

## Assay Summary

### **Microsatellite Instability Analysis Immunohistochemistry Analysis (MLH1, MSH2, MSH6, and PMS2) Hereditary Nonpolyposis Colorectal Cancer**

#### *Synopsis*

Inherited (germline) mutations in several genes involved in DNA mismatch repair are the major cause of hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. A characteristic of HNPCC tumors is microsatellite instability (MSI). Detection of microsatellite instability in a tumor sample will increase the probability of detecting a germline mutation in a DNA mismatch repair gene from the patient sample. Thus, MSI analysis is usually performed prior to proceeding with full mutation analysis of the mismatch repair genes, MLH1, MSH2, MSH6 and PMS2.

Immunohistochemical (IHC) analysis of suspected HNPCC tumor samples has come into favor as a means of screening patients who may carry a mutation or a deletion in one of the mismatch repair (MMR) genes. The most common of these are MLH1, MSH2, MSH6 and PMS2. Recent studies have shown that IHC has a sensitivity of approximately 89%, and can also help predict which MMR gene is mutated<sup>1</sup>. This could help guide the clinician or counselor in choosing which MMR gene to analyze first. Additionally, cases have been reported where IHC testing indicated a loss of expression while no mutation was detected by sequencing. This could represent a large deletion in one of the MMR genes which would be missed by direct sequencing. Not only has IHC become a useful tool in its own right, but it has also complemented the utility of microsatellite instability testing very nicely. Christensen et al. found that about 92% of patients with an MMR mutation in MLH1 or MSH2 had an abnormality on either IHC or MSI<sup>2</sup>.

Please also see the assay summary for MLH1, MSH2, MSH6 and PMS2 gene mutation analysis.

#### *Indications for testing*

Individuals with a clear or suspected diagnosis of HNPCC [meeting the revised guidelines established by a National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome (“Revised Bethesda Guidelines”)<sup>3</sup>] are candidates for MSI and IHC testing. Those individuals meeting Bethesda Guidelines whose tumors show MSI and/or absence of one of the mismatch repair proteins by IHC testing are very likely to carry germline mutations in one of the mismatch repair genes. IHC testing can also be offered to patients with sporadic colorectal cancer who have demonstrated microsatellite instability. This test should be offered in the context of genetic counseling before and after testing.

#### *Methodology*

MSI: Amplification of five mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) and two pentanucleotide repeat markers (Penta C and Penta D). The mononucleotide markers are used for MSI determination, and the pentanucleotide markers are only used to detect potential sample mix-ups and/or contamination but NOT used in MSI classification. The amplification products are separated by fragment analysis on an automated fluorescent sequencer. MSI-high is defined as appearance of new alleles for at least two microsatellite markers. Microsatellite stability (MSS) is defined as no instability observed with at least five markers amplified successfully. Instability observed for only one marker when five markers are amplified successfully will be reported as a low level of microsatellite instability (MSI-low).

Immunohistochemistry: Slides are stained with antibodies against one of three mismatch repair gene proteins, MLH1, MSH2, MSH6 and/or PMS2. The slides are analyzed for the presence or absence of each of the proteins.

### **Performance**

MSI analysis is performed using Promega MSI Analysis System (Version 1.2), a commercial available fluorescent PCR-based assay to detect microsatellite instability<sup>4</sup>.

### **Limitations**

Both MSI and IHC analyses are dependent upon specimen quality and purity. For MSI, the determination of instability requires, among other things, comparison of matched normal and tumor samples and presence of instability in the markers used. The presence of MSI in a tumor is not necessarily due to HNPCC; nor does the absence of MSI rule out the possibility of HNPCC.

### **Specimen Requirements**

#### **Microsatellite Instability (MSI)**

We request formalin-fixed, paraffin-embedded tissue samples. Please submit:

- 6 slides of tumor sample (5 micron serial sections, unstained; 1 H/E stained)
- 6 slides of matched normal tissue (bordering tissue, normal lymph node(s), unstained; 1 H/E stained) -

Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes.

(blood sample is used as a back-up normal control in case normal control from tissue sample is unsuccessful) Ensure that the slides are clearly labeled with the patient name or identifier, date of birth and type of sample (tumor, normal). Place slides in appropriate containers to ensure against breakage. Alternatively, the paraffin blocks may be submitted.

#### **Immunohistochemistry (IHC)**

We request formalin-fixed, paraffin-embedded tissue samples. Ensure that the blocks are clearly labeled with the patient name or identifier, date of birth and type of sample (tumor, normal). A copy of the pathology report should accompany the tissue block. We prefer to receive tissue blocks, but if they are not available, we will accept unstained slides (please submit one slide per antibody to be tested plus one for H&E, cuts must be 4 microns thick). Place slides in appropriate containers to ensure against breakage.

### **Test Request Form (TRF)**

- a) A completed CMDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- b) [HNPCC Patient Information Form](#): Include a completed HNPCC Patient Information .

<i>Order Codes</i>	<i>CPT Codes</i>	<i>TAT</i>
MSI-P (Microsatellite Instability Analysis)	81301, 88381, G0452	4 wks
IHC-MLH1 (MLH1 Immunohistochemistry)	88323, 88342, G0452	2 wks
IHC-MSH2 (MSH2 Immunohistochemistry)	88323, 88342, G0452	2 wks
IHC-MSH6 (MSH6 Immunohistochemistry)	88323, 88342, G0452	2 wks
IHC-PMS2 (PMS2 Immunohistochemistry)	88323, 88342, G0452	2 wks
IHC-HNPCC (HNPCC Immunohistochemistry- MLH1, MSH2, MSH6, PMS2)	88323, 88342(x4) , G0452	2 wks

### **References**

1. Hendriks et al., (2003) Am. J. of Path. 162(2):469-477
2. Christensen et al., (2002) Cancer 95:2422-2430
3. Umar, A. et al., (2004) J. Natl. Cancer Inst. 96:261-268
4. Bacher J.W. et al. (2004) Dis Markers. 20(4-5):237-250