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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<th>Always Performed</th>
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<tbody>
<tr>
<td>PBMLH</td>
<td>MLH1 Hypermethylation/BRAF Mutation</td>
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Reflex Tests

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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 MLH1 hypermethylation analysis will be performed. If an endometrial tumor does not show loss of MLH1 by immunohistochemistry, we could still run BRAF and not do MLH1 hypermethylation.

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**
Polymerase Chain Reaction (PCR)

**NY State Available**
Yes

**Specimen**

**Specimen Type**
Varies

**Advisory Information**

This test is not recommended as a first-tier screening measure for hereditary nonpolyposis colon cancer (HNPCC). Please refer to MSI / Microsatellite Instability (MSI), Tumor and IHC / Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor. Testing will only be performed on colon tumors demonstrating microsatellite instability or immunohistochemistry results indicating loss of MLH1 protein expression. Extracted DNA from tissues is not an acceptable specimen type.

**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**

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

**Specimen Stability Information**
Test Definition: BRMLH
MLH1 Hypermethylation/BRAF Mutation

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Clinical and Interpretive

Clinical Information

Hereditary nonpolyposis colon cancer (HNPCC), also known as Lynch syndrome, is an inherited cancer syndrome caused by a germline mutation in 1 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 HNPPC/Lynch syndrome, including testing tumor tissue for the presence of microsatellite instability (MSI-H) and loss of protein expression for any 1 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) mutation, 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 mutation 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 mutation is rarely identified in cases with germline MLH1 mutations. Thus, direct assessment of MLH1 promoter methylation status and testing for the BRAF V600E mutation can be used to help distinguish between a germline mutation and epigenetic/somatic inactivation of MLH1. Tumors that have the BRAF V600E mutation and demonstrate MLH1 promoter hypermethylation are almost certainly sporadic, whereas tumors that show neither are most often caused by an inherited mutation.

Although testing for the BRAF V600E mutation 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 HNPCC/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 or endometrial for MLH1 hypermethylation has not been fully evaluated, and these specimens are not accepted for testing.

Testing tumors other than colon (in the evaluation of HNPPC) for BRAF 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 an HNPCC family. Therefore, evaluation of other family members should still be considered in cases with \textit{MLH1} promoter hypermethylation and absence of the \textit{BRAF} V600E mutation if there is high clinical suspicion of HNPCC.

**Clinical Reference**


**Performance**

**Method Description**

A PCR-based assay is used to test tumor DNA for the presence of hypermethylation of the \textit{MLH1} promoter and for the presence of the V600E mutation within the \textit{BRAF} gene. The \textit{MLH1} hypermethylation method is a modification of the method described by Grady et al.(Domingo E, Laiho P, Ollikainen M, et al: \textit{BRAF} screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet 2004;41:664-668; Grady WM, Rajput A, Lutterbaugh JD, Markowitz S: Detection of aberrantly methylated \textit{hMLH1} promoter DNA in the serum of patients with microsatellite unstable colon cancer. Cancer Res 2001;61:900)

**PDF Report**

No

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

Weekly; Varies

**Analytic Time**

7 days

**Maximum Laboratory Time**

14 days

**Specimen Retention Time**

Extracted DNA: 3 months

**Performing Laboratory Location**

Rochester

**Fees and Codes**
Test Definition: BRMLH
MLH1 Hypermethylation/BRAF Mutation

Fees
- 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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