Overview

Useful For
As an adjunct to positive hypermethylation in tumor to distinguish between somatic and germline hypermethylation

As an adjunct to negative MLH1 germline testing in cases where colon or endometrial tumor demonstrates microsatellite instability-H (MSI-H) and loss of MLH1 protein expression

Testing Algorithm
See Lynch Syndrome Testing Algorithm in Special Instructions.

Special Instructions
- Molecular Genetics: Inherited Cancer Syndromes Patient Information
- Informed Consent for Genetic Testing
- Lynch Syndrome Testing Algorithm
- Informed Consent for Genetic Testing (Spanish)

Method Name
Polymerase Chain Reaction (PCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Shipping Instructions
Specimen preferred to arrive within 96 hours of draw.

Specimen Required

Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Container/Tube:

Preferred: Lavender top (EDTA) or yellow top (ACD)

Acceptable: Any anticoagulant

Specimen Volume: 3 mL

Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube.

**Forms**

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available in Special Instructions:
   - **Informed Consent for Genetic Testing** (T576)
   - **Informed Consent for Genetic Testing-Spanish** (T826)

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

**Specimen Minimum Volume**

1 mL

**Reject Due To**

All specimens will be evaluated by Mayo Clinic Laboratories for test suitability.

**Specimen Stability Information**

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<th>Time</th>
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**Clinical and Interpretive**

**Clinical Information**

Lynch syndrome/hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant hereditary cancer syndrome associated with germline mutations in the mismatch repair genes, MLH1, MSH2, MSH6, and PMS2. Deletions within the 3-prime end of the EPCAM gene have also been associated with Lynch syndrome/HNPCC, as this leads to inactivation of the MSH2 promoter.

Lynch syndrome/HNPCC is predominantly characterized by significantly increased risks for colorectal and endometrial cancer. The lifetime risk for colorectal cancer is highly variable and dependent on the gene involved. The risk for colorectal cancer associated MLH1 and MSH2 mutations (approximately 50%-80%) is generally higher than the risks associated with mutations in the other Lynch syndrome/HNPCC-related genes and the lifetime risk for endometrial cancer (approximately 25%-60%) is also highly variable. Other malignancies within the tumor spectrum include gastric cancer, ovarian cancer, hepatobiliary and urinary tract carcinomas, and small bowel cancer. The lifetime risks for these cancers are <15%. Of the 4 mismatch repair genes, mutations within the PMS2 gene confer the lowest risk for any of the tumors within the Lynch syndrome/HNPCC spectrum.

Several clinical variants of Lynch syndrome/HNPCC have been defined. These include Turcot syndrome, Muir-Torre syndrome, and homozygous mismatch repair mutations (also called constitutional mismatch repair deficiency syndrome). Turcot syndrome and Muir-Torre syndrome are associated with increased risks for cancers within the tumor spectrum described, but also include brain and central nervous system malignancies and sebaceous carcinomas, respectively. Homozygous mismatch repair mutations, characterized by the presence of biallelic deleterious mutations within a mismatch repair gene, are associated with a different clinical phenotype defined by
hematologic and brain cancers, cafe au lait macules, and childhood colon or small bowel cancer.

There are several strategies for evaluating individuals whose personal or family history of cancer is suggestive of Lynch syndrome/HNPCC. One such strategy involves testing the tumors from suspected individuals for microsatellite instability (MSI) and/or immunohistochemistry (IHC) for the presence or absence of defective DNA mismatch repair. 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 mismatch repair 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.

However, individuals with tumor hypermethylation may additionally have \( MLH1 \) promoter hypermethylation consistent with germline inactivation. Individuals with germline inactivation of \( MLH1 \) by promoter hypermethylation are at an increased risk for Lynch syndrome/HNPCC-related tumors. In contrast to sequence mutations in \( MLH1 \), current evidence suggests that the risk of transmitting germline \( MLH1 \) promoter hypermethylation is <50%.

Reference Values
Interpretive report will be provided.

Interpretation
An interpretive report will be provided.

Cautions
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Errors in our interpretation of results may occur if information given is inaccurate or incomplete.

Clinical Reference


Performance

Method Description
A PCR-based assay is used to test normal DNA for the presence of hypermethylation of the \( MLH1 \) promoter. This is
Test Definition: MLHPB
MLH1 Hypermethylation Analys, Blood

a modification of the method described by Grady et al.(Grady WM, Rajput A, Lutterbaugh JD, Markowitz SD: Detection of aberrantly methylated hMLH1 promoter DNA in the serum of patients with microsatellite unstable colon cancer. Cancer Res 2001;61:900-902)

PDF Report
No

Day(s) and Time(s) Test Performed
Friday; 3 p.m.

Analytic Time
8 days

Maximum Laboratory Time
12 days

Specimen Retention Time
Whole Blood: 2 weeks (if available) Extracted DNA: 3 months

Performing Laboratory Location
Rochester

Fees and Codes

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
81288

LOINC® Information

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<td>MLH1 Hypermethylation Analys, Blood</td>
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## Test Definition: MLHPB
MLH1 Hypermethylation Analys, Blood

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