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

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.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA) or yellow top (ACD)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**.
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information.

Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA is met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

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 (T576)
2. Molecular Genetics: Inherited Cancer Syndromes Patient Information (T519)
3.If not ordering electronically, complete, print, and send an Oncology Test Request (T729) with the specimen.

Specimen Minimum Volume

See Specimen Requirements

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	Varies		

Clinical & Interpretive

Clinical Information

The lifetime risk of colorectal cancer in the general population is 4% to 6%.(1) Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer) is an autosomal dominant hereditary cancer syndrome accounting for 2% to 4% of all colorectal cancer cases.(2,3)

Lynch syndrome is associated with germline variants in the mismatch repair (MMR) genes, *MLH1, MSH2, MSH6,* and *PMS2,* or deletions of the *EPCAM* gene. It is predominantly characterized by significantly increased risks for colorectal and endometrial cancer.(2,3) The lifetime risk for cancer is highly variable and dependent on the gene involved. Other malignancies within the tumor spectrum include gastric, ovarian, and small bowel cancers and hepatobiliary and upper urinary tract carcinomas.(2,3)

Several laboratory-based strategies may be utilized to screen for Lynch syndrome, including testing tumor tissue for the presence of microsatellite instability (MSI-H) and assessment of protein expression of MMR proteins (*MLH1, MSH2, MSH6, PMS2*) by immunohistochemistry (IHC).

Defective MMR in sporadic colon cancer is most often due to molecular variation in *MLH1*, of which promoter hypermethylation (epigenetic silencing) constitutes the most common cause of *MLH1* inactivation. A somatic-occurring variant in the *BRAF* gene (V600E) is present in approximately 70% of tumors with hypermethylation of the *MLH1* promoter. Importantly, the V600E variant is rarely identified in cases with disease-causing germline *MLH1* variants.

While most *MLH1* promoter hypermethylation occurs sporadically, some individuals with tumor hypermethylation may have germline inactivation of the *MLH1* gene via constitutional promoter hypermethylation. This condition is known as constitutional *MLH1* promoter hypermethylation, which is consistent with a diagnosis of Lynch syndrome.(4-7) In contrast to sequence variants in *MLH1*, current evidence suggests that the risk of transmitting constitutional *MLH1* promoter hypermethylation is less than50%. As such, these individuals may not have a strong family history of Lynch-related cancers and often test negative on traditional germline sequencing panels. Thus, testing for constitutional *MLH1* promoter hypermethylation may be considered if there is still suspicion for an inherited etiology following negative germline testing for patients with *MLH1* promoter methylated tumors.

For more information see Lynch Syndrome Testing Algorithm

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. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review. 1975-2018. National Cancer Institute. Updated April 2021. Accessed March 24, 2025. Available at: https://seer.cancer.gov/csr/1975_2018
2. 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
3. Idos G, Valle L. Lynch syndrome. In: Adam MP, Everman DB, Mirzaa GM, et al, eds. GeneReviews (Internet). University of Washington, Seattle; 2004. Updated February 4, 2021. Accessed March 24, 2025. Available at www.ncbi.nlm.nih.gov/books/NBK1211/
4. 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
5. 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
6. 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
7. 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

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;61[3]:900-902)

PDF Report
No

Day(s) Performed
Varies

Report Available
8 to 12 days

Specimen Retention Time
Whole blood: 30 days (if available); 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

81288

LOINC® Information

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

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