MAYO CLINIC LABORATORIES

Test Definition: MLHPB

MLH1 Hypermethylation Analysis, Blood

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

For information see Lynch Syndrome Testing Algorithm.

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 collection.

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 whole blood specimen in original tube. **Do not aliquot.**

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file.
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. Molecular Genetics: Inherited Cancer Syndromes Patient Information (T519)

Specimen Minimum Volume

1 mL

Reject Due To

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

Specimen Stability Information

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

Clinical & 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' end of *EPCAM* 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 *PMS2* confer the lowest risk for any tumor 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. However, it is important to note 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 *BRAF* (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

The report will include specimen information, assay information, and interpretation of test results.

Absence of hypermethylation is reported as not providing evidence for germline (constitutional) MLH1 promoter hypermethylation. Presence of hypermethylation is reported as consistent with germline (constitutional) inactivation of MLH1 by promoter hypermethylation.

Cautions

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

Clinical Reference

1. Hitchins MP, Ward RL: Constitutional (germline) *MLH1* epimutation as an aetiological mechanism for hereditary non-polyposis colorectal cancer. J Med Genet. 2009;46(12):793-802
2. Hitchins M, Williams R, Cheong K, et al: *MLH1* germline epimutations as a factor in hereditary nonpolyposis colorectal cancer. Gastroenterology. 2005;129(5):1392-1399
3. Niessen RC, Hofstra RM, Westers H, et al: Germline hypermethylation of *MLH1* and *EPCAM* deletions are a frequent cause of Lynch syndrome. Genes Chromosomes Cancer. 2009;48(8):737-744
4. Valle L, Carbonell P, Fernandez V, et al: *MLH1* germline epimutations in selected patients with early-onset non-polyposis colorectal cancer. Clin Genet. 2007;71(3):232-237
5. Idos G, Valle L: Lynch syndrome. In: Adam MP, Mirzaa GM, Pagon RA, et al, eds. GeneReviews (Internet). University of

Washington, Seattle; 2004. Updated February 2, 2021. Accessed June 27, 2023. Available at www.ncbi.nlm.nih.gov/books/NBK1211/

Performance

Method Description
A polymerase chain reaction-based assay is used to test normal DNA for the presence of hypermethylation of the *MLH1* promoter.(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 Feb;61(3):900-902)

PDF Report
No

Day(s) Performed
Varies

Report Available
8 to 12 days

Specimen Retention Time
Whole blood: 2 weeks (if available) 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 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
81288

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MLHPB	MLH1 Hypermethylation Analys,	97760-3

	Blood	

Result ID	Test Result Name	Result LOINC® Value
52906	Result Summary	50397-9
52907	Result	82939-0
52908	Interpretation	69047-9
52909	Reason for Referral	42349-1
52910	Specimen	31208-2
52911	Source	31208-2
52912	Released By	18771-6