Overview

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

Identifying colon tumors with a previously negative KRAS mutation analysis result that may respond to epidermal growth factor receptor-targeted therapies

Identifying melanoma tumors that may respond to anti-BRAF targeted therapies (This test is not FDA-approved for this purpose)

Additional Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLIRV</td>
<td>Slide Review in MG</td>
<td>No, (Bill Only)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Testing Algorithm
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) Analysis

NY State Available
Yes

Specimen

Specimen Type
Varies

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

This test is not recommended as a first-tier screening measure for hereditary nonpolyposis colorectal cancer
Test Definition: BRAFT

BRAF Mutation Analysis(V600E), Tumor

(HNPCC). Please refer to MSI / Microsatellite Instability (MSI), Tumor and IHC / Mismatch Repair (MMR) Protein Immunohistochemistry Only, Tumor. Testing is best performed in the context of immunohistochemistry results indicating loss of MLH1 protein expression.

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

Specimen Required

Preferred

Specimen Type: Formalin-fixed, paraffin-embedded tissue block

Acceptable

Specimen Type: Tissue

Slides: 1 stained and 10 unstained

Collection Instructions: Submit 1 slide stained with hematoxylin and eosin and 10 unstained, nonbaked slides with 5-micron thick sections of the tumor 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

<table>
<thead>
<tr>
<th>Hemolysis</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipemia</td>
<td>NA</td>
</tr>
<tr>
<td>Icterus</td>
<td>NA</td>
</tr>
<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</td>
</tr>
</tbody>
</table>

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varies</td>
<td>Ambient (preferred)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 HNPCC/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 has not been identified to date 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.

Assessment for the BRAF V600E mutation has alternative clinical utilities. BRAF is part of the epidermal growth factor receptor (EGFR) signaling cascade, which plays a role in cell proliferation. Dysregulation of this pathway is a key factor in tumor progression. Targeted therapies directed to components of this pathway have demonstrated some success (increased progression-free and overall survival) in treating patients with certain tumors. Effectiveness of these therapies, however, depends in part on the mutation status of the pathway components.

Reference Values
An interpretative report will be provided.

Interpretation
An interpretive report will be provided.

Cautions
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 MLH1 promoter hypermethylation and absence of the BRAF V600E mutation if there is high clinical suspicion of HNPCC.

Not all patients with wild-type BRAF colon tumors respond to epidermal growth factor receptor -targeted therapies.

Not all melanoma patients with a BRAF mutation will respond to anti-BRAF targeted therapies.

Metastatic and corresponding primary lesions may have discordant results.

Clinical Reference
**Test Definition: BRAFT**

**BRAF Mutation Analysis(V600E), Tumor**


**Method Description**


**PDF Report**

No

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

Monday, Wednesday, Friday; 10 a.m.

**Analytic Time**

14 days

**Maximum Laboratory Time**

20 days

**Specimen Retention Time**

Extracted DNA will be stored indefinitely.

**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
81210- BRAF (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant
88381-Microdissection, manual

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAFT</td>
<td>BRAF Mutation Analysis(V600E), Tumor</td>
<td>58415-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>53217</td>
<td>Result Summary</td>
<td>50397-9</td>
</tr>
<tr>
<td>53218</td>
<td>Result</td>
<td>58415-1</td>
</tr>
<tr>
<td>53219</td>
<td>Interpretation</td>
<td>69047-9</td>
</tr>
<tr>
<td>53220</td>
<td>Specimen</td>
<td>31208-2</td>
</tr>
<tr>
<td>53221</td>
<td>Source</td>
<td>31208-2</td>
</tr>
<tr>
<td>54439</td>
<td>Tissue ID</td>
<td>80398-1</td>
</tr>
<tr>
<td>53222</td>
<td>Released By</td>
<td>18771-6</td>
</tr>
</tbody>
</table>