Overview

Useful For
An adjunct to MSI / Microsatellite Instability (MSI), Tumor and IHC / Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor testing, when colon tumor demonstrates microsatellite instability (MSI-H) and loss of MLH1 protein expression, to help distinguish a somatic versus germline event prior to performing expensive germline testing
An adjunct to negative MLH1 germline testing in cases where colon tumor from the same patient demonstrates MSI-H and loss of MLH1 protein expression

Genetics Test Information
If this test is ordered in conjunction with the MLH1 immunostain (IHC / Mismatch Repair [MMR] Protein Immunohistochemistry Only, Tumor) and MSI (MSI / Microsatellite Instability [MSI], Tumor), this test will only be performed when clinically indicated.

Profile Information

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<th>Test ID</th>
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<tbody>
<tr>
<td>PBMLH</td>
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Reflex Tests

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<td>BBRAF</td>
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Additional Tests

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Testing Algorithm

When this test is ordered, BRAF analysis and MLH1 hypermethylation analysis will always be performed. The exception would be if the tissue origin is an endometrial tumor; in those cases only the MLH1 hypermethylation analysis component will be performed.

When this test is ordered, slide review will always be performed at an additional charge.
See Lynch Syndrome Testing Algorithm in Special Instructions.
Special Instructions

- Molecular Genetics: Inherited Cancer Syndromes Patient Information
- Lynch Syndrome Testing Algorithm

Method Name

Methylation-Specific Polymerase Chain Reaction (PCR) and Digital Droplet Polymerase Chain Reaction (ddPCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Advisory Information

This test is **not recommended** as a first-tier screening measure for Lynch syndrome. Please refer to MSI / Microsatellite Instability (MSI), Tumor and IHC / Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor.

This test will only be performed on colon tumors demonstrating loss of MLH1 protein expression.

Necessary Information

Pathology report **must** accompany specimen in order for testing to be performed.

Specimen Required

Specimen Type: Tissue block or slide

Collection Instructions:

1. Submit formalin-fixed, paraffin-embedded tissue block (preferred) or 1 slide stained with hematoxylin and eosin and 10 unstained, nonbaked slides (5 micron-thick sections) of the tumor tissue.

2. Sections should contain both tumor and normal tissue.

Forms

1. Molecular Genetics: Inherited Cancer Syndromes Patient Information (T519) in Special Instructions

2. If not ordering electronically, complete, print, and send an Oncology Test Request (T729) with the specimen.

Reject Due To

| Specimens that have been decalcified (all methods); specimens that have not been formalin-fixed, paraffin-embedded Extracted DNA | Reject |

Specimen Stability Information
Clinical and Interpretive

Clinical Information

Lynch syndrome is an inherited cancer syndrome caused by a germline pathogenic variant in one of several genes involved in DNA mismatch repair (MMR), including MLH1, MSH2, MSH6, and PMS2. There are several laboratory-based strategies that help establish the diagnosis of Lynch syndrome, including testing tumor tissue for the presence of microsatellite instability (MSI-H) and loss of protein expression for any one of the MMR proteins by immunohistochemistry (IHC). It is important to note, however, that the MSI-H tumor phenotype is not restricted to inherited cancer cases; approximately 20% of sporadic colon cancers are MSI-H. Thus, MSI-H does not distinguish between a somatic (sporadic) and a germline (inherited) etiology, nor does it identify which gene is involved. Although IHC analysis is helpful in identifying the responsible gene, it also does not distinguish between somatic and germline defects.

Defective MMR in sporadic colon cancer is most often due to an abnormality in MLH1, and the most common cause of gene inactivation is promoter hypermethylation (epigenetic silencing). A specific alteration in the BRAF gene (V600E) has been shown to be present in approximately 70% of tumors with hypermethylation of the MLH1 promoter. Importantly, the V600E alteration is rarely identified in cases with germline MLH1 pathogenic variants. Thus, direct assessment of MLH1 promoter methylation status and testing for the BRAF V600E alteration can be used to help distinguish between germline etiology and epigenetic/somatic inactivation of MLH1. Tumors that have the BRAF V600E alteration and demonstrate MLH1 promoter hypermethylation are almost certainly sporadic, whereas tumors that show neither are most often caused by an inherited (germline) pathogenic variant.

Although testing for the BRAF V600E alteration and MLH1 promoter hypermethylation are best interpreted together, they are also available separately to accommodate various clinical situations and tumor types. These tests can provide helpful diagnostic information when evaluating an individual suspected of having Lynch syndrome, especially when testing is performed in conjunction with MSI / Microsatellite Instability (MSI), Tumor and IHC / Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor. It should be noted that these tests are not genetic tests, but rather stratify the risk of having an inherited cancer predisposition and identify patients who might benefit from subsequent genetic testing.

See Lynch Syndrome Testing Algorithm in Special Instructions.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided.

Cautions

Testing tumors other than colon (in the evaluation of Lynch syndrome) for BRAF and MLH1 hypermethylation has not been fully evaluated; therefore, other specimens are not accepted.

Colon cancer is relatively common and it is possible for a sporadic colon cancer to occur in a Lynch syndrome family.
Therefore, evaluation of other family members should still be considered in cases with MLH1 promoter hypermethylation and absence of the BRAF V600E alteration if there is high clinical suspicion of Lynch syndrome.

**Clinical Reference**


**Performance**

**Method Description**

A methylation-specific polymerase chain reaction (PCR)-based assay is used to test tumor DNA for the presence of hypermethylation of the MLH1 promoter, based on a modification of the method described by Grady et al (Grady WM, Rajput A, Lutterbaugh JD, Markowitz S: Detection of aberrantly methylated hMLH1 promoter DNA in the serum of patients with microsatellite unstable colon cancer. Cancer Res 2001;61:900), and digital droplet PCR (ddPCR) is used to test for the presence of the V600E alteration within the BRAF gene. (Unpublished Mayo method)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Varies; Varies

**Analytic Time**

7 days

**Maximum Laboratory Time**

14 days

**Specimen Retention Time**

Extracted DNA: 3 months

**Performing Laboratory Location**

Rochester

**Fees and Codes**

Fees
Test Definition: BRMLH
MLH1 Hypermethylation/BRAF Mutation

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
Slide Review

88381-Microdissection, manual

81210-BRAF (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant, if appropriate

81288-MLH1 promoter methylation analysis, if appropriate

LOINC® Information

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