**H P T Dilrukshi**

**AGE:** 39 years | **Gender:** Female | **Coupon/UID:** NA / AOMG098

**Diagnosis:** Colorectal Neoplasms

MEDGENOME

**MGM527**: Microsatellite Instability (MSI) by fragment analysis

| Report Details | | Specimen Information | | Ordering Clinician | |
|---|---|---|---|---|---|
| **Sample ID / Order ID:** | 9061894 / 1253338 | **Specimen Site:** | Colon | **Clinician:** | Dr. Mahilal Wijekoon |
| **Collection Date:** | NA | **Specimen Received:** | FFPE Tissue Blocks [1] | **Affiliation:** | Aegle Omics Private Limited |
| **Date Received:** | 5th April 2025 | **Specimen Tested:** | 1218/25 block | **Serviced By:** | 18718 |
| **Report Date & Time:** | 12th Apr 2025 16:53 PM | **Tumor Content (%):** | 65 | **Report Status:** | Final |

**Clinical Summary:**Moderately differentiated adenocarcinoma of sigmoid colon, Stage IIA

## TEST RESULT SUMMARY

Microsatellite Instability (MSI) Status -Stable


| Summary of Markers | |
|---|---|
| Count of markers reported Unstable | 0 |
| Count of markers reported Stable | 13 |
| Reported Unstable Rate | 0.00% |
| Unstable Markers | None |

## CLINICAL SIGNIFICANCE

- MSI screening has long been recognized as important in the care of patients with colorectal cancer (CRC) or endometrial cancer (EC).
- High-frequency MSI (MSI-H) is also recognized as a potential marker for germline mutations in certain DNA mismatch repair (MMR) genes associated with Lynch syndrome [PMID: 15872200].
- MSI has been found in several cancer types, including non-small cell lung cancer, melanoma, breast cancer, urothelial cancer, pancreatic ductal adenocarcinoma and brain cancer. The expansion of MSI clinical trials into other cancers may elucidate the prognostic and predictive value of MSI for non-colorectal [PMID: 35955855].
- NCCN® guidelines recommend universal screening for 15+ different cancer types by MSI and/or IHC analysis [www.nccn.org]
- MSI-H status is predictive of a positive response to immunotherapies such as immune checkpoint blockade inhibitors [PMID: 26028255]
- The 2015 paper by Le et al. reported the extended analysis on the efficacy of PD-1 blockade in patients with advanced mismatch repair-deficient cancers of both colorectal cancer and non-colorectal origins. Following 41 patients, the study found that patients with mismatch repair deficient tumors, experienced an objective response rate of 40% and a progression-free survival rate of 78%. In contrast, the objective response rate was 0% and the progression-free survival rate was 11% for mismatch repair-proficient
- The College of American Pathologists (CAP),in collaboration with the Association of Molecular Pathology (AMP),American Society of Clinical Oncology (ASCO), and patient advocacy group Fight Colorectal Cancer (Fight CRC) convened a multidisciplinary expert and advisory panel to develop evidence-based guidelines to identify the optimal clinical laboratory test to identify defects in DNA mismatch repair (dMMR) in patients with solid tumor malignancies who are being considered for immune checkpoint inhibitor (ICI) therapy. MSI by PCR was recommended for colorectal cancer, patients with gastroesophageal and small bowel cancer and other solid malignancies [PMID: 35920830]

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- On June 29, 2020, the Food and Drug Administration approved pembrolizumab (KEYTRUDA, Merck & Co.) for the first-line treatment of patients with unresectable or metastatic microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) colorectal cancer [www.fda.gov].
- The FDA approved pembrolizumab on May 23, 2017, for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H), or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options and for the treatment of unresectable or metastatic MSI-H or dMMR colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan [www.fda.gov].

## DISCLAIMER

- **Decisions regarding treatment action plan should not be solely based on these test results. These findings are highly recommended to be correlated with the patient's clinical, pathological, radiological and family history for decisions on diagnosis, prognosis, or therapeutics.**
- The therapy information provided in this report is based onFDA approved drugs data, NCCN guidelines, peer-reviewed published literature, standard clinical databases, and strength of biomarker results. These therapies may or may not be suitable/beneficial to a particular patient. This clinical report summarizes potentially effective medications, potentially ineffective medications, and medications that may pose a higher risk of adverse reactions by mapping the patient's genetic alterations to the biomedical reference information. The report may also provide prognostic and diagnostic biomarkers detected or shown for the given disease context.
- The identification of a genomic biomarker does not necessarily imply pharmacological effectiveness or ineffectiveness. The medications identified by the treating physician may or may not be suitable for use on a particular patient. Thus, the clinical report does not guarantee that any particular agent will be effective in the treatment of any particular condition. Also, the absence of a treatment option does not determine the effectiveness or predict an ineffective or safety-relevant effect of a medication selected by the treating physician.
- Due to poor quality of FFPE tissue blocks, the QC parameters for extracted DNA may not pass to proceed further with the testing, therefore there is a possibility of assay failure or compromised. However, sample status in such scenarios shall be sent through mail to the ordering clinician.
- This test has been validated at MedGenome Labs as per the CAP guidelines with 100% sensitivity and specificity.
- The results of this test are dependent on the tumor content in the tissue sample provided. A minimum of >10% tumour content is required for a successful testing.
- In case of MSI negative or MSS patients, if there is a co-existing strong personal or family history of HNPCC related cancers for this patient, consider microsatellite instability and IHC testing on a different tumor block to further evaluate the possible role of defective DNA mismatch repair.
- **Additional case specific disclaimer: None**

## TEST METHODOLOGY

This assay detects the presence of microsatellite instability (MSI) in DNA samples through multiplex PCR [1] and fragment analysis and screens for 13 mononucleotide markers listed in table below. Mononucleotide markers like BAT-25, BAT-26 and BAT-40 markers are selected as per the NCI guidelines. A revised guidelines suggests mononucleotide marker panel is more sensitive for MSI-H tumors than other microsatellite markers. Dinucleotide markers are less sensitive, and if only dinucleotide markers are positive, it is mandatory to test additional mononucleotide markers to rule out MSI-L [PMID: 14970275].This kit contains 13 mononucleotide markers for higher resolution and two STR sequences that can be used to track sample identity [PMID: 35884597][PMID: 35982978].

| | | | | | | |
|---|---|---|---|---|---|---|
| ABL-16 | ABL-19 | ABL-20B | BAT-26 | CAT-25 | NR-22 | NR-27 |
| ABL-17 | ABL-20A | BAT-25 | BAT-40 | NR-21 | NR-24 | |

The primers are fluorophore tagged at the 5' end and the end-point PCR product is analyzed by Fluorophore Capillary Electrophoresis. The tumor

tissue is classified as MSS/MSI-L/MSI-H as mentioned in the table below.

| MSI Result | **Interpretation**[PMID: 35884597] |
|---|---|
| **MSI-High** | Unstable marker rate:- 30% - 100% |
| **MSI-Low** | Unstable marker rate:- 5% - 29.99% |
| **MSS(Microsatellite Stable)** | Unstable marker rate:- 0% |

## RECOMMENDATION

Test results should be interpreted in context of clinical findings, family history, and other laboratory data. If results obtained do not match other clinical or laboratory findings, please contact the laboratory for possible interpretation. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

## REFERENCE

1. Application note: TrueMark MSI Assay—a simplified solution for analyzing microsatellite instability in FFPE tumor samples, 2020.

Dr Syed Muqlisur Rehman, MD
Molecular Pathologist
KMC Registration No. 71468

**END OF REPORT**

MEDGENOME

Medgenome Labs Ltd
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Attibele Hobli, Electronic City, Phase-1, Bangalore,
Karnataka, 560100.

# MMR by Immunohistochemistry - Test Report

| | | | |
|---|---|---|---|
| Patient Name | H P T Dilrukshi | Order ID | 1253338 |
| Age / Gender | 39 Years / Female | Sample ID | 9061894 |
| Physician | Dr. Mahilal Wijekoon | Collection Date | NA |
| Customer | MCC18718-Aegle Omics Private Limited | Sample Received Date | 05-04-2025 12:35 PM |
| Report Date | 10-04-2025 09:20 AM | Report Status | Final |

**Lab/Biopsy No** : MBH-1872/25

**Clinical Details** : Ca. Sigmoid colon. IIA.

**Specimen received** : 1 block. Colectomy specimen block.

**Gross examination** : Received one paraffin block bearing No. 1218 / 25 - 3. From Aegle Omics Private Limited. For IHC.

**Immunohistochemistry (IHC) Testing for Mismatch Repair (MMR) Proteins.**

**Immunohistochemistry Microscopy :**

| IHC Markers | Pattern of expression |
|---|---|
| **1. MLH1** | Intact nuclear expression |
| **2. MSH2** | Intact nuclear expression |
| **3. MSH6** | Intact nuclear expression |
| **4. PMS2** | Intact nuclear expression |

Background nonneoplastic tissue / internal control with intact nuclear expression : Yes

## Impression:

**Intact DNA mismatch repair (MMR) function within tumor (MMR proficient).**

## Note:

Appropriate dual control tissue run with the test is satisfactory. Reagents used are as follows **Anti MLH1( GM011 clone)** mouse monoclonal antibody , **Anti MSH2 (RED2 clone)** rabbit monoclonal antibody , **Anti-MSH6 (EP49 clone)** rabbit monoclonal antibody , **Anti PMS2 (EP51 clone)** Rabbit monoclonal antibody .

This assay has not been validated on decalcified tissue and result should be interpreted with caution given the likelihood of false negativity of decalcified specimen. Specimen should be processed by routine tissue processing method. Inappropriate fixation (nonformalin) and processing may give erroneous result. The performance characteristics of these assays have been determined by MedGenome. Performance characteristics refer to the analytical performance of the test.

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## Interpretation of IHC MMR:

**No loss of nuclear expression of mismatch repair (MMR) proteins : Low probability of microsatellite instability-high (MSI-H).**

Loss of nuclear expression of MLH1 and PMS2: testing for methylation of the MLH1 promoter and/or mutation of BRAF is indicated (the presence of a BRAF V600E mutation and/or MLH1 methylation suggests that the tumor is sporadic and germline evaluation is probably not indicated; absence of both MLH1 methylation and of BRAF V600E mutation suggests the possibility of Lynch syndrome, and sequencing and/or large deletion/duplication testing of germline MLH1 may be indicated)*

Loss of nuclear expression of MSH2 and MSH6: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline MSH2 may be indicated, and, if negative, sequencing and/or large deletion/duplication testing of germline MSH6 may be indicated)*

Loss of nuclear expression of MSH6 only: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline MSH6 may be indicated)*

Loss of nuclear expression of PMS2 only: high probability of Lynch syndrome (sequencing and/or large deletion/duplication testing of germline PMS2 may be indicated)*

* There are exceptions to the above IHC interpretations. These results should not be considered in isolation, and clinical correlation with genetic counseling is recommended to assess the need for germline testing.

## Explanatory Notes

### Mismatch Repair Testing: Microsatellite instability and Immunohistochemistry

Detection of defective mismatch repair in colorectal carcinomas is important for detection of Lynchsyndrome (hereditary nonpolyposis colorectal cancer syndrome [HNPCC]), which accounts for approximately 2% to 3% of all colorectal carcinomas and has clinical implications for treatment of the affected patient and family members. Microsatellite instability (MSI) testing can be used to cost-effectively screen colorectal cancer patients for possible Lynch syndrome. Patients with a microsatellite instability-high (MSI-H) phenotype that indicates mismatch repair deficiency in their cancer may have a germline mutation in one of several DNA mismatch repair (MMR) genes (eg, MLH1, MSH2, MSH6, or PMS2) or an altered EPCAM (TACSTD1) gene. After appropriate genetic counseling, patients may want to consider testing to identify the causative heritable abnormality.

An MSI-H phenotype is more frequently observed in sporadic colorectal cancer (about 15% of cases) due to somatic abnormalities, usually hypermethylation of the MLH1 gene promoter. The specificity of MSI testing can be increased by using it primarily on at-risk populations, such as colorectal cancer patients younger than 50 years, or patients with a strong family history of Lynch associated tumors (eg, colorectal, endometrial, gastric, or upper urinary tract urothelial carcinoma), but with sacrifice of sensitivity, since a sizeable minority of cases lacks these clinical characteristics.

MSI testing of tumor DNA is generally performed with at least 5 microsatellite markers, generally mononucleotide or dinucleotide repeat markers. In 1998, a National Institutes of Health consensus panel proposed that laboratories use a 5-marker panel consisting of 3 dinucleotide and 2 mononucleotide repeats for MSI testing. Recent data suggests that dinucleotide repeats may have lower sensitivity and specificity for identifying tumors with an MSI-H phenotype. As a consequence, there has been a move towards including more mononucleotides and fewer dinucleotides in MSI testing panels. Many laboratories now use a commercially available kit for MSI testing that utilizes 5 mononucleotide markers.

MSI testing is frequently done in conjunction with immunohistochemical (IHC) testing for DNA MMR protein expression (ie, MLH1, MSH2, MSH6, and PMS expression). If DNA MMR IHC has not been performed, this testing should be recommended for any case that shows an MSI-H phenotype, because this information will help identify the gene that is most likely to have a germline mutation (eg, a patient whose tumor shows loss of MSH2 and MSH6

## MMR by Immunohistochemistry - Test Report

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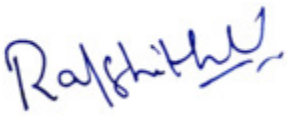
expression, but retention of MLH1 and PMS2 expression, is likely to have an MSH2 germline mutation). If the results of DNA MMR IHC and MSI testing are discordant (eg, MSI-H phenotype with normal IHC or abnormal IHC with MSS phenotype), then the laboratory should make sure that the same sample was used for MSI and IHC testing and that there was no sample mix-up. However, MSI-H may not occur in colorectal cancers of patients with germline MSH6 mutation.

Intact expression of all 4 proteins indicates that MMR enzymes tested are intact but does not entirely exclude Lynch syndrome, as approximately 5% of families may have a missense mutation (especially in MLH1) that can lead to a nonfunctional protein with retained antigenicity. Defects in lesser-known MMR enzymes may also lead to a similar result, but this situation is rare.

Any positive reaction in the nuclei of tumor cells is considered as intact expression (normal), and it is common for intact staining to be somewhat patchy. An interpretation of expression loss in tumor cells should be made only if a positive reaction is seen in internal control cells, such as the nuclei of stromal, inflammatory, or nonneoplastic epithelial cells. Loss of expression of MLH1 may be due to Lynch syndrome or methylation of the MLH1 promoter region (as occurs in sporadic MSI colorectal carcinoma). Genetic testing is ultimately required for this distinction, although a specific BRAF gene mutation (V600E) is present in many sporadic cases, but not familial cancers. Loss of MSH2 expression strongly suggests Lynch syndrome. PMS2 loss is often associated with loss of MLH1 and is only independently meaningful if MLH1 is intact. MSH6 is similarly related to MSH2. One should also keep in mind that nucleolar staining or complete loss of MSH6 staining has been described in colorectal cancer cases with prior radiation or chemotherapy, and a significant reduction of MSH6 staining has been described in a small percentage of colorectal carcinomas with somatic mutations of the coding region microsatellites of the MSH6 gene in MLH1/PMS2-deficient carcinomas.

**Enclosed:** 1 block.



**Approved By**

**Dr. Rakshith V**
**Consultant Histopathologist**
**KMC95334**

*****End of Report*****

## CONDITIONS OF LABORATORY TESTING AND REPORTING

Medgenome Labs Ltd, Bangalore, Karnataka, India

- Laboratory results should be used with other clinical information to determine a final diagnosis.
- In case of unexpected test results please contact the laboratory. We will investigate and repeat analysis if possible.
- The medical report must be viewed and reproduced as a whole
- This medical report is not intended for medico-legal purposes.
- The medical report is to be interpreted and used by medical personnel only
- Assays are performed and reported in accordance with the stated schedule.
- There may be circumstances beyond our control that delay results, e.g., invalid assay run.
- The results of a laboratory test are dependent on the quality of the sample as well as the assay procedure.
- A requested test may not be carried out if:
- Sample is insufficient or inappropriate
- Sample quality is unsatisfactory
- Request for testing is withdrawn by the ordering doctor or patient
- There is discord between the labelling of the sample container and the name on the test requisition.
- For any query contact customer support : +91(0)8067154932/33

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