EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR

Ventana MMR IHC Panel

DECISION SUMMARY

A. De Novo Number:

DEN170030

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation of the VENTANA Mismatch Repair Immunohistochemistry (MMR IHC) Panel.

C. Measurand:

Mismatch repair proteins MLH1, PMS2, MSH2, MSH6 and V600E mutated BRAF protein.

D. Type of Test:

Immunohistochemistry

E. Applicant:

Ventana Medical Systems, Inc.

F. Proprietary and Established Names:

VENTANA MMR IHC Panel

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.1866

2. Classification:

   Class II (Special Controls)

3. Product code:

   PZJ

4. Panel:

88- Pathology

H. Indications for use:

1. Indications for use:

The VENTANA MMR IHC Panel is a qualitative immunohistochemistry (IHC) test intended for use in the light microscopic assessment of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, and MSH6) and BRAF V600E proteins in formalin-fixed, paraffin-embedded colorectal cancer (CRC) tissue sections. The OptiView DAB IHC Detection Kit is used with MLH1, MSH2, MSH6 and BRAF V600E, and the OptiView DAB IHC Detection Kit with OptiView Amplification Kit is used for PMS2 detection. The VENTANA MMR IHC Panel is for use on the VENTANA BenchMark ULTRA instrument. The VENTANA MMR IHC Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody, and VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody.

The VENTANA MMR IHC Panel is indicated in patients diagnosed with colorectal cancer (CRC) to detect mismatch repair (MMR) proteins deficiency as an aid in the identification of probable Lynch syndrome and to detect BRAFV600E protein as an aid to differentiate between sporadic CRC and probable Lynch syndrome.

Results from the Ventana MMR IHC Panel should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

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

2. Special conditions for use statement(s):

Intended for in vitro diagnostic (IVD) use.

Prescription use only.

3. Special instrument requirements:

Ventana BenchMark Ultra instrument

I. Device Description:

The Ventana MMR IHC panel is comprised of five primary antibodies used to detect the MMR proteins MLH1, PMS2, MSH2 and MSH6 and mutated BRAF V600E protein in CRC tissue specimens. The primary antibodies are used in combination with individually optimized detection reagents and in conjunction with ancillary reagents common to all

immunohistochemistry test systems in order to complete specimen testing. The MMR IHC panel and BRAF V600E are optimized to run on the VENTANA BenchMark Ultra platform with OptiView DAB detection kit or in the case of PSM2 antibody the OptiView DAB detection Kit with the OptiView Amplification Kit. The presence or absence of target proteins is determined by visual examination of the specimen slide under light microscope by a qualified pathologist.

The Ventana MMR IHC panel antibodies are packaged as individual products in single ready to use reagent dispensers. The MMR IHC panel test is run on five separate CRC tissue slides and stained on BenchMark Ultra instrument. The primary antibody reagents are listed in Table 1 below.

Table 1. VENTANA MMR IHC Panel

Primary Antibody	Antibody Concentration
VENTANA anti-MLH1(M1)	~1 µg /mL
VENTANA anti-PMS2 (A16-4)	~1 µg/mL
VETNANA anti-MSH2 (G219-1129)	~20 µg/mL
VENTANA anti-MSH6 (SP93)	~1 µg/mL
VENTANA anti-BRAF V600E (VE1)	~12 µg/mL

Detection and ancillary reagents required but not provided with Ventana MMR IHC panel are listed below:

- OptiView DAB IHC detection Kit containing the following components
  - OptiView peroxidase Inhibitor
  - OptiView HQ universal Linker
  - OptiView HRP Multimer
  - OptiView H_2O_2
  - OptiView DAB
  - OptiView Copper
- OptiView Amplification kit
  - OptiView Amplification (0.003% HQ conjugated tyramide complex)
  - OptiView H_2O_2
  - OptiView Multimer
- Hematoxylin II
- Bluing Reagent
- Reaction Buffer (10x)
- EZ Prep Reagent (10x)
- ULTRA Cell Conditioning (CC1) Pre Dilute
- ULTRA Liquid Cover Slip (LCS) (Pre-dilute)

Control Tissue:

MMR antibodies: Pre-qualified CRC tissue with a MMR status of intact may be used as a

positive system-level control for MMR antibodies to detect the intact protein. Alternatively, pre-qualified normal colon tissue fixed and processed in the same manner as the patient tissue can also be used as a positive system-level control. Normal colon will stain positive for all antibodies in the MMR IHC panel. Since the MLH1, PMS2, MSH2, and MSH6 proteins are expressed in all tissues, a normal negative tissue control does not exist for these biomarkers. For a negative system level control, CRC with loss of an MMR protein can be used as an appropriate tissue control for mismatch repair protein deficiency status. However, lymphocytes, fibroblast and epithelial cells should exhibit staining and serve as positive internal control cells in CRC samples with MMR protein deficiency (dMMR).

BRAF V600E: A case of CRC positive for BRAF V600E mutated protein by VENTANA anti-BRAF V600E (VE1) IHC is an appropriate positive control. A negative system-level control is achieved with the fibroblasts and lymphocytes in normal adjacent epithelium.

Instrumentation and Software:

The MMR IHC panel tests are fully automated**.** The MMR IHC panel antibodies are for use on the BenchMark ULTRA instrument using Ventana System Software (VSS) software version 12.3 or earlier.

J. Standard/Guidance Document Referenced (if applicable):

CLSI guideline I/LA28-A2: Quality Assurance for Design Control and Implementation of ImmunohistochemistryAssays; Approved Guideline – Second Edition

Guidance for Submission of Immunohistochemistry Applications to the FDA. 1998

K. Test Principle:

The MMR IHC panel is an immunohistochemistry test system used to stain FFPE colorectal cancer (CRC) specimens to detect expression of the MMR proteins -MLH1, PMS2, MSH2 and MSH6- and the BRAF V600E mutated protein. The 5 antibodies of MMR IHC panel have individualized staining protocols that are created using available staining parameters provided in staining procedures in the VSS software that drives the BenchMark ULTRA automated staining platform. The panel test is run individually on 5 separate tissue sections and the test process involves sequential application of specific primary antibodies against the panel protein, followed by detection reagents and chromogen deposition for visualization of the target protein expression.

Briefly the assay steps are as follows 1) anti MMR/ BRAF V600E antibody binds to the epitope in the target protein; 2) a Haptenated (HQ) secondary antibody binds to the primary antibody; 3) a tertiary horseradish peroxidase (HRP)-labeled antibody directed against HQ binds to the HQ-labeled secondary antibody; and 4) the resulting complex is visualized with hydrogen peroxide and DAB, due to the formation of a visible brown precipitate at the antigen site. The PMS2 test uses the OptiView amplification in addition of to the OptiView DAB detection system for signal amplification by addition of hydrogen peroxide and Tyramide-HQ. The specimen slide is then counterstained with hematoxylin and cover slipped. Results are interpreted using a light

microscope by a pathologist.

L. Interpretation of Results

1. Staining Interpretation

   Clinical status for MMR proteins (MLH1, PMS2, MSH2 and MSH6) is assigned by a trained pathologist based on their evaluation for 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.

   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.

   BRAF V600E test interpretation is based on the evaluation of presence of immunohistochemical staining in cytoplasm of CRC neoplastic cells. Tumors with unequivocal cytoplasmic staining of any intensity in viable tumor cells are considered to be positive for BRAF V600E mutations.

   Interpretation for MMR proteins and the BRAF V600E status is detailed in Table 2 below

Table 2. Interpretation of staining for VENTANA MMR IHC Panel antibodies

Clinical Status	Description
Intact MMR protein 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 MMR Protein Expression	Unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls
Note: 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.	
Positive for BRAF V600E mutation	Unequivocal cytoplasmic staining of any intensity in viable tumor cells above background.

Clinical Status	Description
Negative for BRAF V600E mutation	No staining or Equivocal cytoplasmic staining in viable tumor cells. Note: Nuclear staining, weak to strong staining of isolated viable tumor cells/or small tumor clusters should be considered negative.

2. Result Conclusions
   - Detection of all four proteins in the tumor indicates normal (i.e., Intact) mismatch repair status.
   - Loss of MLH1 expression is accompanied with the loss of its heterodimer partner, PMS2. Loss of MLH1 may be either sporadic or evidence of probable Lynch syndrome. In sporadic occurrences of CRC, expression of the MLH1 gene may be suppressed by hypermethylation of its promoter. The presence of the BRAF V600E mutation in CRC cases is tightly linked to MLH1 promoter hypermethylation and loss of MLH1 protein. Loss of MLH1 and positive for BRAF V600E mutated proteins status are likely the result of a sporadic occurrence. Tumors negative for BRAF V600E mutated protein status is suggestive of Lynch syndrome.
   - The loss of PMS2, in the presence of intact MLH1 expression, or loss of MSH2 or MSH6 expression designates the tumor as Loss of the respective MMR protein and is consistent with probable Lynch syndrome.
   - All individuals with suspected Lynch syndrome should be referred for genetic counseling and further genetic testing to confirm the presence of the suspected germline mutation.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision:* Assay precision was evaluated for each of the 5 panel antibodies individually using an identical study design. The following precision parameters were evaluated in the precision studies

      - Within Day
      - Between day
      - Between Instrument
      - Lot to Lot
      - Reader Precision

      i. *Within Day Precision:*
      Within-day precision was evaluated using 10 CRC cases consisting of 5 cases with intact protein status and 5 cases with loss status for the panel proteins except MSH2 study that included 6 MSH2 intact and 5 MSH2 loss cases. Five (5) slides were stained using each panel antibody and one slide of each case was stained with Negative Control (Monoclonal). All slides were run on the BenchMark ULTRA using the OptiView DAB detection kit except for PSM2 slides which were run using the OptiView Amplification kit. Data was obtained from 50 total observations (10

cases X 5 replicates x 1 instrument) for each panel antibody. Within-day precision was 100%. The study met pre-specified acceptance criteria of ≥85% lower bound confidence interval. Results for individual panel antibodies are shown in Table 3 below.

Table 3. Within day Precision

Antibody	Agreement			
	Type	n/N	%	95% CI
anti MLH1 (M1) antibody	PPA	25/25	100.0	(86.7,100.0)
	NPA	25/25	100.0	(86.7,100.0)
	OPA	**50/50**	**100.0**	**(92.9, 100)**
anti-PMS2 (A16-4) antibody	PPA	25/25	100.0	(86.7,100.0)
	NPA	25/25	100.0	(86.7,100.0)
	OPA	**50/50**	**100.0**	**(92.9,100.0)**
anti MSH6 (SP93) antibody	PPA	25/25	100.0	(86.7,100.0)
	NPA	25/25	100.0	(86.7,100.0)
	OPA	**50/50**	**100.0**	**(92.9,100.0)**
anti-MSH2 (G219-1129) Antibody	PPA	30/30	100.0	(88.6,100.0)
	NPA	25/25	100.0	(86.7,100.0)
	OPA	**55/55**	**100.0**	**(93.5,100.0)**
anti BRAF V600E (VE1) antibody	PPA	25/25	100.0	(86.7,100.0)
	NPA	25/25	100.0	(86.7,100.0)
	OPA	**50/50**	**100.0**	**(92.9,100.0)**

ii. Between Day Precision:

Between-day repeatability was evaluated using the same CRC cases as within-day testing. Two slides were stained using each primary antibody and one slide of each case was stained with Negative Control (Monoclonal) across 5 non-consecutive days. For Day 1, the first two slides from each case from Within-day precision study were used. Data was obtained from 100 total observations (10 cases X 2 replicates X 5 days) for each of the panel antibody (Note: MSH2 evaluation was conducted with 6 intact and 5 loss status cases for a total of 110 observations). Between-day precision was observed to be 100%. The study met pre-specified acceptance criteria.

Results for individual panel antibody are shown in Table 4 below.

Table 4. Between day Precision

Antibody	Agreement			
	Type	n/N	%	95% CI
anti-MLH1 (M1) antibody	PPA	50/50	100.0	(92.9, 100.0)
	NPA	50/50	100.0	(92.9, 100.0)
	OPA	**100/100**	**100.0**	**(96.3, 100)**
anti-PMS2 (A16-4)	PPA	50/50	100.0	(92.9,100.0)

	NPA	50/50	100.0	(92.9,100.0)
	OPA	**100/100**	**100.0**	**(96.3,100.0)**
anti MSH-6 (SP93) antibody	PPA	50/50	100.0	(92.9,100.0)
	NPA	50/50	100.0	(92.9,100.0)
	OPA	**100/100**	**100.0**	**(96.3,100.0)**
Anti-MSH2 (G219-1129)	PPA	60/60	100.0	(94.0,100.0)
	NPA	50/50	100.0	(92.9,100.0)
	OPA	**110/110**	**100.0**	**(96.6,100.0)**
BRAF V600E	PPA	50/50	**100.0**	(92.9,100.0)
	NPA	50/50	**100.0**	(92.9,100.0)
	OPA	**100/100**	**100.0**	**(96.3,100.0)**

iii. Between instrument:

Instrument to instrument reproducibility was evaluated using the same CRC cases as Within-day testing. Two slides were stained using panel antibody and one slide of each case was stained with Negative Control (Monoclonal) across 3 different ULTRA instruments. Data was obtained from 60 total observations (10 cases X 2 replicates X 3 instruments) for each panel antibody (Note: MSH2 evaluation was conducted with 6 intact and 5 loss status cases for a total of 66 observations). The study met pre-specified acceptance criteria. Results for individual panel antibody are shown in Table 5 below.

Table 5. Between Instrument Precision

Day	**Agreement**			
	Type	**n/N**	**%**	**95% CI**
All ULTR MLH1 (M1) antibody As	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)**
anti-PMS2 (A16-4)	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)**
anti-MSH2 (G219-1129) antibody	PPA	36/36	100.0	(90.4,100.0)
	NPA	30/30	100.0	(88.6,100.0)
	OPA	**66/66**	**100.0**	**(94.5,100.0)**
anti MSH6 (SP93) antibody	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)**
BRAF V600E	PPA	30/30	100.0	(88.6,100.0)

antibody	NPA	30/30	100.0	(88.6,100.0)
	OPA	**60/60**	**100.0**	**(94.0,100.0)**

iv. Lot to lot Precision:

The study evaluated Lot to lot precision with 3 final lots of the 5 panel antibodies with 10 qualified CRC cases consisting of 5 cases Intact for the panel protein expression and 5 cases Loss. All cases were run in triplicate for each lot test and one slide of each case was stained with the negative control reagent. All slides were run on the BenchMark ULTRA using OptiView DAB detection kit. Data was obtained from 90 total observations (10 cases X 3 replicates X 3 lots). Overall, 90 out of 90 slides were evaluable for MLH1, MSH2, MSH6 and BRAF V600E antibodies, while 3 PMS2 stained slides could not be evaluated due to unacceptable background scores >0.5 and therefore could not be evaluated. The study demonstrated an overall percent agreement of 100% for each of the panel antibodies. Results for individual panel antibody are shown in Table 6 below. The results met the pre-specified acceptance criteria.

Table 6. Between Lot Precision

Antibody	**Agreement**			
	Type	**n/N**	**%**	**95% CI**
MLH1 (M1) antibody	PPA	45/45	100.0	(92.1, 100.0)
	NPA	45/45	100.0	(92.1, 100.0)
	OPA	**90/90**	**100.0**	**(95.9, 100.0)**
anti-PMS2 (A16-4)	PPA	44/44	100.0	(92.9,100.0)
	NPA	43/43	100.0	(92.9,100.0)
	OPA	**87/87**	**100.0**	**(96.3,100.0)**
anti MSH6 (Sp93) antibody	PPA	45/45	100.0	(92.1, 100.0)
	NPA	45/45	100.0	(92.1, 100.0)
	OPA	**90/90**	**100.0**	**(95.9, 100.0)**
Anti-MSH2 (G219-1129)	PPA	45/45	100.0	(92.1, 100.0)
	NPA	45/45	100.0	(92.1, 100.0)
	OPA	**90/90**	**100.0**	**(95.9, 100.0)**
BRAF V600E	PPA	45/45	100.0	(92.1, 100.0)
	NPA	45/45	100.0	(92.1, 100.0)
	OPA	**90/90**	**100.0**	**(95.9, 100.0)**

v. Reader Precision:

Between-Reader and Within-Reader precision were assessed by evaluating concordance of marker status across 3 readers and among individual readers using 20 cases of CRC. These 20 CRC specimens consisted of varying number of cases with loss or intact MMR protein status and BRAF V600E positive or negative status as shown in Table 7. Each reader scored all 20 cases in two rounds that were separated by a two week wash out period. Scores were analyzed for agreement between and within readers. Between reader precision ranged 97.5 to 100%. The results are shown

in Table 8. Within reader precision ranged 98.3 to 100%. Within-reader precision is shown in Table 9.The studies met the pre-specified acceptance criteria.

Table 7. Case Distribution across Markers in Reader Precision

Marker	# of Cases with Intact Status	# of Cases with Loss Status
MLH1	11	9
PMS2	13	7
MSH2	12	8
MSH6	11	9
BRAF V600E	10	10

Table 8. Between Reader Precision by marker

Marker	Agreement			
	Type	**n/N**	**%**	**95% CI**
MLH-1	PPA	66/66	100.0	(94.5,100.0)
	NPA	53/54	98.1	(90.2,99.7)
	OPA	**119/120**	**99.2**	**(95.4,99.9)**
PMS2	PPA	78/78	100.0	(95.3,100.0)
	NPA	42/42	100.0	(91.6,100.0)
	OPA	**120/120**	**100.0**	**(96.9,100.0)**
MSH6	PPA	66/66	100.0	(94.5,100.0)
	NPA	53/54	98.1	(90.2,99.7)
	OPA	**119/120**	**99.2**	**(95.4,99.9)**
MSH2	PPA	72/72	100.0	(94.9,100.0)
	NPA	48/48	94.4	(92.6,100)
	OPA	**120/120**	**97.5**	**(96.9,100)**
BRAF V600E	PPA	60/60	100.0	(94.0,100.0)
	NPA	60/60	100.0	(94.0,100.0)
	OPA	**120/120**	**100.0**	**(96.9,100.0)**

Table 9. Within reader precision

Marker	Agreement			
	Type	**n/N**	**%**	**95% CI**
MLH-1	APA	66/67	98.5	(88.0,100.0)
	ANA	52/53	98.1	(83.3,100.0)
	OPA	**59/60**	**98.3**	**(85.0,100.0)**
PMS2	APA	72/72	100	(95.3100.0)
	ANA	48/48	100	(91.2,100.0)
	OPA	**60/60**	**100**	**(94.0,100.0)**
MSH6	APA	66/67	98.5	(88.0, 100.0)
	ANA	52/53	98.1	(83.3,100.0)
	OPA	**59/60**	**98.3**	**(83.3,100.0)**
MSH2	APA	72/72	100	(94.9100.0)

	ANA	48/48	100	(92.3,100.0)
	OPA	**60/60**	**100**	**(94.0,100.0)**
BRAF V600E	APA	60/60	100.0	(93.9,100.0)
	ANA	60/60	100.0	(93.9,100.0)
	OPA	**60/60**	**100.0**	**(94.0,100.0)**

b. Reproducibility:

The reproducibility of the Ventana MMR IHC panel was assessed at 3 sites with 40 archival FFPE CRC tissue specimens. For the 4 antibodies against MMR protein in the panel (anti-MLH1, anti-PMS2, anti-MSH2, and anti-MSH6), 6 CRC tissue specimens (3 intact and 3 loss cases) were included in the study, resulting in 24 cases total for these 4 antibodies. In addition, for the anti-BRAF V600E antibody, 16 CRC tissue specimens -8 positive and 8 negative cases- with adequate tumor content (at least 50 viable tumor cells) were included in the study. Multiple tissue sections were cut from each case. Three (3) external clinical sites stained all cases with the designated antibody and the appropriate negative reagent control (NRC) antibody on each of 5 non-consecutive days spanning a period of at least 20 days. Cases were stained on a BenchMark ULTRA instrument in a different randomized order each day.

Specimen sets consisting of H&E, NRC, and antibody stained slide from all four antibodies in the MMR IHC panel staining were combined and randomized into a day-specific reading set in order to minimize recall bias within the study. The case slide triplets for the anti-BRAF V600E antibody were combined into a separate, randomized, day-specific reading set.

Two pathologists at each site independently evaluated the reading set for the MMR IHC panel to determine the status (intact or loss) for each case. The same two pathologists at each site independently evaluated the separate reading set for BRAF V600E to determine the BRAF V600E status (positive or negative) for each case. The pathologists also evaluated all of the case slides for staining artifacts and staining failures affecting their ability to interpret the slides.

There were 720 observations for the four MMR markers (4 markers * 6 cases per marker * 5 days* 2 readers * 3 sites =720 observations). There were 480 observations for the BRAF marker (16 cases*5days*2 readers* 3 sites= 480 observations). When pooling the observations of all five markers, there were 1200 observations.

MMR-intact cases and BRAF V600E-positive cases were used to calculate PPA, and MMR-loss cases and BRAF V600E-negative cases were used to calculate NPA. Point estimates for PPA, NPA, and OPA were and their 95% confidence intervals (CI) for calculated by using the generalized linear mixed model (GLMM) approach. Modal case reference status for calculation PPA, NPA and OA was derived on the most often observed status of 30 observations.

The overall performance of the Ventana MMR IHC panel plus the anti-BRAF V600E antibody was to be considered acceptable if both the PPA and NPA rates across all observations exhibited a lower bound of the 2-sided 95% confidence interval (LBCI) ≥ 85%, when using the modal result for each case as the reference for that case.

Summary of pooled agreement statistics between modal case reference status and individual observation are summarized in Table 10. PPA was 99.8% for all proteins and NPA was 98.9% for all proteins. There were 9 discordant cases, one each for MSH2 and MSH6 four for PMS2 and 3 for BRAF V600E status. Additionally, pair wise comparison for between site, between day and between readers was assessed by individual panel maker and 95% Confidence intervals calculated with Wilsons' score method. The data is summarized in Tables 11-15.

Table 10. Inter laboratory Reproducibility for all proteins

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)

Table 11. Pairwise Agreement rates for Ventana MLH-1 (M1) Antibody

Inter-Laboratory Reproducibility MLH1 (M1) Ab		Agreement			
		Type	n/N	%	95% CI
Between-Site (3 sites)		APA	360/360	100.0	(98.9,100.0)
		ANA	360/360	100.0	(98.9,100.0)
		OPA	360/360	100.0	(98.9,100.0)
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	120/120	100.0	(96.9,100.0)
		ANA	120/120	100.0	(96.9,100.0)
		OPA	120/120	100.0	(96.9,100.0)
Between-Reader		APA	90/90	100.0	(95.9,100.0)

Inter-Laboratory Reproducibility MLH1 (M1) Ab	Agreement			
	Type	n/N	%	95% CI
(2 pathologists per site)	ANA	90/90	100.0	(95.9,100.0)
	OPA	90/90	100.0	(95.9,100.0)

Table 12. Pairwise Agreement rates for Ventana PMS2 (A16-4) Antibody

Inter-Laboratory Reproducibility PMS2(A16-4) Ab		Agreement			
		Type	n/N	%	95% CI
Between-Site (3 sites)		APA	344/360	95.6	(90.7,100.0)
		ANA	344/360	95.6	(90.7,100.0)
		OPA	344/360	95.6	(91.1,100.0)
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	104/120	86.7	(69.2,100.0)
		ANA	104/120	86.7	(69.2,100.0)
		OPA	104/120	86.7	(73.3,100.0)
Between-Reader (2 pathologists per site)		APA	90/90	100.0	(95.9,100.0)
		ANA	90/90	100.0	(95.9,100.0)
		OPA	90/90	100.0	(95.9,100.0)

Table 13. Pairwise Agreement rates for Ventana anti MSH2 (G219-1129) Antibody

Inter-Laboratory Reproducibility MSH2 (G219-1129) Ab		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	Site A	APA	120/120	100.0	(96.9,100.0)
		ANA	120/120	100.0	(96.9,100.0)

Inter-Laboratory Reproducibility MSH2 (G219-1129) Ab		Agreement			
		Type	n/N	%	95% CI
(5 non-consecutive days)		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)

Table 14. Pairwise Agreement rates for Ventana MSH6 (SP93) Antibody

Inter-Laboratory Reproducibility MSH6 (SP93) AB		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	120/124	96.8	(90.9,100.0)
		ANA	112/116	96.6	(88.9,100.0)
		OPA	116/120	96.7	(90.0,100.0)
	Site C	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)
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)

Table 15. Pairwise Agreement rates for Ventana anti BRAF V600E (VE1) Antibody

Inter-Laboratory	Agreement

Reproducibility BRAF V600E (VE1) Ab		Type	n/N	%	95% CI
Between-Site (3 sites)		APA	960/972	98.8	(97.2,100.0)
		ANA	936/948	98.7	(97.0,100.0)
		OPA	948/960	98.8	(97.1,100.0)
Between-Day (5 non-consecutive days)	Site A	APA	320/320	100.0	(98.8,100.0)
		ANA	320/320	100.0	(98.8,100.0)
		OPA	320/320	100.0	(98.8,100.0)
	Site B	APA	320/320	100.0	(98.8,100.0)
		ANA	320/320	100.0	(98.8,100.0)
		OPA	320/320	100.0	(98.8,100.0)
	Site C	APA	320/332	96.4	(92.0,100.0)
		ANA	296/308	96.1	(90.4,100.0)
		OPA	308/320	96.3	(91.3,100.0)
Between-Reader (2 pathologists per site)		APA	242/243	99.6	(98.8,100.0)
		ANA	236/237	99.6	(98.7,100.0)
		OPA	239/240	99.6	(98.8,100.0)

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability (controls, calibrators, or methods), Stability, Expected values:*

i. Controls: See section I for description of controls.

ii. Stability: Product expiration dating is based on testing 3 lots of MMR antibodies in accelerated stability studies.

a) *Reagent Stability Studies*:

MLH1, MSH2, MSH6, PMS2: The stability of MMR panel antibodies was tested by subjecting 3 production equivalent lots of MMR antibodies to heat stress in an accelerated stability model. Staining performance for the antibodies was assessed after subjecting the antibodies to 243 hours at 45^0C or 531 hours at 37^0C to support a stability claim of 24 months. Interim stability after antibody storage at 45^0C and 243 hours (12 month equivalent) was also assessed. The staining was performed using an OptiView DAB IHC Detection Kit on the BenchMark ULTRA with 3 tissue blocks, including 2 intact CRC (colorectal carcinoma) cases and 1 dMMR CRC case. The staining intensities were compared with case slides stained at baseline (0 hours) on a 0-4 scale and determined that the staining intensity did not vary more than 1.0 point and background by 0.5 points when compared to staining at base line (0 hours of stress) on a 0-4 scale .

Study met acceptance criteria for MLH-1, MSH2 and MSH6 antibodies to support expiration dating of 24 months for these 3 antibodies. Real time stability studies in support of the 24 month claim are on-going.

PMS2 antibody passed accelerated stability testing on storage at 37^0C for 287 hours to support a 12 month dating and at 30^0C for 603 and 1081.5 hours. Results from real time stability for one lot of PMS2 antibody was provided to support stability claims based on accelerated stability testing at lower temperatures (30^0C instead of 37^0C and 45^0C). One lot of antibody was tested for intended storage condition (2-8 ^{0}C); ship stress (15^0C and 30^0C for 192 hours) and cold ship stress (-20^0C, 192 hours) at 4 and 6 months with 6 PMS2 Intact and 3 Loss cases and tonsil controls specimens. The PMS2 antibody lot passed the 6 month time point with PMS2 specific staining within 1 point and background staining within 0.5 point when compared to baseline on a 0-4 point intensity scale. The data supports a stability claim of 12 months for PMS2. Real time stability studies in support of the claim are on-going

BRAF V600E*:* Real time stability testing was conducted using 3 Stability Master Lots (SMLs). Each SML consisted of a 1 lot of anti-BRAF V600E (VE1) antibody and 1 lot of OptiView detection kit. Real time stability was assessed for the 3 stability master lots beginning of the study (Day 0) and subsequently assessed for intended storage, ship stress and freeze thaw cold ship stability for a total of 37 months with testing at 3, 4, 6, 8, 9, 12, 14, 18, 20, 24, 26 and 37 months. Intended storage (2-8 ^{0}C) was tested through 37 months, heated ship stress (30^0C for 192 hours) was tested to 26 months and Freeze thaw cold ship stress (-20^0C for 192 hours) for 37 months. Initial assessment of stability was performed with thyroid papillary carcinoma cases, and was subsequently confirmed with 6 CRC cases. Testing included ship stress, freeze thaw and cold ship stability parameters. Data supports expiration dating of 24 months for the anti BRAF V600E antibody.

b) *Cut slide Stability*: Specimen stability with storage time and temperature was assessed on cut sections stored prior to staining for each of the panel proteins in separate studies. Studies for each of the MMR panel protein used 1 normal colon and 2 CRC cases with expression of MMR (Intact) in tumors. For BRAF V600 E section stability 1 WT and 3 BRAF V600E mutation positive cases were included. Slides were stored at 5±3°C or at 30±5°C. Two (2) slides were stained with Ventana MMR antibody and 1 slide with negative reagent control at different time points starting from the beginning of the experiment (Day 0) up to six months (Week 26). Cut slides for

PMS2 included 5 PMS 2 intact cases and stability was tested to 8 weeks. The anti-MMR panel antibody stained slides were evaluated by pathologist for MMR protein status (Intact/Loss), stain intensity and background. The negative reagent control stained slides were also evaluated. The study required that the stain intensity will not vary by more than 1.0 point when compared to Day 0 scores for a minimum of four weeks after storage at 5±3°C and at 30±5°C. Table 16 lists specimen stability as cut sections stored at 5±3°C and at 30±5°C for each of the panel markers. The specimen block stability claim is the same as the cut-section stability claim.

Table 16. Cut Section Stability by marker

Marker	Cut section stability*
MLH1	26 Weeks
PMS2	8 weeks
MSH2	26 Weeks
MSH6	26 Weeks
BRAF V600E	26 Weeks

* When Stored at 5±3°C and at 30±5°C

d. Detection Limit:
Not applicable

e. Analytical specificity:

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

i. Western Blot and IHC: Western blots analyses were conducted to demonstrate that the antibodies specifically detect the proteins of predicted molecular weight for each of the 5 Ventana MMR panel antibodies using cell lines with known MMR loss or intact status. Cell lines used in the study were matched pair of human cell line HD PAR595 that expressed wild type MMR proteins or were engineered with frame shift knockouts for PMS2 and MLH1 and complete knockout for MSH6 and MSH2. BRAFV600E containing cell lines were engineered to express moderate and high levels of the V600E protein. Western Blots confirmed presence of reactive bands at expected molecular weighs for each of the 5 panel markers.

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

The combined results from western blots and cell line IHC demonstrated

specific antibody reactivity for each of the 5 markers included in the Ventana MMR IHC panel.

ii. Immunoreactivity: Immunoreactivity testing to demonstrate Ventana MMR panel and BRAFV600 E antibodies (M1) staining across multiple cases of normal and tumor tissue types was performed on commercially available tissues and tissue arrays were obtained for Tour of Body (TOB) and Tour of Tumor (TOT) studies. Note: Mismatch repair proteins are present in all actively proliferating cells. For all tissues, positive/negative MMR staining was determined for tissue specific elements in the presence of positive staining in normal control cells (lymphocytes, fibroblasts and epithelial cells). For all tissues, BRAF V600E positive/negative staining was determined for tissue specific elements and such cases should not be considered as positive for BRAF V600E Clinical Status. The summary of staining results with the panel antibodies is shown in Table 17 and Table 18.

Table 17. Tour of Body Immunoreactivity (Normal Tissue)

	Positive/Total Cases				
Tissue	**MLH1**	**PMS2-**	**MSH2**	**MSH6**	**BRAF V600E**
Adrenal Gland	3/3	3/3	3/3	3/3	0/3
Bladder	3/3	3/3	3/3	3/3	0/3
Bone Marrow	3/3	3/3	3/3	3/3	0/3
Ovary	5/5	4/4	5/5	5/5	0/3
Breast	3/3	3/3	3/3	3/3	0/3
Cerebellum	3/3	3/3	3/3	3/3	1/3*
Cerebrum	3/3	3/3	3/3	3/3	0/3
Cervix	3/3	3/3	3/3	3/3	0/3
Colon	3/3	3/3	3/3	3/3	5/12*
Endometrium	3/3	3/3	3/3	2/3	0/3
Esophagus	3/3	3/3	3/3	3/3	0/3
Heart	3/3	2/3	1/3	3/3	0/3
Hypophysis	3/3	3/3	3/3	3/3	3/3**
Intestine	3/3	3/3	3/3	3/3	2/4*
Kidney	3/3	3/3	3/3	3/3	0/3
Liver	3/3	3/3	3/3	3/3	0/3
Lung	4/4	3/3	3/3	4/4	0/3
Lymph node	3/3	3/3	3/3	3/3	0/3
Mesothelium	4/4	2/3	3/3	3/3	0/3
Pancreas	3/3	3/3	3/3	3/3	2/3*
Parathyroid Gland	3/3	3/3	3/3	3/3	0/3
Peripheral Nerve	5/5	4/4	5/5	5/5	0/5
Prostate	3/3	3/3	3/3	3/3	0/3
Skeletal Muscle	3/3	2/3	3/3	3/3	0/3

Skin	3/3	3/3	3/3	3/3	0/3
Spleen	3/3	3/3	3/3	3/3	0/3
Stomach	3/3	3/3	3/3	3/3	0/3
Testis	3/3	3/3	3/3	3/3	2/3*
Thymus	3/3	3/3	3/3	3/3	0/3
Thyroid	4/4	4/4	3/3	4/4	0/3
Tongue/Salivary Gland	3/3	3/3	2/3	3/3	0/3
Tonsil	3/3	3/3	3/3	3/3	0/3

For BRAF V600E, *Weak cytoplasmic and nuclear staining in Purkinje cells of cerebellum, smooth muscle and epithelial cells of normal colon, glandular cells of intestine, acinar structures of pancreas, and interstitial cells of testis.
**Moderate staining observed in neuroendocrine cells in hypophysis.

Table 18. Ventana MMR IHC Panel Staining in Tumor Tissues

Pathology	MLH1-positive / total cases	PMS2-positive / total cases	MSH2-positive / total cases	MSH6-positive / total cases	BRAF V600E-positive / total cases
Glioblastoma	1/1	1/1	1/1	1/1	0/1
Atypical meningioma	1/1	n.e.*	1/1	1/1	0/1
Malignant ependymoma	1/1	1/1	1/1	1/1	0/1
Malignant oligodendroglioma	1/1	1/1	1/1	1/1	0/1
Serous adenocarcinoma (ovary)	1/1	1/1	1/1	1/1	0/1
Adenocarcinoma (ovary)	1/1	1/1	1/1	1/1	0/1
Islet cell carcinoma	1/1	1/1	1/1	1/1	0/1
Adenocarcinoma of pancreas	n.e.	n.e.	1/1	n.e	0/1
Seminoma	2/2	2/2	2/2	2/2	0/2
Thyroid medullary carcinoma	1/1	1/1	1/1	1/1	0/1
Thyroid papillary carcinoma	1/1	1/1	1/1	1/1	3/3
Intraductal carcinoma (breast)	1/1	1/1	1/1	1/1	0/1
Intraductal carcinoma with early infiltrate (breast)	1/1	1/1	1/1	1/1	0/1
Invasive ductal carcinoma (breast)	1/1	1/1	1/1	1/1	1/1
Diffuse B-cell lymphoma	1/1	n.e.	1/1	1/1	0/1
Lung small cell undifferentiated carcinoma	1/1	1/1	1/1	1/1	0/1

Pathology	MLH1-positive / total cases	PMS2-positive / total cases	MSH2-positive / total cases	MSH6-positive / total cases	BRAF V600E-positive / total cases
Lung squamous cell carcinoma	1/1	1/1	1/1	1/1	0/1
Neuroendocrine carcinoma (esophagus)	1/1	1/1	1/1	1/1	0/1
Adenocarcinoma (esophagus)	1/1	n.e.	1/1	1/1	0/1
Signet ring carcinoma	1/1	1/1	1/1	1/1	0/1
Adenocarcinoma (small intestine)	1/1	1/1	1/1	1/1	0/1
Stromal sarcoma (small intestine)	1/1	1/1	1/1	1/1	1/1
Adenocarcinoma (colon)	1/1	1/1	1/1	1/1	0/1
Interstitialoma (abdominal cavity)	1/1	n.e.	n.e.	n.e.	0/1
Adenocarcinoma (rectum)	1/1	1/1	1/1	1/1	0/1
Moderate malignant interstitialoma (rectum)	1/1	1/1	1/1	1/1	0/1
Hepatocellular carcinoma	n.e.	n.e.	n.e.	1/1	0/1
Hepatoblastoma	1/1	1/1	1/1	1/1	0/1
Clear cell carcinoma (kidney)	1/1	1/1	1/1	1/1	0/1
Adenocarcinoma (Prostate, Gleason grade:4, Gleason score:4+5)	1/1	1/1	1/1	1/1	0/1
Adenocarcinoma (Prostate)	1/1	n.e.	1/1	1/1	0/1
Leiomyoma (uterus)	1/1	n.e.	1/1	n.e.	n.e.
Adenocarcinoma (uterus)	1/1	n.e.	1/1	1/1	0/1
Clear cell carcinoma (endometrium)	n.e.	n.e.	n.e.	1/1	n.e.
Squamous cell carcinoma (cervix)	2/2	1/1	2/2	2/2	0/2
Embryonal rhabdomyosarcoma of left leg	1/1	1/1	1/1	1/1	0/1
Squamous cell carcinoma of chest wall	1/1	1/1	1/1	1/1	0/1
Neurofibroma	1/1	n.e.	n.e.	n.e.	0/1
Neuroblastoma	1/1	1/1	1/1	1/1	0/1

Pathology	MLH1-positive / total cases	PMS2-positive / total cases	MSH2-positive / total cases	MSH6-positive / total cases	BRAF V600E-positive / total cases
Malignant mesothelioma	1/1	1/1	1/1	1/1	0/1
Diffuse B-cell lymphoma of lymph node	1/1	1/1	1/1	1/1	0/1
Diffuse B-cell lymphoma of right thigh	1/1	1/1	1/1	1/1	0/1
Hodgkin's lymphoma left groin	1/1	1/1	1/1	1/1	1/1
Transitional cell carcinoma of bladder	n.e.	n.e.	n.e.	n.e.	0/1
Low grade malignant leiomyosarcoma (bladder)	1/1	1/1	1/1	1/1	0/1
Osteosarcoma of right femur	1/1	1/1	1/1	1/1	0/1
Spindle cell rhabdomyosarcoma	1/1	n.e.	n.e.	1/1	0/1
Moderate malignant leiomyosarcoma of left buttock	1/1	1/1	1/1	1/1	0/1

Note: Mismatch repair proteins are present in all actively proliferating cells. For all tissues, MMR positive/negative staining was determined for tumor cells in the presence of positive staining in normal control cells (lymphocytes, fibroblasts and epithelial cells).
For BRAF V600E, for all tissues, BRAF V600E positive/negative staining was determined for tumor cells.
*n.e.: non-evaluable either due to tissue loss or lack of internal control staining.

f. Assay cut-off:

No Assay cut off is employed in the assessment of MMR status or in the assessment of BRAF V600E protein status in FFPE CRC tissue.

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

The device is only validated for formalin fixed paraffin embedded (FFPE) colorectal cancer tissue.

3. Clinical Performance:

The clinical validity of the Ventana MMR IHC panel was determined in a study that assessed agreement between test results obtained from the Ventana MMR IHC panel and a DNA sequencing panel validated for detecting pathogenic lynch syndrome variants and BRAF V600E mutations in colorectal carcinoma (CRC) specimens. The DNA sequencing panel was used to detect variants in the MMR genes MLH-1, MSH2, PMS2 and MSH6, along with EPCAM, and BRAF gene that are associated with MMR deficiency in CRC. The purpose of the study was to estimate the ability of the panel to correctly aid in the identification of patients needing additional Lynch syndrome genetic testing.

A sequential series of CRC specimens were procured and enriched with a second set of specimens with known Lynch syndrome variants due to the rarity of the variants. Specimens from the two studies were combined and randomized for testing with Ventana MMR IHC panel. Concordance (PPA, NPA and OPA) between the two methods was calculated contingent on the DNA sequencing results. MMR status (Intact/Loss) was stratified by BRAF V600E status and DNA sequencing results were stratified by presence or absence of known pathogenic mutations.

CRC cases were procured and assessed for quality (e.g., presence of tumor and internal control cells) prior to use in the study. Specimens were required to have 50% tumor content to meet specimen requirements for DNA sequencing. Of the sequential CRC cases, 7 cases were excluded from the specimen set due to insufficient viable tumor (i.e., adequate cellularity or lack of tumor content), 3 cases due to misclassification as CRC, and 1 due to clerical error. Following review, 111 sequential cases meeting the study criteria were enrolled into the study. A total of 15 CRC cases with confirmed loss status for MMR protiens by IHC brought the total to 126 specimens. Assessment of the demographic data associated with the study specimens determined that it was consistent intended use population.

Results: Of 126 specimens tested by the IHC panel and DNA sequencing, 7 cases were excluded from final analysis due failure of DNA sequencing. Of the remaining 119, one failed IHC testing. The point estimates for overall agreement between Ventana MMR IHC panel and DNA sequencing were 77.8% PPA, 97.0% NPA and 94.1% OPA. The comparisons of IHC and sequencing status for all specimens with MMR IHC results stratified by BRAFV600E status is summarized in Table 19. Table 20A and Table 20B summarizes results by sequential and enrichment study sets respectively.

Table 19. Agreement between VENTANA MMR IHC Panel Results and DNA Sequencing Results: All Specimens

MMR IHC Panel Results	DNA Sequencing Results			
	Pathogenic Mutation	No Pathogenic Mutation	Invalid	Total

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

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

Note: Invalids are defined as failure to produce results by IHC and/or DNA sequencing. Only Invalids resulting from a failure by IHC are included in the analysis. 95% CIs were calculated using the (Wilson) Score method.

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

Table 20A. Agreement between VENTANA MMR IHC Panel and DNA Sequencing Results: Sequential Cohort

Sequential Study Set					
VENTANA MMR IHC Panel Results		DNA Sequencing Results			
		Pathogenic Mutation	No Pathogenic Mutation	Invalid	Total
MMR Loss	BRAF V600E +	1	18	0	19
	BRAF V600E -	4	2	0	6

Sequential Study Set					
VENTANA MMR IHC Panel Results		**DNA Sequencing Results**			
		Pathogenic Mutation	**No Pathogenic Mutation**	**Invalid**	**Total**
MMR Intact	BRAF V600E +	0	3	1	4
	BRAF V600E -	1	76	5	82
Invalid		0	0	0	0
Total		6	99	6	111

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

Table 20B. Agreement between VENTANA MMR IHC Panel and DNA Sequencing Results: Enrichment Cohort

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

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

Accuracy by MMR proteins:
The concordance for MMR gene mutation status by sequencing and MMR protein loss by IHC was also compared individually. For MLH1 and PMS2 loss cases, results were stratified by BRAF V600E. The OPA of each MMR protein, when compared to the results of the DNA sequencing colon panel, was 95.8% for VENTANA anti-MLH1 (M1) antibody, 94.1% for VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, 98.3% for VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody and 96.6% for VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary antibody.

MLH1/PMS2: A total of 26 cases were identified as MLH-1 and PMS2 loss cases, of which 20 were BRAFV600E positive and therefore sporadic and 19 of these did not carry any pathogenic mutation in the MLH-1 gene. Out of three cases containing a potential pathogenic mutation in the *MLH1* gene, one was also BRAF V600E positive and a low allele frequency in sequencing suggesting sporadic CRC. 3 cases carried potential pathogenic PMS2 mutations. One MLH-1/PMS2 loss case was BRAF V600E negative and had no pathogenic variants for either gene.

MSH2/MSH6: Six specimens with loss for MSH2/MSH6 and one for MSH6 alone were identified by MMR IHC panel. Of these three cases contained potential pathogenic mutation in MSH2 gene and 3 in MSH6 gene and the one MSH6 alone case carried potential pathogenic mutation in MSH6. Additional four cases that contained a potential pathogenic mutation affecting MSH6 expression demonstrated MSH6 Intact status by IHC. Of these, two contained *POLE* mutations which variably affect the expression of MMR protein and do not represent Lynch syndrome mutations. One case demonstrates MSH6 IHC staining in a small portion of the tumor and was designated intact, but DNA sequencing showed several mutations in the *MSH6* gene which likely result from somatic mutation.

BRAF V600E: The ability of the VENTANA anti-BRAF V600E (VE1) antibody to stratify CRC cases showing a loss of MLH1 protein expression was verified in the study. Of the 23 positive BRAF V600E cases, 20 cases had loss of MLH1 protein by IHC. The remaining three cases were pMMR (intact for all MMR proteins). All 23 BRAF V600E positive specimens were identified as sporadic CRC and were confirmed to carry the V600E mutation by sequencing. The results verified that the VENTANA anti-BRAF V600E (VE1) antibody can correctly differentiate between sporadic and probable Lynch syndrome CRC in the absence of MLH1 expression.
A breakdown of the agreements between the MMR IHC status-stratified by BRAFV600E results for MLH-1 and PMS2 loss cases- and DNA sequencing results for the MMR genes by individual MMR marker is captured in table 21A and in 21B and 21C summarizes agreement by marker for sequential and enrichment study set respectively.

Table 21A. Agreement between each protein in the VENTANA MMR IHC Panel and DNA Sequencing Results all specimens.

IHC Results	DNA Sequencing Results

		Pathogenic Mutation	No Pathogenic Mutation	Total
MLH1 Loss	BRAF V600E +	1	19	20
	BRAF V600E -	2	4	6
MLH1 Intact		0	92	92
Total		3	115	118
PMS2 Loss	BRAF V600E +	0	20	20
	BRAF V600E -	3	7	10
PMS Intact		0	88	88
Total		3	115	118
MSH2 Loss		3	2	5
MSH2 Intact		0	113	113
Total		3	115	118
MSH6 Loss		4	0	4
MSH6 Intact		4	110	114
Total		8	110	118

Agreement				
Protein	Type	n/N	%	95% CI
MLH1	PPA	2/3	66.7	(20.8, 93.9)
	NPA	111/115	96.5	(91.4, 98.6)
	OPA	113/118	95.8	(90.5, 98.2)
PMS2	PPA	3/3	100.0	(43.9, 100.0)
	NPA	108/115	93.9	(88.0, 97.0)
	OPA	111/118	94.1	(88.3, 97.1)
MSH2	PPA	3/3	100.0	(43.9,100.0)
	NPA	113/115	98.3	(93.9,99.5)
	OPA	116/118	98.3	(94.0,99.5)

IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MSH6	PPA	4/8	50.0	(21.5,78.5)
	NPA	110/110	100.0	(96.6,100.0)
	OPA	114/118	96.6	(91.6, 98.7)

Table 21B. Agreement by Marker for Sequential Cohort

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

Agreement				
Protein	Type	n/N	%	95% CI
MLH1	PPA	1/2	50.0	(9.5, 90.5)
	NPA	99/103	96.1	(90.4, 98.5)

Sequential Study Set				
IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
	OPA	100/105	95.2	(89.3, 97.9)
PMS2	PPA	n.e.	n.e.	n.e.
	NPA	100/105	95.2	(89.3, 97.9)
	OPA	100/105	95.2	(89.3, 97.9)
MSH2	PPA	1/1	100.0	(20.7,100.0)
	NPA	104/104	100.0	(96.4, 100.0)
	OPA	105/105	100.0	(96.5, 100.0)
MSH6	PPA	0/3	0.0	(0.0, 56.1)
	NPA	102/102	100.0	(96.4,100.0)
	OPA	102/105	97.1	(91.9, 99.0)

Table 21C. Agreement by Marker for Enrichment set

Enrichment Study Set				
IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MLH1 Loss	BRAF V600E +	0	1	1
	BRAF V600E -	1	0	1
MLH1 Intact		0	11	11
Total		1	12	13
PMS2 Loss	BRAF V600E +	0	1	1
	BRAF V600E -	3	2	5
PMS Intact		0	7	7
Total		3	10	13
MSH2 Loss		2	2	4
MSH2 Intact		0	9	9
Total		2	11	13

Enrichment Study Set				
IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MSH6 Loss		4	0	4
MSH6 Intact		1	8	9
Total		5	8	13
Agreement				
Protein	Type	n/N	%	95% CI
MLH1	PPA	1/1	100.0	(20.7, 100.0)
	NPA	12/12	100.0	(75.8, 100.0)
	OPA	13/13	100.0	(77.2, 100.0)
PMS2	PPA	3/3	100.0	(43.9, 100.0)
	NPA	8/10	80.0	(49.0, 94.3)
	OPA	11/13	84.6	(57.8, 95.7)
MSH2	PPA	2/2	100.0	(34.2,100.0)
	NPA	9/11	81.8	(52.3, 94.9)
	OPA	11/13	84.6	(57.8, 95.7)
MSH6	PPA	4/5	80.0	(37.6, 96.4)
	NPA	8/8	100.0	(67.6,100.0)
	OPA	12/13	92.3	(66.7, 98.6)

b. Other Clinical supportive data:

Testing CRC patients for possible Lynch Syndrome is well-established as part of the clinical management of these patients and is included in National comprehensive cancer network (NCCN) guidelines for newly diagnosed patients. Ventana submitted literature to support clinical validity of IHC testing of the MMR marker panel consisting of MLH1, PMS2, MSH2, MSH6 and BRAF V600E IHC as an aid in detection of mismatch repair protein deficiency and BRAFV600E status as aid to differentiate between sporadic and probable lynch syndrome identifying

A total of 154 papers were identified as relevant for supporting the clinical validity of MMR IHC test in CRC as aid in Lynch syndrome diagnoses.

The measured sensitivity in these studies to detect germline MLH1, MSH2, MSH6 and PMS2 mutations by IHC (not represented by this panel) was 92% (23/25), 93% (28/30), 100% (8/8) and 100% (3/3), respectively, when data from studies evaluating all 4 MMR

proteins were analyzed. This data supports the use of a four antibody MMR IHC panel as a screening tool for potential Lynch syndrome patients.

BRAF V600E mutation IHC to distinguish Sporadic and Germline CRC:
In 550 MMR mutation carriers, the BRAF V600E mutation frequency was only 1.4% (95% CI: 0.06-2.52). The frequency of BRAF V600E mutations was 5% (95% CI: 3.6-6.9) in 1,623 microsatellite stable (MSS) cases, 36.1% (95% CI: 21.0-52.8) in MSI-H cases without MMR mutations, and 63.5% (95% CI 447.0-78.5) in 332 cases demonstrating MLH1 methylation or MLH1 expression loss.

Conclusion: Literature survey shows that IHC test that include the 4 dMMR markers can identify MSI-H /dMMR phenotype in CRC subjects who can be directed to additional testing for Lynch syndrome diagnoses. The survey presented also shows the effectiveness of BRAF V600E IHC in distinguishing sporadic and germline CRC patients.

4. Clinical cut-off**:**

   The MMR IHC panel and BRAF V600E tests are qualitative in nature and marker status (loss / intact or positive/negative) are determined based on unequivocal staining of FFPE CRC tissue (see section I on staining interpretation and scoring) and do not rely on discreet clinical cut off.

5. Expected values/Reference range:

   Not applicable.

N. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

O. Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

P. Identified Risks to Health and Identified Mitigations

Identified Risks to Health	Identified Mitigations
False positive test result	General controls and special controls (1) and (2)
False negative test result	General controls and special controls (1) and (2)

Q. **Benefit/Risk Determination**

Summary	
Summary of the Benefit(s)	This kit has significant benefit as an adjunct to the physician's evaluation of suspected Lynch syndrome as indicated by the guidelines and as demonstrated in analytical and clinical performance studies. IHC testing for MMR deficiency in CRC patients is a sensitivity method for identification of Lynch syndrome and can lead to better patient management and outcomes.

Summary of the Risk(s)	There are risks associated with false negative and false positive results. However, the risks associated with these misclassifications have to be taken into the context that the test is intended to be used as an adjunct to physician's evaluation of suspected of having Lynch syndrome along with additional clinic-pathological factors and is not intended to be used as a standalone diagnostic. False positive test results for the loss of MMR proteins will result in additional testing for the patients and the family. The risk of a false positive test result is mitigated by special control (1)(iv) that requires identification and inclusion of appropriate positive and negative controls to in the test to ensure accurate performance and is further mitigated by the demonstrated analytical accuracy of the device. Furthermore the test is intended to use as an adjunct to identify patients who will benefit from additional testing, thereby minimizing the risk. A false negative test result can result in missing additional testing and failure to identify Lynch syndrome. In clinical practice, if the clinical or pathological features suggestive of Lynch syndrome still remains high, then the pathologist performs PCR testing, thereby negating the false negative result of this test. The false negative rates in the study were low in the accuracy study except in the case of MSH6, with 4 cases of MSH6 mutation carrying specimens testing as MSH6 intact in the study. However it was determined that 3 of the four were due to somatic mutations of which 2 resulted from mutation to an MMR unrelated POLE genes. Mutations in POLE genes are associated with secondary somatic alteration in MSH6 with no loss in protein expression. The risk of false negatives is mitigated by demonstrated performance of the test.

Conclusions **Do the probable benefits outweigh the probable risks?**	The probable clinical benefits of this device outweigh the potential risks in light of the special controls established for this device type, in combination with applicable general controls, including design controls.

R. Conclusion

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 864.1866. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PZJ
Device Type: Lynch syndrome test systems
Class: II (special controls)
Regulation: 21 CFR 864.1866

(a) Identification: Lynch syndrome test systems are in vitro diagnostic tests for use with tumor tissue to identify previously diagnosed cancer patients at risk for having Lynch syndrome.

(b) Classification: Class II (special controls). A Lynch syndrome test system must comply with the following special controls:

(1) Premarket notification submissions must include the following information, as appropriate:

(i) A detailed description of all test components, including all provided reagents, and required but not provided, ancillary reagents.

(ii) A detailed description of instrumentation and equipment, including illustrations or photographs of non-standard equipment or manuals.

(iii) Detailed documentation of the device software, including, but not limited to, standalone software applications and hardware-based devices that incorporate software.

(iv) A detailed description of quality controls including appropriate positive and negative controls that are recommended or provided.

(v) Detailed specifications for sample collection, processing, and storage.

(vi) A detailed description of methodology and assay procedure.

(vii) A description of the assay cut-off (i.e., the medical decision point between positive and negative results) or other relevant criteria that distinguishes positive and negative results, or ordinal classes of marker expression, including the rationale for the chosen cut-off or other relevant criteria and results supporting validation of the cut-off.

(viii) Detailed specification of the criteria for test result interpretation and reporting.

(ix) Detailed information demonstrating the performance characteristics of the device, including:

(A) Data from an appropriate study demonstrating clinical accuracy using well-characterized clinical specimens representative of the intended use population (i.e., concordance to DNA sequencing results of the Lynch syndrome associated genes or method comparison to the predicate device using samples with known alterations in genes representative of Lynch syndrome). Pre-specified acceptance criteria must be provided and followed.

(B) Appropriate device reproducibility data investigating all sources of variance (e.g., for distributed tests, data generated using a minimum of three sites, of which at least two sites must be external sites). Each site must perform testing over a minimum of 5 nonconsecutive days evaluating a sample panel that spans the claimed measuring range, and includes the clinical threshold. Pre-specified acceptance criteria must be provided and followed.

(C) Data demonstrating reader reproducibility, both within-reader and between-reader, assessed by three readers over three nonconsecutive days at each site, including a two week washout period between reads, as appropriate.

(D) Device precision data using clinical samples spanning the measuring range and controls to evaluate the within-lot, between-lot, within-run, between run, and total variation.

(E) Analytical specificity studies including as appropriate, western blots, peptide inhibition, testing in normal tissues and neoplastic tissues, interference by endogenous and exogenous substances, and cross-reactivity and cross contamination testing.

(F) Device analytical sensitivity data generated by testing an adequate number of samples from individuals with the target condition such that prevalence of the biomarker in the target population is established.

(G) Device stability data, including real-time stability and in-use stability, and stability evaluating various storage times, temperatures, and freeze-thaw conditions, as appropriate.

(H) The staining performance criteria assessed must include overall staining acceptability, background staining acceptability, and morphology acceptability, as appropriate.

(I) Appropriate training requirements for users, including interpretation manual,

as applicable.

(J) Identification of risk mitigation elements used by the device, including a description of all additional procedures, methods, and practices incorporated into the instructions for use that mitigate risks associated with testing.

(2) The device's 21 CFR 809.10(b) compliant labeling must include a detailed description of the protocol, including the information described in paragraphs (b)(1)(i) through (b)(1)(viii) of this section, as appropriate, and a detailed description of the performance studies performed and the summary of the results, including those that relate to paragraph (b)(1)(ix) of this section, as appropriate.