Microsatellite Instability (MSI) Testing - Lynch Syndrome

Lynch Syndrome

Introduction: Microsatellite instability high (MSI-H) phenotype is found in approximately 10-15% of sporadic colon, gastric and endometrial tumors, and in the majority of tumors from patients with Lynch Syndrome including hereditary nonpolyposis colorectal cancer (HNPCC). Inactivation of any of several mismatch repair system (MMR) genes, including MLH1, MSH2, MSH6 and PMS2 can result in microsatellite instability.

MSI has been shown to correlate with germline/inherited defects in MMR genes in families with HNPCC. In sporadic cases, MSI-H phenotype is due to MLH1 promoter methylation, rather than germline or somatic mutations. Hypermethylation of cytosine-phosphorothioate-guanine (CpG) islands of MLH1 promoter has been detected in majority of sporadic MSI-H tumors, and is rarely detected in familial cases with germline mutations. The aberrant hypermethylation of the MLH1 promoter and its consequent transcriptional silencing has been shown to be a primary mechanism of gene inactivation in sporadic MSI-H colorectal cancer.

Human gastrointestinal tumors with inactivated mismatch repair system have distinct molecular and clinicopathologic profiles, and are associated with favorable prognosis.

MMR genes are responsible for maintaining the integrity of DNA sequences during cell replication, consequently MMR defect fails to recognize errors introduced into DNA sequence. Defects in the MMR system lead to accumulation of slippage-induced frame shift mutations in genes with repetitive tracts in their coding sequences. The resulting genetic instability is characterized by variation in the lengths of microsatellite markers between tumor and normal tissue DNA that is called microsatellite instability. Microsatellites are highly polymorphic, repetitive nucleotide sequences widely used to detect loss of heterozygosity (LoH) in human cancers. The observation in a subset of tumors of new microsatellite alleles that were absent in matching normal DNA led to the discovery of "microsatellite instability phenotype," and corresponding inactivation of MMR genes.

Microsatellite unstable (MSI-H) tumors have more frequent loss of MMR protein expression. Immunohistochemistry (IHC) for MMR proteins complements PCR-based MSI testing, and can provide additional information, especially in cases where MMR protein expression is down regulated in absence of MSI-H phenotype.

Determining the MSI status of a colorectal cancer has clinical use for identifying patients with HNPCC/Lynch Syndrome. In addition, MSI status, regardless of whether the causative defect is inherited or sporadic, may have use in prognostic and therapeutic decision-making. MSI-H

colonic and gastric tumors are associated with favorable prognosis. There is also evidence to suggest that colorectal cancer patients with MSI-H tumors respond differently to fluorouracil-based chemotherapy.

Testing: Microsatellite Instability testing is performed on tumor tissue and corresponding normal tissue (as distant from tumor as possible). The testing consists of three parts:

1. **MSI Testing by PCR**: The MSI Analysis System kit (Promega, Madison, WI) consists of five nearly monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) for MSI determination and two polymorphic pentanucleotide markers (Penta C and Penta D) for sample identification. The assay involves fluorescent polymerase chain reaction (PCR) analysis of tumor samples and matching normal samples. Testing is performed on formalin fixed, paraffin embedded tissue sections. The limit of detection of the MSI assay has been determined to be approximately 1 cell in 10-100 total cells (10%).
2. **Immunohistochemistry (IHC):** for four MMR proteins (MLH1, MSH2, MSH6 and PMS2) is performed on formalin-fixed, paraffin-embedded tissue sections.
3. **MLH1 Promoter Methylation Detection:** This is a reflex test that will be performed if only loss of MLH1 is detected and other MMR proteins are intact. MLH1 Promoter Methylation Assay detects hypermethylation of the MLH1 promoter through the use of TaqMan technology and real time PCR of bisulfite-treated tumor DNA. Testing is performed on formalin-fixed, paraffin-embedded tissue sections. The limit of detection of MethyLight protocol was 1% of methylated DNA in the background of normal DNA.

Clinical Correlation: Test results should be interpreted in context of clinical findings, family history and other laboratory data. Negative result of MSI testing on colonic polyps does not entirely exclude possibility of Lynch Syndrome. Negative results cannot rule out the possibility that this individual's tumor is due to inherited defect in another gene not involved in mismatch repair. Genetic counseling may be recommended in some cases.

Reported: 5-7 business days

Sample requirements: The presence of adequate tumor in the material submitted for analysis should be confirmed by a surgical pathologist. A section from archival paraffin material or frozen surgical biopsies should be confirmed to contain > 50% tumor by a surgical pathologist. If the submitted material for analysis contains < 50% of tumor, areas of predominant tumor will be macrodissected using a scalpel to trim away non-neoplastic areas

- Formalin-fixed, paraffin-embedded tissue
- Tissue sections on charged slides (15 slides)

IMPORTANT: Tumor tissue and corresponding non-malignant tissue are tested in parallel.

CPT Codes: MSI: 81301, 88381 may apply; IHC: 88342; MLH1: 81401

Ship Specimens to:

Henry Ford Center for Precision Diagnostics
Henry Ford Hospital
Clinic Building, K6, Core Lab E-655
2799 W. Grand Blvd.
Detroit, MI 48202