# *Lynch Syndrome Colon Cancer*

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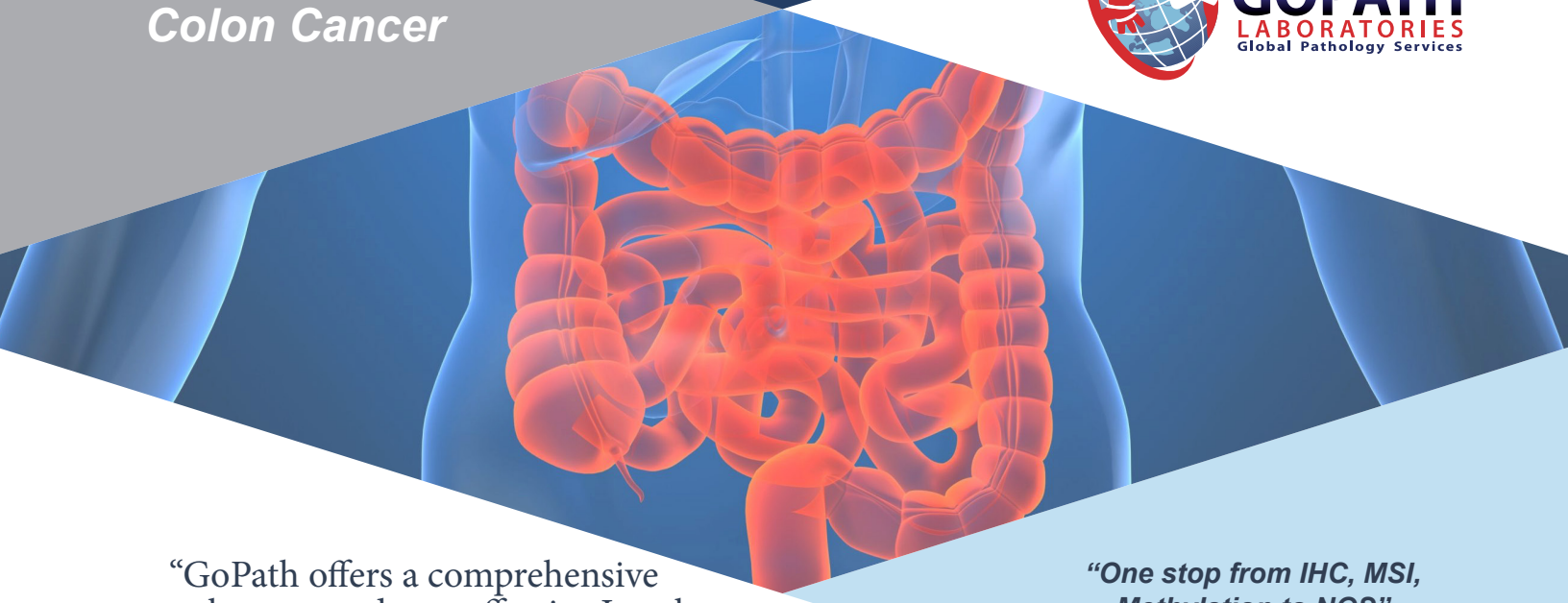
Global Pathology Services



"GoPath offers a comprehensive step-by-step and cost effective Lynch screening program"

***"One stop from IHC, MSI, Methylation to NGS"***

## Lynch Syndrome – Hereditary Nonpolyposis Colon Cancer (HNPCC)

Lynch syndrome, often called hereditary nonpolyposis colorectal cancer (HNPCC), is a type of inherited cancer of the digestive tract, particularly the colon (large intestine) and rectum. People with Lynch syndrome have an increased risk of cancers of the stomach, small intestine, liver, gallbladder ducts, upper urinary tract, brain, skin, and prostate. Women with this disorder also have a high risk of cancer of the endometrium (lining of the uterus) and ovaries. Even though the disorder was originally described as not involving noncancerous (benign) growths (polyps) in the colon, people with Lynch syndrome may occasionally have colon polyps. In individuals with this disorder, colon polyps occur at an earlier age than in the general population. Although the polyps do not occur in greater numbers than in the general population, they are more likely to become cancerous.

Lynch syndrome affects about 3%-5% of all colorectal cancers and is believed to be caused by mutations in DNA mismatched repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2). Those carrying a mutation have a (MMR) 50%-80% higher than normal chance of developing colorectal cancer during his or her lifetime. Endometrial cancer, ovarian cancer, stomach cancer and other various types of aggressive cancers are also more likely to occur, often at a young age.

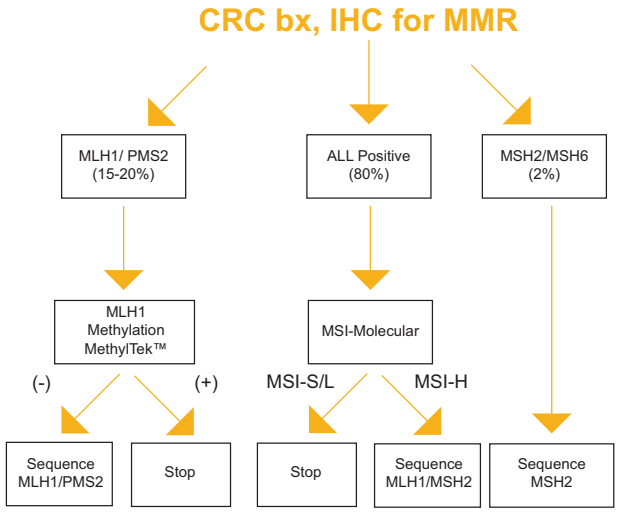
## About the Mismatch Repair Genes (MMR)

Variations in the MLH1, MSH2, MSH6, and PMS2 genes increase the risk for developing Lynch syndrome. All of these genes are involved in the repair of mistakes made when DNA is copied (DNA replication) in preparation for cell division. Mutations in any of these genes prevent the proper repair of DNA replication mistakes. As the abnormal cells continue to divide, the accumulated mistakes can lead to uncontrolled cell growth and possibly cancer. Although mutations in these genes predispose individuals to cancer, not all people who carry these mutations will develop cancerous tumors.

## Lynch Screening Protocol

GoPath Laboratories has several years of experience in dealing with Lynch patients and has developed a comprehensive screening protocol than not only detects the disorder rapidly, but saves unnecessary costs to those parties involved. The protocol, which is largely accepted throughout the medical field, consists of first screening for DNA mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 by way of immunohistochemistry. This process gives results within 3 to 5 days and allows the clinician to make a rapid decision on the next step. Following this, based on whether or not MMR proteins are intact, it will determine what next step to take. See figure 1.

**Figure 1. Lynch Algorithm Protocol**




Modified from Hampel Het al NEJM 352, 1851-2005

## MLH1 Promoter Methylation Test ( MethylTek™)

### Assay Description and Methodology

Formalin-fixed, paraffin-embedded (FFPE) tumor tissues with a tumor content of about 70 percent are micro-dissected and then de-paraffinized. DNA is extracted using a Qiagen kit (Qiagen, Valencia, CA) specific for FFPE specimen types. Promoter hypermethylation of the MLH1 gene is detected using MLH1 MethylTek™, a proprietary, laboratory-developed and validated assay. MLH1 MethylTek™ is an quantitative real-time methylation-specific PCR assay detecting methylated MLH1 promoter sequence in clinical tumor specimens with an analytical sensitivity and specificity of 100%.

### Interpretation

Methylation of MLH1 promoter (MethylTek™) is determined by the score of Methylation index (Mdex). Samples with a Mdex score of 0 to 1 are recorded as negative, and samples with a Mdex score of 3 or higher are recorded as positive. Negative MLH1 promoter methylation indicates that IHC loss of MLH1 staining in the tested tumor is not caused by somatic hypermethylation. Therefore, Lynch Syndrome is suggested. Consultation with a genetic counselor is recommended with possible sequencing of the MLH-1 gene for confirmation.

### Inadequate Samples

Tumor content of less than 20% may be considered inadequate samples.

## Next Generation Sequencing (NGS) Germinal Mutation Detection (LynchNow™)

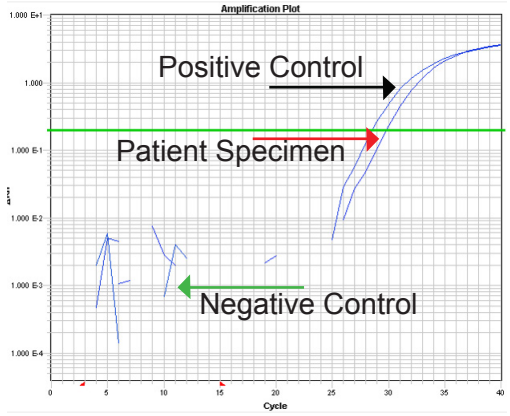
### Assay Description and Methodology

Germline mutations of 15 genes associated with common cancer syndromes including Lynch Syndrome were detected by an NGS method using the GoPath Genetic Gene Panel (gpNGS-15). The assay was developed and validated using Kappa library preparation technology and an Illumina Miseq instrument combined with the NextGene data analysis platform (SoftGenetics). The assay is designed to detect single and multi-nucleotide substitutions, insertions, duplications and small deletions in coding and exon-intron junction of the genes in this NGS panel. The assay provides >1500X average coverage at the targeted genomic regions of the tested genes. Sensitivity and specificity of the assay for detection of mutations in targeted genomic regions is 96.5% and 100% with a negative predictive value (NPV) and positive predictive value of 96.5% and 100%. Targeted regions with inadequate sequencing read coverage (read depth < 200X) from NGS are sequenced by a Sanger DNA Sequencing method. Variant frequency of ≥10% was defined as the value of limit-of-detection (LOD) of the assay. Genes in the panel: APC, AXIN2, BLM, BMPR1A, BRCA1, BRCA2, BUB1B, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, EXO1, FLCN, GREM1, MLH1, MLH3, MSH2, MSH6, MUTYH, NF2, PMS1, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TGFBR2, TP53, VHL.

### Samples For Submission

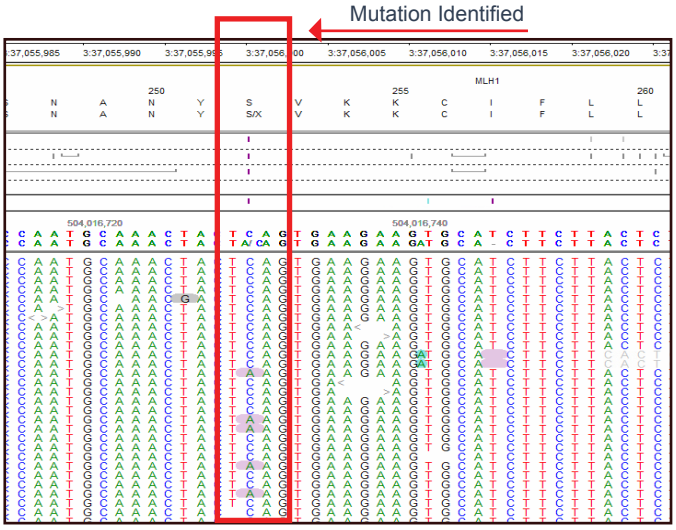
FFPE sections (Formalin-fixed, paraffin-embedded unstained sections: 5 to 10 sections, 10 um thickness from the corresponding surgical case): store at room temperature or 4° C until extraction or EDTA blood tube of 5mL of whole blood: ship over ice packet.

**Figure 2. Example Of A Positive MethylTek™ Result**




Data generated using Applied Biosystems 7900HT Real-Time PCR

**Figure 3. NGS Data For Positive Lynch Patient**




Data generated from Illumina MiSeq Sequencer

### References

1.Raedle J et al. Bethesda guidelines: relation to microsatellite instability and MLH1 promoter methylation in patients with colorectal cancer. Ann Intern Med 2001;135:566-76.

2.Kuismanen SA et al. Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. Am J Pathol 2000;156:1773- 9.

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