

COLARIS[®] Technical Specifications

Myriad Genetic Laboratories, Inc. Updated: February 2009

TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:

Description of Analysis

Full sequence analysis: Full sequence determination of the *MLH1* gene is performed in both forward and reverse directions of approximately 2,300 base pairs comprising 19 exons and approximately 560 adjacent non-coding intronic base pairs. Full sequence determination of *MSH2* is performed in both forward and reverse directions of approximately 2,800 base pairs comprising 16 exons and approximately 470 adjacent non-coding intronic base pairs. Full sequence determination of *MSH6* is performed in both forward and reverse directions of approximately 4,080 base pairs comprising 10 exons and approximately 290 adjacent non-coding intronic base pairs. The non-coding intronic regions of *MLH1*, *MSH2* and *MSH6* that are analyzed by sequence analysis do not extend more than 20 base pairs proximal to the 5' end and 10 base pairs distal to the 3' end of each exon.

COLARIS[®] Rearrangement Test: The *MLH1* and *MSH2* genes are tested for large rearrangements that are not detected by sequence analysis. All coding exons of *MLH1* and *MSH2* and their respective promoters are examined for evidence of deletions and duplications by quantitative multiplexed endpoint PCR analysis. Analysis of both *MLH1* and *MSH2* is performed even if the test request is for *MLH1* or *MSH2* rearrangements only.

Comprehensive COLARIS[®]: Comprehensive COLARIS testing can be performed using full sequence analysis of the *MLH1*, *MSH2* and *MSH6* genes, together with rearrangement testing of *MLH1* and *MSH2* by quantitative multiplex endpoint PCR analysis. Other combinations of *MLH1*, *MSH2*, or *MSH6* testing can be performed as ordered on the test request form

Single Site COLARIS[®]: DNA sequencing analysis is performed for a targeted gene region containing the specified variant in *MLH1*, *MSH2* or *MSH6*. Quantitative multiplex PCR analysis of both *MLH1* and *MSH2* is performed for all single site mutation analyses of large rearrangements.

Description of Method:

Blood samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from white cells isolated from each sample.

Full sequence analysis: Aliquots of patient DNA are each subjected to polymerase chain reaction (PCR) amplification reactions. The amplified products are each directly sequenced in forward and reverse directions using fluorescent dye-labeled sequencing primers. Chromatographic tracings of each amplicon are analyzed by a proprietary computer-based review followed by visual inspection and confirmation. Genetic variants are detected by comparison with a consensus wild-type sequence constructed for each gene. All potential clinically significant variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above.

MLH1 and *MSH2* large rearrangement analysis (COLARIS Rearrangement Test): Genomic DNA from patients is analyzed by multiplexed quantitative PCR assays to determine copy number abnormalities indicative of deletion or duplication mutations across the *MLH1* and *MSH2* genes. Twelve fluorescently labeled multiplex PCR reactions are designed to interrogate all exons and the respective promoters of *MLH1* and *MSH2*, with a minimum of two amplicons per target region. Proprietary software analysis is used to normalize the copy number of individual amplicons in the *MLH1* against the *MSH2* genes, plus two control genes. All potential mutations are independently confirmed.

Performance Characteristics:

Analytical specificity: The incidence of a false report of a genetic variant or mutation resulting from technical error is considered negligible because of independent confirmation of all genetic variants (see above). No false-positive results were identified by the sequencing method described above in a sample set consisting of thirty-two DNA samples obtained from low-risk individuals that were analyzed for *MLH1* and *MSH2* and thirty-six DNA samples obtained from low-risk individuals that were analyzed for *MSH6*. In addition, no false positives results were obtained through the large rearrangement testing process that uses

multiplex quantitative PCR analysis and confirmatory testing on a set of 214 individual samples that were previously examined for deletions and duplications in *MLH1* and *MSH2* by Southern analysis.

Analytical sensitivity: Failure to detect a genetic variant or mutation in the analyzed DNA regions may result from errors in specimen handling and tracking, amplification and sequencing reactions, or computer-assisted analysis and data review. The sequencing method described above accurately identified each of seventy-four mutations in *MLH1* and *MSH2* and twenty-seven mutations in *MSH6* in samples that had been analyzed previously by independent laboratories. In addition, the large rearrangement testing process that uses quantitative multiplex endpoint PCR analysis and confirmatory testing correctly identified all 55 positives among 214 samples that were previously examined by alternative methods for deletions and duplications in *MLH1* and *MSH2*.

Limitations of method: There may be limited portions of *MLH1*, *MSH2*, or *MSH6* for which sequence determination can be performed only in the forward or reverse direction. Unequal allele amplification may result from rare interfering polymorphisms. The COLARIS[®] Rearrangement Test described above will detect unbalanced genomic rearrangements involving the promoter and coding regions of *MLH1* and *MSH2*. These assays will not detect some types of errors in RNA transcript processing.

Description of Nomenclature:

All mutations and genetic variants are named according to the convention of Beaudet and Tsui. (Beaudet AL, Tsui LC. A suggested nomenclature for designating mutations. *Hum Mut* 1993; 2:245-248). Nucleotide numbering starts at the first translated base of *MLH1*, *MSH2*, and *MSH6*.

Interpretive Criteria:

“Positive for a deleterious mutation”: Includes clinically significant nonsense and frameshift mutations that prematurely truncate the protein. In addition, specific missense mutations and non-coding intervening sequence (IVS) mutations are recognized as deleterious on the basis of data derived from linkage analysis of high risk families, functional assays, statistical analysis, biochemical evidence and/or demonstration of abnormal mRNA transcript processing.

Deletions and duplications of an entire exon(s) identified by the COLARIS Rearrangement Test may also be interpreted to be deleterious. Deleterious large genomic rearrangements include single exon and multi exonic deletions and duplications that are out of frame. In frame deletions/duplications are interpreted on an individual basis and the specific evidence supporting the classification of these mutations is included in the individual patient report.

“Genetic variant, suspected deleterious”: Includes genetic variants for which the available evidence indicates a likelihood, but not proof, that the mutation is deleterious. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

“Genetic variant, favor polymorphism”: Includes genetic variants for which available evidence indicates that the variant is highly unlikely to contribute substantially to cancer risk. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

“Genetic variant of uncertain significance”: Includes missense mutations and mutations that occur in analyzed intronic regions whose clinical significance has not yet been determined. These also include nonsense and frameshift mutations that occur very close to the normal stop codon, unless otherwise documented.

A genetic variant of uncertain significance in *MLH1*, *MSH2* or *MSH6* is considered less likely to be deleterious if it has been observed in one or more individuals with a known deleterious mutation.

“No deleterious mutation detected”: Includes genetic variants for which published data demonstrate absence of substantial clinical significance. Also includes variants in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and base pair alterations in non-coding portions of

the gene that have been demonstrated to have no deleterious effect on the length or stability of the mRNA transcript.

There may be uncommon genetic abnormalities in *MLH1*, *MSH2*, and *MSH6* that will not be detected by COLARIS® and *MSH6* analysis (see **Limitations of method**, above). This analysis, however, is believed to rule out the majority of abnormalities in these genes, which are believed to be responsible for most hereditary nonpolyposis colorectal cancer (HNPCC). Data on polymorphic variants are available upon request.

“Specific variant/mutation not identified”: Indicates that specific and designated mutations or variants are not present in the individual being tested.

Change of interpretation and issuance of amended reports:

Whenever there is a change in the interpretation of a patient’s test result, an amended report will automatically be provided by Myriad Genetic Laboratories.