Test Definition: ML1HM

MLH1 Hypermethylation Analysis, Tumor

Overview

Useful For

An adjunct to tumor microsatellite instability and mismatch repair protein immunohistochemistry testing 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

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

Testing Algorithm

When this test is ordered, slide review will always be performed at an additional charge.

For more information see Lynch Syndrome Testing Algorithm.

Special Instructions

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

Method Name

Polymerase Chain Reaction (PCR)

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. For more information see TMSI / Microsatellite Instability, Tumor and IHC / 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 Clinic's preferred screening test includes both *MLH1* promoter hypermethylation and *BRAF* V600E testing. Order BRMLH / *MLH1* Hypermethylation and *BRAF* Mutation Analysis, Tumor.

Extracted DNA from tissues is **not** an acceptable specimen type.

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 and mismatch repair immunohistochemistry results must accompany specimen in order for testing to be performed.

Specimen Required

This assay requires at least 20% tumor nuclei.
-Preferred amount of tumor area with sufficient percent tumor nuclei: tissue 216 mm(2)
-Minimum amount of tumor area: tissue 36 mm(2)
-These amounts are cumulative over up to 10 unstained slides and must have adequate percent tumor nuclei.
-Tissue fixation: 10% neutral buffered formalin, not decalcified
-For specimen preparation guidance, see Tissue Requirement for Solid Tumor Next-Generation Sequencing. In this document, the sizes are given as 4 mm x 4 mm x 10 slides as preferred: approximate/equivalent to 144 mm(2) and the minimum as 3 mm x 1 mm x 10 slides: approximate/equivalent to 36 mm(2).

Preferred: Submit 2, if available, of the following specimens.
Acceptable: Submit **at least one** of the following specimens**.**

Specimen Type: Tissue block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block with acceptable amount of tumor tissue.

Specimen Type: Tissue slide
Slides: 1 Hematoxylin and eosin-stained and 10 unstained
Collection Instructions:
Submit the followings slides:
1 Slide stained with hematoxylin and eosin
AND
10 Unstained, nonbaked slides with 5-micron thick sections of the tumor tissue.
Note: The total amount of required tumor nuclei can be obtained by scraping up to 10 slides from the same block.
Additional Information:
1. Hematoxylin and eosin-stained and unstained slides will not be returned.
2. Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

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 Test Request (T728)
-Oncology Test Request (T729)

Specimen Minimum Volume

See Specimen Required

Reject Due To

Broken or cracked unstained slides	Reject
Broken or cracked stained slides that cannot be repaired	Reject

Specimen Stability Information

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

Clinical & Interpretive

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 *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 one of the MMR proteins by immunohistochemistry (IHC). 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 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 *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.

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 TMSI / Microsatellite Instability, Tumor and IHC / 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 may benefit from subsequent genetic testing.

Reference Values

An interpretative report will be provided.

Interpretation

An interpretive report will be provided. The likelihood of a germline (inherited) mutation is very low in those cases where the tumor demonstrates *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 *MLH1* promoter hypermethylation. In cases where the tumor and normal tissue demonstrate *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 *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 a family with hereditary nonpolyposis colorectal cancer (HNPCC). Therefore, evaluation of other family members should still be considered in cases with *MLH1* promoter hypermethylation if there is high clinical suspicion of HNPCC.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

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(4):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(17):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(9):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(11):3221-3228

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 tumor DNA for the presence of hypermethylation of the *MLH1* promoter.(Modification of 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[3]:900)

PDF Report

No

Day(s) Performed

Varies

Report Available

7 to 14 days

Specimen Retention Time

Tissue blocks: Unused portions of blocks will be returned; Tissue slides: Hematoxylin and eosin-stained and unstained slides will not be returned. Unused slides are stored for at least 5 years; 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.

Test Definition: ML1HM

MLH1 Hypermethylation Analysis, Tumor

CPT Code Information

81288
88381

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ML1HM	MLH1 Hypermethylation Analys, Tumor	97761-1

Result ID	Test Result Name	Result LOINC® Value
53299	Result Summary	50397-9
53300	Result	82939-0
53301	Interpretation	69047-9
53302	Reason for Referral	42349-1
53303	Specimen	31208-2
53304	Source	85298-8
54447	Tissue ID	80398-1
53305	Released By	18771-6