Test Definition: MLH1

MLH1 Immunostain, Technical Component Only

Overview

Useful For

Evaluation of tumor tissue to identify patients at high risk for having hereditary nonpolyposis colorectal cancer, also known as Lynch syndrome

Evaluation of tumor tissue to identify patients at risk for having hereditary endometrial carcinoma

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
IHTOI	IHC Initial, Tech Only	No	No
IHTOA	IHC Additional, Tech Only	No	No

Testing Algorithm

For the initial technical component only immunohistochemical (IHC) stain performed, the appropriate bill-only test ID will be reflexed and charged (IHTOI). For each additional technical component only IHC stain performed, an additional bill-only test ID will be reflexed and charged (IHTOA).

Method Name

Immunohistochemistry (IHC)

NY State Available

Yes

Specimen

Specimen Type

TECHONLY

Ordering Guidance

This test includes only technical performance of the stain; **no pathologist interpretation is provided**.

Technical component only stains **should not** be ordered with PATHC / Pathology Consultation. If ordered with PATHC, the technical component stains **will be canceled**. Any immunohistochemistry (IHC)/in situ hybridization (ISH) stain performed as a part of the PATHC will be performed at the reviewing pathologist's discretion at an additional charge.

Shipping Instructions

Attach the green "Attention Pathology" address label (T498) and the pink Immunostain Technical Only label included in the kit to the outside of the transport container.

Necessary Information

If sending normal and tumor blocks; indicate the block number to be stained in performing lab notes (electronic orders) or on the enclosed paperwork (manual orders).

Specimen Required

Specimen Type: Tissue
Supplies: Immunostain Technical Only Envelope (T693)
Container/Tube: Immunostain Technical Only Envelope
Submit:
-Formalin-fixed, paraffin-embedded tissue block
OR
-2 Unstained, positively charged glass slides (25- x 75- x 1-mm) per test ordered; sections 4-microns thick
Additional Information: 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.

Digital Image Access

1. Information on accessing digital images of immunohistochemical (IHC) stains and the manual requisition form can be accessed through this website: https://news.mayocliniclabs.com/pathology/digital-imaging/
2. Clients ordering stains using a manual requisition form will not have access to digital images.
3. Clients wishing to access digital images must place the order for IHC stains electronically. Information regarding digital imaging can be accessed through this website: https://news.mayocliniclabs.com/pathology/digital-imaging/#section3

Forms

If not ordering electronically, complete, print, and send a Immunohistochemical (IHC)/In Situ Hybridization (ISH) Stains Request (T763) with the specimen.

Reject Due To

Wet/frozen tissue	Reject
Cytology smears	Reject
Non-formalin fixed tissue	Reject
Nonparaffin embedded tissue	Reject
Noncharged, ProbeOn or Snowcoat slides	Reject
Broken or cracked	Reject

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

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
TECHONLY	Ambient (preferred)		
	Refrigerated		

Clinical & Interpretive

Clinical Information

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is an autosomal dominant hereditary cancer syndrome associated with germline variants in the mismatch repair genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2*.

Lynch syndrome 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* variants (approximately 50%-80%) is generally higher than the risks associated with variants in the other Lynch syndrome-related genes and the lifetime risk for endometrial cancer (approximately 25%-60%) is also highly variable. Other malignancies within the tumor spectrum include sebaceous neoplasms, gastric cancer, ovarian cancer, hepatobiliary and urinary tract carcinomas, and small bowel cancer. The lifetime risks for these cancers are less than 15%. Of the 4 mismatch repair genes, variants within the *PMS2* gene confer the lowest risk for any of the tumors within the Lynch syndrome spectrum.

Several clinical variants of Lynch syndrome 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 or compound heterozygous 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. Testing tumors from individuals at risk for Lynch syndrome for microsatellite instability (MSI) indicates the presence or absence of defective DNA mismatch repair phenotype within the tumor but does not suggest in which gene the abnormality rests. Tumors from individuals affected by Lynch syndrome usually demonstrate an MSI-H phenotype

(MSI >30% of microsatellites examined). The MSI-H phenotype can also be seen in individuals whose tumors have somatic *MLH1* promoter hypermethylation. Tumors from individuals that show the MSS/MSI-L phenotype (MSI at <30% of microsatellites examined), are not likely to have Lynch syndrome or somatic hypermethylation of *MLH1*. Immunohistochemistry (IHC) is a complementary testing strategy to MSI testing. In addition to identifying tumors with defective DNA mismatch repair, IHC analysis is helpful for identifying the gene responsible for the defective DNA mismatch repair within the tumor, because the majority of MSI-H tumors show a loss of expression of at least 1 of the 4 mismatch repair genes described above.

Testing is typically first performed on the tumor of an affected individual and in the context of other risk factors, such as young age at diagnosis or a strong family history of Lynch syndrome-related cancers. If defective DNA mismatch repair is identified within the tumor, mutation analysis of the associated gene can be performed to identify the causative germline variant and allow for predictive testing of at-risk individuals.

Of note, MSI-H phenotypes and loss of protein expression by IHC have also been demonstrated in various sporadic cancers, including those of the colon and endometrium. Absence of MLH1 and PMS2 protein expression within a tumor, for instance, is most often associated with a somatic alteration in individuals with an older age of onset of cancer than typical Lynch syndrome families. Therefore, an MSI-H phenotype or loss of protein expression by IHC within a tumor does not distinguish between somatic mutations and germline variants. Genetic testing of the gene indicated by IHC analysis can help to distinguish between these 2 possibilities. In addition, when absence of MLH1/PMS2 is observed, BRMLH / *MLH1* Hypermethylation and *BRAF* Mutation Analyses, Tumor or ML1HM / *MLH1* Hypermethylation Analysis, Tumor may also help to distinguish between a sporadic and germline etiology.

It should be noted that this Lynch syndrome screen is not a genetic test but rather stratifies the risk of having an inherited cancer predisposition syndrome and identifies patients who might benefit from subsequent genetic testing.

Interpretation

This test does not include pathologist interpretation, only technical performance of the stain.

The positive and negative controls are verified as showing appropriate immunoreactivity.

Interpretation of this test should be performed in the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Cautions

Age of a cut paraffin section can affect immunoreactivity. Stability thresholds vary widely among published literature and are antigen dependent. Best practice is for paraffin sections to be cut within 6 weeks.

The charge of glass slides can be affected by environmental factors and subsequently may alter slide staining. Sending unsuitable glass slides can result in inconsistent staining due to poor slide surface chemistry.

Best practices for storage of positively charged slides:
-Minimize time slides are stored after being unpackaged
-Limit exposure to high humidity and heat
-Minimize exposure to plastics

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. Burgart LJ. Testing for defective DNA mismatch repair in colorectal carcinoma: a practical guide. Arch Pathol Lab Med 2005;129(11):1385-1389
2. Klarskov L, Ladelund S, Holck S, et al. Interobserver variability in the evaluation of mismatch repair protein immunostaining. Hum Pathol. 2010;41(10):1387-1396
3. Lanza G, Gafa R, Maestri I, Santini A, Matteuzzi M, Cavazzini L. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mod Pathol. 2002;15(7):741-749
4. Modica I, Soslow RA, Black D, Tornos C, Kauff N, Shia J. Utility of immunohistochemistry in predicting microsatellite instability in endometrial carcinoma. Am J Surg Pathol. 2007;31(5):744-751
5. Mojtahed A, Schrijver I, Ford JM, Longacre TA, Pai RK. A two-antibody mismatch repair protein immunohistochemistry screening approach for colorectal carcinomas, skin sebaceous tumors, and gynecologic tract carcinomas. Mod Pathol. 2011;24(7):1004-1014
6. Rigau V, Sebbagh N, Olschwang S, et al. Microsatellite instability in colorectal carcinoma. The comparison of immunohistochemistry and molecular biology suggests a role for hMLH6 [correction of hMLH6] immunostaining. Arch Pathol Lab Med. 2003;127(6):694-700
7. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn. 2008;10(4):293-300
8. Magaki S, Hojat SA, Wei B, So A, Yong WH. An introduction to the performance of immunohistochemistry. Methods Mol Biol. 2019;1897:289-298. doi:10.1007/978-1-4939-8935-5_25

Performance

Method Description

Immunohistochemistry on sections of paraffin-embedded tissue.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

1 to 3 days

Specimen Retention Time

Until staining is complete.

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

88342-TC, primary
88341-TC, if additional IHC

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MLH1	MLH1 IHC, Tech Only	Order only;no result

Result ID	Test Result Name	Result LOINC® Value
70822	MLH1 IHC, Tech Only	Bill only; no result