assay should be considered unacceptable and repeated. Punctate nuclear staining of tumor cells should be considered negative (Loss). In cases with focal tumor cell staining, the intensity of the nuclear staining should be at least that of the internal positive controls along with the confluent/continuous staining of the nuclei in a few epithelial glands or nests for the case to be given a Clinical Status of Intact. In the absence of these conditions, a Clinical Status of Loss is given to the case.

Table 3. Staining interpretation for VENTANA anti-MSH2 (G219-1129) antibody.

Clinical Status	Description
Intact MSH2 Expression	Unequivocal nuclear staining in viable tumor cells, in the presence of internal positive controls (nuclear staining in lymphocytes, fibroblasts, or normal colonic epithelium in the vicinity of the tumor)
Loss of MSH2 Expression	Unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls. Punctate nuclear staining will be considered negative.

VENTANA anti-MSH2 (G219-1129) antibody stained cases are categorized as Intact or Loss according to the presence or absence of specific staining in the tumor.

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.

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-MSH2 (G219-1129) 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-MSH2 (G219-1129) antibody IHC interpretation.
- Focal Staining: Some specimens may exhibit focal staining in the tumor cells and staining intensity may vary from weak to strong. As specifiedby VENTANA anti-MSH2 (G219-1129) antibody IHC scoring algorithm, focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls 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-MSH2 (G219-1129) 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.

Slides should be desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories should validate cut slide stability within their own environment when storing beyond 45 days.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Positive staining is nuclear unless otherwise specified. No unexpected staining was observed with VENTANA anti-MSH2 (G219-1129) 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 4. Sensitivity/Specificity of VENTANA anti-MSH2 (G219-1129) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	3/3	Esophagus	3/3
Cerebellum	3/3	Stomach	3/3
Adrenal gland	3/3	Small intestine	3/3
Ovary	5/5	Colon	3/3
Pancreas	3/3	Liver	3/3
Lymph node	3/3	Tongue/salivary gland	2/3
Pituitary gland	3/3	Kidney	3/3
Testis	3/3	Prostate	3/3
Thyroid	3/3	Bladder	3/3
Breast	3/3	Parathyroid gland	3/3
Spleen	3/3	Endometrium	3/3
Tonsil	3/3	Cervix	3/3
Thymus	3/3	Skeletal muscle	3/3
Bone marrow	3/3	Skin	3/3
Lung	3/3	Nerve	5/5
Heart	1/3	Mesothelium	3/3

Note: Mismatch repair proteins such as MSH2 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 5. Sensitivity/Specificity of VENTANA anti-MSH2 (G219-1129) antibody was determined by testing 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
Neuroendocrine neoplasm (Pancreas)	1/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	2/2
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Microinvasive ductal carcinoma (Breast)	1/1
Ductal carcinoma in situ (Breast)	1/1

Pathology	# positive / total cases
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 cell 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
Hepatoblastoma (Liver)	1/1
Clear cell carcinoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Leiomyoma (Uterus)	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
Leiomyosarcoma (Bladder)	1/1
Osteosarcoma (Bone)	1/1
Leiomyosarcoma (Smooth muscle)	1/1

Note: Mismatch repair proteins such as MSH2 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

Precision studies for VENTANA anti-MSH2 (G219-1129) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT, BenchMark GX, BenchMark ULTRA, instrument.

All studies met their acceptance criteria.

Within-Run Repeatability and Between-Day Intermediate Precision

The repeatability and precision of VENTANA anti-MSH2 (G219-1129) antibody were evaluated on the BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit.

Within-Run Repeatability was evaluated using 11 CRC specimens (6 Intact and 5 Loss for MSH2 expression). Five replicate slides from each of the CRC specimens were stained with VENTANA anti-MSH2 (G219-1129) antibody on a single BenchMark ULTRA instrument within a single day. Each VENTANA anti-MSH2 (G219-1129) 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 11 CRC specimens (6 Intact and 5 Loss for MSH2 expression). Replicate slides from each of the CRC specimens were stained with VENTANA anti-MSH2 (G219-1129) antibody on a BenchMark ULTRA instrument on each of 5 non-consecutive days. Each VENTANA anti-MSH2 (G219-1129) 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 can be found in Table 6.

Table 6. Within-Run Repeatability and Between-Day Intermediate Precision of VENTANA anti-MSH2 (G219-1129) antibody as measured by Clinical Status (Intact or Loss).

Repeatability/ Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Run Repeatability	Intact	PPA	30/30	100.0	(88.6, 100.0)
	Loss	NPA	25/25	100.0	(86.7, 100.0)
	Total	OPA	55/55	100.0	(93.5, 100.0)
Between-Day Intermediate Precision	Intact	PPA	60/60	100.0	(94.0, 100.0)
	Loss	NPA	50/50	100.0	(92.9, 100.0)
	Total	OPA	110/110	100.0	(96.6, 100.0)

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

Between-Instrument Intermediate Precision

BenchMark ULTRA Instrument Between-Instrument Intermediate Precision of VENTANA anti-MSH2 (G219-1129) antibody was determined by staining replicate slides of 11 CRC specimens (6 Intact and 5 Loss for MSH2 expression) across 3 BenchMark ULTRA instruments with VENTANA anti-MSH2 (G219-1129) antibody using OptiView DAB IHC Detection Kit.

Each VENTANA anti-MSH2 (G219-1129) 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 7.

Table 7. BenchMark ULTRA Instrument Between-Instrument Intermediate Precision of VENTANA anti-MSH2 (G219-1129) antibody as measured by Clinical Status (Intact or Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Between-Instrument Intermediate Precision	Intact	PPA	36/36	100.0	(90.4, 100.0)
	Loss	NPA	30/30	100.0	(88.6, 100.0)
	Total	OPA	66/66	100.0	(94.5, 100.0)

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

In addition, Between-Instrument Intermediate Precision of VENTANA anti-MSH2 (G219-1129) antibody was determined by staining replicate slides of 6 CRC specimens (4 Intact and 2 Loss for MSH2 expression) across 3 BenchMark XT and 3 BenchMark GX instruments with VENTANA anti-MSH2 (G219-1129) antibody using 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 VENTANA anti-MSH2 (G219-1129) antibody was determined by staining CRC specimens with VENTANA anti-MSH2 (G219-1129) antibody using OptiView DAB IHC Detection Kit. All slides were evaluated for Clinical Status (Intact/Loss) by a single pathologist.

Pairwise comparisons of CRC specimens were made between platforms including BenchMark GX to BenchMark ULTRA instruments (136 Intact and 23 Loss cases), BenchMark GX to BenchMark XT instruments (134 Intact and 23 Loss cases), and BenchMark ULTRA to BenchMark XT instruments (137 Intact and 23 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 CRC specimens (12 Intact and 8 Loss cases) stained with VENTANA anti-MSH2 (G219-1129) antibody and OptiView DAB IHC Detection Kit. Each VENTANA anti-MSH2 (G219-1129) 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 MSH2 Clinical Status. Pathologists were blinded to the case diagnosis. Following a two-week washout period, the VENTANA anti-MSH2 (G219-1129) antibody-stained slides were re-randomized for a second evaluation of the MSH2 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 CRC slide comparisons per pathologist. Comparisons from the 3 pathologists were pooled and demonstrated 100.0% APA, 100.0% ANA, and 100.0% OPA for Within-Reader precision. A summary of the results can be found in Table 8.

Between-Reader precision compared all slide evaluations (20 CRC x 2 evaluations/case x 3 pathologists = 120 slide evaluations) to a modal case status for each CRC case. The results demonstrate 100.0% PPA, NPA, and OPA for Between-Reader precision. A summary of the results can be found in Table 8.

Table 8. Within-Reader and Between-Reader Precision of VENTANA anti-MSH2 (G219-1129) antibody on CRC cases as measured by MSH2 Clinical Status (Intact/Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Reader	Intact	APA	72/72	100.0	(94.9, 100.0)
	Loss	ANA	48/48	100.0	(92.3, 100.0)
	Total	OPA	60/60	100.0	(94.0, 100.0)
Between-Reader	Intact	PPA	72/72	100.0	(94.9, 100.0)
	Loss	NPA	48/48	100.0	(92.6, 100.0)
	Total	OPA	120/120	100.0	(96.9, 100.0)

Note: For Within-Reader, the APA and ANA 95% CIs 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-MSH2 (G219-1129) antibody was determined by testing 3 production lots of VENTANA anti-MSH2 (G219-1129) antibody each on triplicate slides of 10 CRC specimens (5 Intact and 5 Loss for MSH2 expression) on a BenchMark ULTRA instrument using OptiView DAB IHC Detection Kit.

Each VENTANA anti-MSH2 (G219-1129) 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-MSH2 (G219-1129) 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-MSH2 (G219-1129) antibody Lot-to-Lot Precision, all slide evaluations were compared to a modal case status for each CRC case. The OPA between VENTANA anti-MSH2 (G219-1129) antibody lots was 100%; demonstrating that VENTANA anti-MSH2 (G219-1129) antibody staining is reproducible across antibody lots.

A summary of the results for Lot-to-Lot Precision of VENTANA anti-MSH2 (G219-1129) antibody is shown in Table 9.

Table 9. Lot-to-Lot Precision of VENTANA anti-MSH2 (G219-1129) 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: 95% CIs were calculated using the Wilson Score method.

Inter-Laboratory Reproducibility Study

An Inter-Laboratory Reproducibility Study of VENTANA MMR IHC Panel was completed to demonstrate reproducibility of each VENTANA MMR IHC Panel assay to determine Clinical Status. The study included 6 CRC tissue specimens (3 Intact and 3 Loss) for each MMR protein and 16 CRC 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 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 10.

Table 10. Agreement between 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: Clinical Status is defined as Intact or Loss for protein expression for MMR proteins and Positive or Negative for BRAF V600E protein. 95% CIs were calculated using a generalized linear mixed model (GLMM) approach.

In addition, pairwise comparisons were made Between-Site, Between-Day, and Between-Reader for VENTANA anti-MSH2 (G219-1129) antibody. For MSH2, this study set included a total of 180 observations. A summary of the results can be found in Table 11. The data indicate assay reproducibility across 5 days, 3 sites, and 6 readers.

Table 11. Inter-Laboratory Reproducibility Pairwise Agreement Rates for VENTANA anti-MSH2 (G219-1129) 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: 95% CIs were calculated using the percentile bootstrap method; in instances where the point estimate was 100%, Wilson Score method was used.

PERFORMANCE OF VENTANA ANTI-MSH2 (G219-1129) ANTIBODY ON THE BENCHMARK ULTRA PLUS INSTRUMENT

Concordance Between BenchMark ULTRA PLUS and BenchMark ULTRA Instruments for MSH2 (G219-1129) 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 VENTANA anti-MSH2 (G219-1129) Antibody, with equal distribution between MSH2 Loss and MSH2 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-MSH2 (G219-1129) 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 MSH2 (G219-1129) staining protocol. Two pathologists per site, blinded to case status, evaluated the slides stained on the BenchMark ULTRA PLUS instrument and determined the MSH2 status. After a two week washout period, corresponding case slides previously stained at RTD 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 RTD. The OPA, LPA and IPA rates were 98.0% (818/835), 97.2% (s416/428), and 98.8% (402/407, respectively. The results were summarized in Table 12.

Table 12. Pooled Agreement of MSH2 status for Cases Stained with VENTANA anti-MSH2 (G129-1129) Antibody on the BenchMark ULTRA PLUS versus ULTRA Instrument.

BenchMark ULTRA PLUS MSH2 (G219-1129) Status	BenchMark ULTRA MSH2 (G219-1129) Status		Total
	Loss	Intact	
Loss	416	5	421
Intact	12	402	414
Total	428	407	835
	n/N	% (95% CI)	
LPA	416/428	97.2 (95.3, 98.8)	
IPA	402/407	98.8 (97.4, 99.8)	
OPA	818/835	98.0 (96.8, 99.0)	

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 VENTANA MMR RxDx Panel was completed to determine 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 if 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 13.

Table 13. Inter-laboratory reproducibility of 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 14, the say was reproducible across 3 days, 3 sites, and 6 readers.

Table 14. Inter-laboratory reproducibility pairwise agreement rates for VENTANA MMR RxDx Panel in solid pan tumor.

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. CIs for 100% ADPA and APPA were calculated using the transformed Wilson Score method. CIs for 100% OPA were calculated using Wilson Score method.

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 VENTANA MMR IHC Panel to molecular testing including a comprehensive DNA sequencing colon panel for the identification of CRCs 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 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 molecular testing. Following review, 105 sequential cases meeting these criteria were enrolled into the study. In addition, 13 CRC 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 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 CRC 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 15.

A pooled analysis comparing the four MMR IHC markers (without 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 16.

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 17 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 CRCs obtained by IHC using 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 VENTANA anti-BRAF V600E (VE1) antibody using DNA sequencing as the reference all were 100% (Table 18). Additional testing was performed to verify the ability of 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 CRC. The results verified that VENTANA anti-BRAF V600E (VE1) antibody correctly identifies CRCs 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 CRC in the absence of MLH1 expression.

Table 15. 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)

* 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. 95% CIs were calculated using the percentile bootstrap method.

Table 16. 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)

* 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. 95% CIs were calculated using the percentile bootstrap method.

Table 17. 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)

* 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. 95% CIs were calculated using the Wilson Score method.

Table 18. 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)

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. 95% CIs were calculated using the Wilson Score method.

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013.
2. Geiersbach KB, Samowitz WS. Microsatellite instability and colorectal cancer. Arch Pathol Lab Med. 2011;135(10):1269-1277.
3. Wright CL, Stewart ID. Histopathology and mismatch repair status of 458 consecutive colorectal carcinomas. Am J Surg Pathol. 2003;27(11):1393-1406.
4. Tiwari AK, Roy HK, Lynch HT. Lynch syndrome in the 21st century: clinical perspectives. QJM. 2016;109(3):151-158.
5. Buza N, Ziai J, Hui P. Mismatch repair deficiency testing in clinical practice. Expert Rev Mol Diagn. 2016;16(5):591-604.
6. Silva FCC, Torrezan GT, Ferreira JRO, Oliveira LP, Begnami M, et al. Germline Mutations in MLH1 Leading to Isolated Loss of PMS2 Expression in Lynch Syndrome: Implications for Diagnostics in the Clinic. Am J Surg Pathol. 2017;41(6):861-864.
7. Boyer JC, Umar A, Risinger JI, Lipford JR, Kane M, et al. Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. Cancer Res. 1995;55(24):6063-6070.
8. Lawes DA, Pearson T, Sengupta S, Boulos PB. The role of MLH1, MSH2 and MSH6 in the development of multiple colorectal cancers. Br J Cancer. 2005;93(4):472-477.
9. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med. 2003;348(10):919-932.
10. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol. 2003;21(6):1174-1179.
11. Lynch HT, Smyrk T. Hereditary nonpolyposis colorectal cancer (Lynch syndrome). An updated review. Cancer. 1996;78(6):1149-1167.
12. Caldes T, Godino J, Sanchez A, Corbacho C, De la Hoya M, et al. Immunohistochemistry and microsatellite instability testing for selecting MLH1, MSH2 and MSH6 mutation carriers in hereditary non-polyposis colorectal cancer. Oncol Rep. 2004;12(3):621-629.
13. Shia J, Klimstra DS, Nafa K, Offit K, Guillem JG, et al. Value of immunohistochemical detection of DNA mismatch repair proteins in predicting germline mutation in hereditary colorectal neoplasms. Am J Surg Pathol. 2005;29(1):96-104.
14. Cunningham JM, Tester DJ, Thibodeau SN. Mutation detection in colorectal cancers: direct sequencing of DNA mismatch repair genes. Methods Mol Med. 2001;50:87-98.
15. Domingo E, Laiho P, Ollikainen M, Pinto M, Wang L, et al. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet. 2004;41(9):664-668.
16. Jin M, Hampel H, Zhou X, Schunemann L, Yearsley M, et al. BRAF V600E mutation analysis simplifies the testing algorithm for Lynch syndrome. Am J Clin Pathol. 2013;140(2):177-183.
17. Deng G, Bell I, Crawley S, Gum J, Terdiman JP, et al. BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. Clin Cancer Res. 2004;10(1 Pt 1):191-195.

18. Giardiello FM, Allen JI, Axilbund JE, Boland CR, Burke CA, et al. Guidelines on Genetic Evaluation and Management of Lynch Syndrome: A Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer. Diseases of the Colon & Rectum. 2014;57(8):1025-1048.
19. Egoavil C, Alenda C, Castillejo A, Paya A, Peiro G, et al. Prevalence of Lynch syndrome among patients with newly diagnosed endometrial cancers. PLoS One. 2013;8(11):e79737.
20. Connell LC, Mota JM, Braghiroli MI, Hoff PM. The Rising Incidence of Younger Patients With Colorectal Cancer: Questions About Screening, Biology, and Treatment. Curr Treat Options Oncol. 2017;18(4):23.
21. Provenzale D, Gupta S, Ahnen DJ, Bray T, Cannon JA, et al. Genetic/Familial High-Risk Assessment: Colorectal Version 1.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2016;14(8):1010-1030.
22. Balmana J, Balaguer F, Cervantes A, Arnold D, Group EGW. Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines. Ann Oncol. 2013;24 Suppl 6:vi73-80.
23. Evaluation of Genomic Applications in P, Prevention Working G. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. Genet Med. 2009;11(1):35-41.
24. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261-268.
25. Parsons MT, Buchanan DD, Thompson B, Young JP, Spurdle AB. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. J Med Genet. 2012;49(3):151-157.
26. Shia J. Evolving approach and clinical significance of detecting DNA mismatch repair deficiency in colorectal carcinoma. Semin Diagn Pathol. 2015;32(5):352-361.
27. Thiel A, Heinonen M, Kantonen J, Gylling A, Lahtinen L, et al. BRAF mutation in sporadic colorectal cancer and Lynch syndrome. Virchows Arch. 2013;463(5):613-621.
28. Toon CW, Chou A, DeSilva K, Chan J, Patterson J, et al. BRAFV600E immunohistochemistry in conjunction with mismatch repair status predicts survival in patients with colorectal cancer. Mod Pathol. 2014;27(5):644-650.
29. Koinuma K, Shitoh K, Miyakura Y, Furukawa T, Yamashita Y, et al. Mutations of BRAF are associated with extensive hMLH1 promoter methylation in sporadic colorectal carcinomas. Int J Cancer. 2004;108(2):237-242.
30. Carson FL, Hladik C, Cappellano CH, Pathology ASfC. Histotechnology: A Self-Instructional Text: American Society for Clinical Pathology; 2015.
31. CLSI. Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays: Approved Guideline-Second Edition. CLSI document I/LA28-A2 (ISBN 1-56238-745-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2011.
32. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
33. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
34. Roche PC, Hsi ED, Firfer BL. Immunohistochemistry: Principles and Advances. Manual of Molecular and Clinical Laboratory Immunology, 7th Edition: American Society of Microbiology; 2006.
35. Rabinovitch A. The College of American Pathologists laboratory accreditation program. Accreditation and Quality Assurance. 2002;7(11):473-476.

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:

https://ec.europa.eu/tools/eudamed

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
UDI	Unique Device Identification

REVISION HISTORY

Rev	Updates
F	Updates to the Intended Use, Performance of Ventana anti-MSH2 (G219-1129) Antibody on the BenchMark ULTRA PLUS Instrument, Symbols, Intellectual Property, and Contact Information sections.

INTELLECTUAL PROPERTY

VENTANA, BENCHMARK, OPTIVIEW, and the VENTANA logo are trademarks of Roche. All other trademarks are the property of their respective owners.

© 2024 Ventana Medical Systems, Inc.

CONTACT INFORMATION

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606

www.roche.com