Overview

Useful For
An adjunct to MSI / Microsatellite Instability (MSI), Tumor and Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor when colon or endometrial 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 or endometrial tumor demonstrates MSI-H and loss of MLH1 protein expression

Additional Tests

<table>
<thead>
<tr>
<th>Test Id</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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<tbody>
<tr>
<td>SLIRV</td>
<td>Slide Review in MG</td>
<td>No, (Bill Only)</td>
<td>Yes</td>
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Testing Algorithm
When this test is ordered, slide review will always be performed at an additional charge.

See Lynch Syndrome Testing Algorithm

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

Method Name
Polymerase Chain Reaction (PCR) Analysis

NY State Available
Yes

Specimen

Specimen Type
Varies

Ordering Guidance
This test is not recommended as a first-tier screening measure for hereditary nonpolyposis colon cancer (HNPCC). Refer to MSI / Microsatellite Instability (MSI), Tumor and Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor.

Testing will only be performed on colon or endometrial tumors demonstrating loss of MLH1 protein expression by
immunohistochemistry.

Mayo's preferred screening test (BRMLH / MLH1 Hypermethylation and BRAF Mutation Analysis, Tumor) includes both MLH1 promoter hypermethylation and BRAF V600E testing.

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 tumor tissue.

**Forms**
1. [Molecular Genetics: Inherited Cancer Syndromes Patient Information](#) (T519)
2. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
   - [Gastroenterology and Hepatology Client Test Request](#) (T728)
   - [Oncology Test Request](#) (T729)

**Reject Due To**

<table>
<thead>
<tr>
<th>Hemo lysis</th>
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<tbody>
<tr>
<td>Lipemia</td>
<td>NA</td>
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<tr>
<td>Icterus</td>
<td>NA</td>
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<tr>
<td>Other</td>
<td>Specimens that have been decalcified (all methods); specimens that have not been formalin-fixed, paraffin-embedded; bone marrow in EDTA, extracted DNA</td>
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**Specimen Stability Information**

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<th>Special Container</th>
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**Clinical Information**

Hereditary nonpolyposis colon cancer (HNPCC), also known as Lynch syndrome, is an inherited cancer syndrome caused by a
germline mutation in one of several genes involved in DNA mismatch repair (MMR), including \textit{MLH1, MSH2, MSH6,} and \textit{PMS2}. There are several laboratory-based strategies that help establish the diagnosis of HNPCC/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) 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 \textit{MLH1}, and the most common cause of gene inactivation is promoter hypermethylation (epigenetic silencing). A specific mutation in the \textit{BRAF} gene (V600E) has been shown to be present in approximately 70\% of tumors with hypermethylation of the \textit{MLH1} promoter. Importantly, the V600E mutation is rarely identified in cases with germline \textit{MLH1} mutations. Thus, direct assessment of \textit{MLH1} promoter methylation status and testing for the \textit{BRAF} V600E mutation can be used to help distinguish between a germline mutation and epigenetic/somatic inactivation of \textit{MLH1}. Tumors that have the \textit{BRAF} V600E mutation and demonstrate \textit{MLH1} promoter hypermethylation are almost certainly sporadic, whereas tumors that show neither are most often caused by an inherited mutation.

Although testing for the \textit{BRAF} V600E mutation and \textit{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 Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor studies. 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 \textit{Lynch Syndrome Testing Algorithm}

**Reference Values**

An interpretative report will be provided.

**Interpretation**

An interpretative report will be provided. The likelihood of a germline (inherited) mutation is very low in those cases where the tumor demonstrates \textit{MLH1} promoter hypermethylation and the normal tissue is unmethylated. The likelihood of a germline mutation is high in those cases where the tumor and normal tissue lack \textit{MLH1} promoter hypermethylation. In cases where the tumor and normal tissue demonstrate \textit{MLH1} promoter hypermethylation, this result will be interpreted as equivocal and a blood sample will be requested to confirm potential germline hypermethylation.

**Cautions**

Testing tumors other than colon or endometrial for \textit{MLH1} hypermethylation has not been fully evaluated, and these specimens are not accepted for testing.

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 if there is high clinical suspicion of HNPCC.

**Clinical Reference**

2. Wang L, Cunningham JM, Winters JL, et al: \textit{BRAF} mutations in colon cancer are not likely attributable to defective DNA

Performance

Method Description
A PCR-based assay is used to test tumor DNA for the presence of hypermethylation of the MLH1 promoter. This is 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)

PDF Report
No

Day(s) Performed
Varies

Report Available
7 to 14 days

Specimen Retention Time
Extracted DNA: 3 months

Performing Laboratory Location
Rochester

Fees & 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 US Food and Drug Administration.

CPT Code Information
## MLH1 Hypermethylation Analysis, Tumor

### Test Definition: ML1HM

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