

# Lynch Syndrome/Hereditary Nonpolyposis Colorectal Cancer

## Indications for Ordering

Microsatellite instability (MSI) and/or immunohistochemistry (IHC) tumor analyses

- Universal screening for Lynch syndrome (LS) in all individuals with newly diagnosed colorectal cancer (CRC) (NCCN, 2016)
- Screening for LS in individuals with endometrial cancer (ASCO, 2015)

Germline mismatch repair (MMR) gene testing

- Order following abnormal screening test result
- Confirms diagnosis of LS

## Test Description

Mismatch repair by IHC

- Determines MMR protein expression (MLH1, MSH2, MSH6, and PMS2) in tumor tissue

MSI testing

- Genotyping of 5 mononucleotide repeats (BAT-25, BAT-26, MONO-27, NR-21, and NR-24)
- Allele size within tumor sample is compared to allele size within normal tissue from the same individual

*BRAF* codon 600 mutation

- Pyrosequencing at codon 600

*MLH1* promoter methylation

- Uses bisulfite conversion followed by real-time polymerase chain reaction (PCR)

MMR gene pathogenic germline variants

- PCR amplification followed by bidirectional sequencing of coding regions and intron/exon boundaries of *MLH1*, *MSH2*, *MSH6*, and *PMS2*
- Multiplex ligation-dependent probe amplification (MLPA) for large *MLH1*, *MSH2*, *MSH6*, and *PMS2* exonic deletions/duplications, 10 Mb inversion of *MSH2* exons 1-7, and exon 9 deletions in *EPCAM*

## Tests to Consider

### Screening studies (requires pathological tissue)

#### [Mismatch Repair by Immunohistochemistry 0049302](#)

- First-line screening test for LS
- Highly recommended prior to ordering germline MMR mutation analysis gene testing
  - Directs subsequent genetic diagnostic testing

#### [Microsatellite Instability \(MSI\), HNPCC/Lynch Syndrome, by PCR 0051740](#)

- First-line screening test for LS
- Directs subsequent genetic diagnostic testing for LS

#### [Mismatch Repair by Immunohistochemistry with Reflex to \*BRAF\* Codon 600 Mutation and \*MLH1\* Promoter Methylation 2002327](#)

- Preferred screening test for LS in individuals with CRC
- If *MLH1* IHC is abnormal, evaluations of *BRAF* codon 600 and, possibly, *MLH1* methylation are performed
- Definitive diagnosis of LS requires additional targeted MMR germline mutation studies
- Do not use in endometrial cancer

#### [BRAF Codon 600 Mutation Detection with Reflex to \*MLH1\* Promoter Methylation 0051750](#)

- Recommended reflex test to differentiate between LS and sporadic CRC in tumors showing loss of *MLH1*
- If no *BRAF* mutation is detected, *MLH1* promoter methylation is evaluated

#### [Mismatch Repair by Immunohistochemistry with Reflex to \*MLH1\* Promoter Methylation 2005270](#)

- Preferred reflex screening test for LS in non-CRC tumors (eg, endometrial carcinoma)
- If *MLH1* expression is lost, *MLH1* methylation is performed

#### [MLH1 Promoter Methylation, Paraffin 2002499](#)

- Recommended test to distinguish between LS and sporadic non-CRC tumors with loss of *MLH1*

### Diagnostic germline genetic studies (peripheral blood)

- Germline genetic testing is available for all 4 MMR genes known to cause LS

#### [HNPCC/Lynch Syndrome \(\*MLH1\*\) Sequencing and Deletion/Duplication 0051650](#)

- Detect germline *MLH1* variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of *MLH1* and *PMS2* proteins), negative for the *BRAF* codon 600 pathogenic variant, and with normal *MLH1* promoter methylation studies

### [HNPCC/Lynch Syndrome \(MSH2\) Sequencing and Deletion/Duplication 0051654](#)

- Detect germline *MSH2* variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of MSH2 and MSH6 proteins)
- Includes evaluation of *EPCAM* exon 9 deletions and 10 Mb inversion of *MSH2* exons 1-7

### [HNPCC/Lynch Syndrome \(MSH6\) Sequencing and Deletion/Duplication 0051656](#)

- Detect germline *MSH6* variants
- Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of MSH6 protein)

### [HNPCC/Lynch Syndrome \(PMS2\) Sequencing and Deletion/Duplication 0051737](#)

- Detect germline *PMS2* variants
- Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of PMS2 protein)

### [Gastrointestinal Hereditary Cancer Panel, Sequencing and Deletion/Duplication, 16 Genes 2013449](#)

- Confirm a diagnosis of hereditary gastrointestinal (GI) cancer in individuals with a personal or family history of GI cancer and/or polyposis
- Includes all 4 MMR genes known to cause LS

### [HNPCC/Lynch Syndrome Deletion/Duplication 2001728](#)

- Order if sequencing studies have been performed previously at another laboratory
- Order if there is a known familial deletion or duplication
- Both sequencing and deletion/duplication testing are necessary to detect all pathogenic variants in MMR genes

### [Familial Mutation, Targeted Sequencing 2001961](#)

- Useful when a pathogenic familial variant identifiable by sequencing is known

## Disease Overview

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### Incidence

- Accounts for 2-4% of CRC and ~1-2% of endometrial cancers (NCCN, 2016; Chadwick, 2001)
- Most common hereditary CRC syndrome
- Most common cause of hereditary endometrial cancer

### Risk estimates

- The following lifetime risk estimates apply to individuals with *MLH1* and *MSH2* pathogenic variants; risks for variants in *MSH6* or *PMS2* may be lower (NCCN, 2016)
  - CRC – 52-82%
  - Endometrial – 25-60%
  - Ovarian – 4-24%
  - Gastric – 6-13%
  - Urinary tract – 1-7%
  - Brain/central nervous system – 1-3%
  - Hepatobiliary tract – 1-4%
  - Small bowel – 3-6%
  - Pancreatic – 1-6% (*MLH1* and *MSH2* only)

## Genetics

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**Genes** – *MLH1*, *MSH2*, *MSH6*, and *PMS2*

**Inheritance** – autosomal dominant

## Test Interpretation

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### Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR

#### Sensitivity/specificity

- Clinical sensitivity – 90% (NCCN, 2016)
- Analytical sensitivity/specificity – >99%

#### Results

- High MSI (MSI-H)
  - MMR deficiency, either sporadic or LS
- Indeterminate MSI (MSI-I)
  - Instability in even 1 mononucleotide repeat can be associated with LS
  - Follow-up IHC studies are recommended
- Microsatellite stable (MSS)
  - LS unlikely

#### Limitations

- 15% of sporadic CRCs are also MSI-H (NCCN, 2016)
- Preoperative chemoradiation of rectal cancer
  - May complicate IHC interpretation and/or decrease tumor mass
  - May make MSI testing difficult
  - Evaluation of pretreatment biopsies will avoid this limitation
- Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

### Mismatch Repair by IHC

**Clinical sensitivity** – 90% (NCCN, 2016)

#### Results

- Normal – MMR proteins are normally expressed
  - MMR deficiency is unlikely
  - LS unlikely

- Abnormal – MMR protein expression is abnormal
  - Loss of expression of 1 or more proteins is highly predictive of MMR deficiency
  - Absence of both MLH1 and PMS2
    - *MLH1* germline pathogenic variant is possible
    - Consider *MLH1* methylation ± *BRAF* V600E studies
    - If methylation and *BRAF* studies are negative, follow with *MLH1* germline genetic testing
  - Absence of PMS2 only
    - *PMS2* germline pathogenic variant likely
    - Consider *PMS2* germline testing
    - If *PMS2* testing does not identify a germline pathogenic variant, consider *MLH1* germline testing
  - Absence of both MSH2 and MSH6
    - *MSH2* germline pathogenic variant likely
    - Consider *MSH2* germline testing
  - Absence of MSH6 only
    - *MSH6* germline pathogenic variant likely
    - Consider *MSH6* germline testing
    - If *MSH6* testing does not identify a germline pathogenic variant, consider *MSH2* germline testing

#### Limitations

- ~10% of individuals with LS will have IHC tests that show normal staining of the MMR proteins
- Because the correlation of MSI with IHC is not 100%, direct testing of MSI by PCR may be helpful
- Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

#### ***BRAF* Codon 600 Mutation Detection with Reflex to *MLH1* Promoter Methylation**

**Analytical sensitivity** – methylation levels >10% are reported as positive

#### Results

- *BRAF* V600E detected
  - Presence in MMR-deficient CRC indicates the tumor is probably sporadic and not associated with LS
  - Further germline testing not typically indicated
- *MLH1* promoter methylation detected
  - Presence in an MSI CRC indicates the tumor is probably sporadic and not associated with LS
  - Further germline testing not typically indicated
- No mutations detected
  - In MSI-H tumors with loss of MLH1 protein by IHC, *MLH1* germline testing indicated

#### **Germline genetic studies (*MLH1*, *MSH2*, *MSH6*, or *PMS2*)**

##### Sensitivity/specificity

- Proportion of LS attributed to pathogenic variants in specific MMR gene (Peltomaki, 2003; Berends, 2002; Senter, 2008)
  - *MLH1* – 45-50%
  - *MSH2* – 40%
  - *MSH6* – 5-10%
  - *PMS2* – <5%
- Analytical sensitivity/specificity – 99%

#### Results

- Positive – 1 pathogenic variant detected
  - Predicted to be causative for LS
- Negative – no pathogenic variants detected
  - Diagnosis of LS unlikely, but not excluded
- Inconclusive – variant detected, but whether it is benign or pathogenic is unknown

#### Limitations

- Not evaluated
  - Regulatory region and deep intronic variants
  - Sequence variants and large deletion/duplications in genes other than *MLH1*, *MSH2*, *MSH6*, *PMS2*, and large deletions in *EPCAM* exon 9
  - Large gene inversions, other than the *MSH2* 10 Mb exons 1-7 inversion
  - Causes of hereditary CRC or endometrial cancer other than LS
- Diagnostic errors can occur due to rare sequence variations

#### References

- Berends M, Wu Y, et al. Molecular and clinical characteristics of *MSH6* variants: an analysis of 25 index carriers of a germline variant. *Am J Hum Genet.* 2002;70(1):26-37
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