Test Definition: BRMLH

MLH1 Hypermethylation and BRAF Mutation Analysis, Tumor

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

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
PBMLH	MLH1 Hypermethylation/BRAF Mutation	No	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
BMLHH	MLH1 Hypermethylation Analysis	Yes, (order ML1HM)	No
BBRAF	BRAF Analysis	Yes, (order BRAFD)	No

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.

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
SLIRV	Slide Review in MG	No, (Bill Only)	Yes

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

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

Ordering Guidance

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

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

If the MMR immunohistochemistry (IHC) results for MLH1 and/or PMS2 suggest possible tumor heterogeneity, are ambiguous, or unusual, the physical IHC stains will be required to optimize the area of tissue selected for testing and for interpretation of the results. If IHC stains are required and not sent with the specimen, a request will be submitted to provide the IHC stains which will result in a slight delay.

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)
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

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Refrigerated		
	Frozen		

Clinical & 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 etiologyand 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

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
1. Cunningham JM, Kim CY, Christensen ER, et al: The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet. 2001;69:780-790
2. Wang L, Cunningham JM, Winters JL, et al: *BRAF* mutations in colon cancer are not likely attributable to defective DNA mismatch repair. Cancer Res. 2003;63:5209-5212
3. Domingo E, Laiho P, Ollikainen M, et al: *BRAF* screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet. 2004;41:664-668
4. Bettstetter M, Dechant S, Ruemmele P, et al: Distinction of hereditary nonpolyposis colorectal cancer and sporadic microsatellite-unstable colorectal cancer through quantification of *MLH1* methylation by real-time PCR. Clin Cancer Res. 2007;13:3221-3228
5. Gupta S, Provenzale D, Llor X, et al: NCCN Guidelines Insights: Genetic/familial high-risk assessment: colorectal, version 2.2019. J Natl Compr Canc Netw. 2019;17(9):1032-1041

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) Performed
Varies

Report Available
7 to 14 days

Specimen Retention Time
Extracted DNA: 3 months

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

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 account representative. 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. It has not been cleared or approved by the US 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

Test ID	Test Order Name	Order LOINC® Value
BRMLH	MLH1 Hypermethylation/BRAF Mutation	97761-1

Test Definition: BRMLH

MLH1 Hypermethylation and BRAF Mutation Analysis, Tumor

Result ID	Test Result Name	Result LOINC® Value
53223	Result Summary	50397-9
53224	Result	82939-0
53225	Interpretation	69047-9
53226	Specimen	31208-2
53227	Source	31208-2
53228	Tissue ID	80398-1
54921	BRAF Analysis	No LOINC Needed
54440	MLH1 Hypermethylation Analysis	No LOINC Needed
53229	Released By	18771-6
55139	Method	85069-3
621822	Disclaimer	62364-5