

## Lynch Syndrome: *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* Sequencing and Deletion/Duplication Panel

**Test Code:** MM381

**Turnaround time:** 6 weeks

**CPT Codes:** 81295 x1, 81297 x1, 81298 x1, 81300 x1, 81317 x1, 81403 x1, 81292 x1, 81294 x1

### Condition Description

Lynch syndrome, caused by a germline mutation in a mismatch repair gene or associated with tumors exhibiting MSI, is characterized by an increased risk of colon cancer and other cancers (e.g., of the endometrium, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, skin). Individuals with Lynch syndrome have an approximately 80% lifetime risk for colon cancer. The average age of colorectal cancer diagnosis is 61 years. Women with Lynch syndrome have a 20%-60% lifetime risk of endometrial cancer. The average age of diagnosis of endometrial cancer is age 46-62 years. Among women with Lynch syndrome who develop both colon cancer and endometrial cancer, approximately 50% present first with endometrial cancer. In Lynch syndrome, the mean age of diagnosis of gastric cancer is age 56 years, with intestinal-type adenocarcinoma being the most commonly reported pathology. Lynch syndrome-associated ovarian cancers have a mean age of diagnosis of 42.5 years; approximately 30% are diagnosed before age 40 years.

The diagnosis of Lynch syndrome can be made on the basis of the Amsterdam Clinical Criteria or by molecular genetic testing for germline mutations in one of several mismatch repair (MMR) genes. The Amsterdam Criteria, first established in 1990 for research purposes, were later modified to include the other Lynch syndrome-related cancers for clinical diagnostic purposes. The Amsterdam Criteria are 1) Three or more family members, one of whom is a first-degree relative of the other two, with a confirmed diagnosis of colorectal cancer 2) Two successive affected generations 3) One or more colorectal cancers diagnosed before age 50 years. The modified Amsterdam Criteria replace "colorectal cancer" with "any Lynch syndrome-related cancers." The sensitivity and specificity of the Amsterdam Criteria for identifying a mutation in the mismatch repair genes *MSH2* and *MLH1* have been reported to be 61% and 67%, respectively. The sensitivity is increased to 78% using the modified Amsterdam Criteria. However, broadening the criteria decreases the specificity.

Lynch syndrome is known to be associated with mutations in four genes involved in the mismatch repair pathway (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). Germline mutations in *MLH1* and *MSH2* account for approximately 90% of detected mutations in families with Lynch syndrome. Mutations in *MSH6* have been reported in approximately 7%-10% of families with Lynch syndrome. Mutations in *PMS2* account for fewer than 5% of mutations in families with Lynch syndrome. Up to 39% of families with mutations in a Lynch syndrome gene do not meet the Amsterdam Criteria. Therefore, families found to have a deleterious mutation in a Lynch syndrome gene should be considered to have Lynch syndrome regardless of the extent of the family history. At least 20% of mutations in *MSH2* and 5% of mutations in *MLH1* are large deletions or genetic rearrangements

Lynch syndrome is inherited in an autosomal dominant manner. The majority of individuals diagnosed with Lynch syndrome have inherited the condition from a parent. However, because of incomplete penetrance, variable age of cancer development, cancer risk reduction as a result of screening or prophylactic surgery, or early death, not all individuals with a Lynch syndrome gene mutation have a parent who had cancer.

Deletions of the 3' end of the *EPCAM* gene (2q21) (also known as the *TACSTD1* gene) cause Lynch syndrome by methylating and inactivating the *MSH2* gene. Kovacs et al. (2009) identified deletions of the *EPCAM* gene in five of 27 families tested that did not have a mutation in the *MLH1* or *MSH2* genes. The phenotype of individuals with deletions of the *EPCAM* gene differs slightly from the classic Lynch syndrome phenotype. These individuals tend to be diagnosed with colorectal cancer only. The age at disease onset (25-63 years) is similar to those with mutations of the *MSH2* gene.

[Click here](#) for the GeneTests summary on this condition.

Visit [www.ThinkGenetic.com](http://www.ThinkGenetic.com) for patient-friendly information on [Lynch syndrome](#).

### Genes

[EPCAM](#), [MLH1](#), [MSH2](#), [MSH6](#), [PMS2](#)

### Indications

This test is indicated for:

- Confirmation of a clinical diagnosis of Lynch syndrome.
- Individuals at-risk for Lynch syndrome due to family history.

### Methodology

**Next Generation Sequencing:** In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members. Long template PCR amplification is used to isolate the coding gene for *PMS2* before standard PCR amplification. Direct sequencing of amplification products is performed in both forward and reverse directions, using automated fluorescence dideoxy sequencing methods. *EPCAM* is not sequenced.

**Deletion/Duplication Analysis:** DNA isolated from peripheral blood is hybridized to a CGH array to detect deletions and duplications. The targeted CGH array has overlapping probes which cover the entire genomic region. Deletion/duplication analysis is performed on *MLH1*, *MSH2*, *MSH6*, and *EPCAM*.

## Detection

Clinical Sensitivity: Germline mutations in *MLH1* and *MSH2* account for approximately 90% of detected mutations in families with Lynch syndrome. Mutations in *MSH6* have been reported in approximately 7%-10% of families with Lynch syndrome. Mutations in *PMS2* account for fewer than 5% of mutations in families with Lynch syndrome. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's biochemical phenotype.

Kovacs et al. (2009) identified deletions of the *EPCAM* gene in five of 27 families tested that did not have a mutation in the *MLH1* or *MSH2* genes. Detection is limited to duplications and deletions. The CGH array will not detect point or intronic mutations. Results of molecular analysis must be interpreted in the context of the patient's clinical and/or biochemical phenotype.

Analytical Sensitivity: ~99%.

## Specimen Requirements

**Submit only 1 of the following specimen types**

### Type: DNA, Isolated

#### Specimen Requirements:

Microtainer

10µg

Isolation using the Perkin Elmer™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

#### Specimen Collection and Shipping:

Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

### Type: Whole Blood (EDTA)

#### Specimen Requirements:

EDTA (Purple Top)

Infants and Young Children ( 2 years of age to 10 years old: 3-5 ml

Older Children & Adults: 5-10 ml

Autopsy: 2-3 ml unclotted cord or cardiac blood

#### Specimen Collection and Shipping:

Ship sample at room temperature for receipt at EGL within 24 hours of collection. Do not refrigerate or freeze.

## Special Instructions

Please submit MSI or IHC results, if available. Contact the laboratory if further information is needed.

## Related Tests

- Sequencing and deletion/duplication analysis is available separately for genes on this panel.
- Microsatellite instability testing is available.
- Immunohistochemistry for *MLH1*, *MSH2*, *MSH6*, and *PMS2* proteins is available, either in a panel or individually.
- Custom diagnostic mutation analysis (KM) is available to family members if mutations are identified by targeted mutation testing or sequencing analysis.
- Prenatal testing is available to adults who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.