COLARIS® Technical Specifications
Myriad Genetic Laboratories, Inc. Updated: February 5, 2013

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

Description of Analysis

COLARIS®: COLARIS® testing can be performed using full sequence analysis of the *MLH1*, *MSH2*, *MSH6, PMS2,* and *MYH* genes, together with rearrangement testing of *MLH1, MSH2, MSH6, MYH* and *EPCAM* by microarray-CGH analysis, and MLPA analysis for *PMS2*. Other combinations of testing can be performed as ordered on the test request form.

Full sequence analysis: Full sequence determination of *MLH1* 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. Full sequence determination of *PMS2* is performed in both forward and reverse directions of approximately 3039 base pairs comprising 15 exons and approximately 450 adjacent non-coding intronic base pairs. Full sequence determination of *MYH* is performed in both forward and reverse directions of approximately 1608 base pairs comprising 16 exons and approximately 450 adjacent non-coding intronic base pairs. The non-coding intronic regions of *MLH1*, *MSH2*, *MSH6, PMS2,* and *MYH* 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.

Large rearrangement analysis: The *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM* (also known as *TACSTD1*), and *MYH* genes are tested for large rearrangements that are not detected by sequence analysis. All coding exons of *MLH1*, *MSH2*, *MSH6*and their respective promoters and the clinically relevant 3' region of the *EPCAM* gene are examined for evidence of deletions and duplications by microarray comparative genomic hybridization analysis (microarray-CGH). All coding exons of *MYH* and its limited flanking intron regions are examined for evidence of deletions and duplications by microarray comparative genomic hybridization analysis (microarray-CGH). Exons of the *PMS2* gene are examined for evidence of deletions and duplications by Multiplex Ligation-Dependent Probe Amplification (MLPA). The MLPA reagents used for these analyses have not been approved or cleared by the FDA, however Myriad Genetic Laboratories has validated the performance characteristics of these tests in its lab facility.

Single Site COLARIS®: DNA sequencing analysis is performed for a targeted gene region containing the specified variant in *MLH1*, *MSH2, MSH6, PMS2* or *MYH*. Sequence analysis of exons 1-5 and 11-15 will be performed for all *PMS2* single site mutations in these regions; however, results will only be reported for the single site ordered unless other reportable variants are identified during analysis. Microarray-CGH analysis is performed for all single site large rearrangements in *MLH1*, *MSH2*, *MYH, MSH6* and the 3' terminal region of *EPCAM*. MLPA is performed for all single site mutation analyses of large rearrangements in the *PMS2* gene. In some cases, long range PCR analysis and/or sequencing of the resulting PCR product is used to detect specific, previously reported insertions in *MLH1* and *MSH2*.

Description of Method:

Patient samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from peripheral blood samples or buccal mouthwash samples, submitted for molecular testing.

Full sequence analysis: Aliquots of patient DNA are each subjected to Polymerase Chain Reaction (PCR) amplification to generate exon-specific amplicons that can be sequenced directly. A long range and nested PCR approach is utilized for portions of *PMS2* to avoid pseudogenes. The amplified products are each sequenced in forward and reverse directions using fluorescent dye-labeled sequencing primers. Electropherogram tracings of each amplicon are analyzed by a proprietary computer-based review system followed by visual inspection and confirmation of all clinically significant variants. 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, ***MSH2***, ***MSH6, MYH,*** **and** ***EPCAM*** **Large Rearrangement Analysis:** Genomic DNA from patients is analyzed by microarray-CGH analysis to determine copy number abnormalities indicative of deletion or duplication mutations across the *MLH1*, *MSH2, MSH6,* and *MYH* genes as well as the 3' terminal region of the *EPCAM* gene.

Approximately 1200 probes have been designed to interrogate all coding exons, limited flanking intron regions, and the respective promoters of *MLH1, MSH2* and *MSH6*; and exons 2-3, 8-9 and the 3'UTR of *EPCAM*. Approximately 220 probes have been designed to interrogate all coding exons, and limited flanking intron regions of *MYH*. Each probe is analyzed using proprietary software that compares the ratio of bound patient DNA to that of a reference DNA to indicate regions of altered copy number. The microarray design includes probes to detect deletions and duplications in multiple genes tested by MGL; however, a data masking feature is used to limit the analysis only to specific genes for which testing has been requested.

Patient samples positive for deletions or duplications are confirmed by repeat microarray analysis of the genes.

PMS2 **Large Rearrangement Analysis:** Aliquots of patient DNA are analyzed by MLPA. All potential mutations are confirmed by repeated analysis.

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 32 DNA samples obtained from low-risk individuals that were analyzed for *MLH1* and *MSH2,* 36 DNA samples obtained from low-risk individuals that were analyzed for *MSH6,* and 305 DNA samples obtained from individuals that were analyzed for *PMS2* . No false positive results were obtained through the large rearrangement testing process that used microarray-CGH on a set of 327 individual samples. For *MYH* large rearrangement analysis, no false positive results were obtained through the large rearrangement testing process that uses microarray-CGH on a set of 309 individual DNA samples. In addition, no false positive results were identified through MLPA analysis on a set of 36 individual samples for *PMS2* that were previously examined for deletions and duplications.

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 74 mutations/variants in *MLH1* and *MSH2*, 27 mutations/variants in *MSH6,* and 5 deleterious mutations among 1535 total sequence variants in *PMS2* in samples that had been analyzed previously by independent laboratories or Myriad Genetic Laboratories, Inc. The large rearrangement testing process using microarray-CGH correctly identified all 88 positives among 327 samples that were previously examined for deletions and duplications in *MLH1, MSH2, MSH6* and the 3'terminal region of *EPCAM* by quantitative multiplex PCR and/or MLPA. In addition, the large rearrangement testing processes that use MLPA analysis correctly identified all 5 *PMS2* positives among 36 samples that were previously analyzed by independent laboratories or Myriad Genetic Laboratories, Inc. The large rearrangement testing process, using microarray-CGH correctly identified a homozygous *MYH* deletion (previously detected by long range PCR analysis and sequencing), tested in duplicate, and a synthetic positive sample tested in four replicates, among 309 samples tested in our validation study. The

synthetic positive sample was created from genomic DNA that was digested with specific restriction enzymes.

Limitations of method: There may be limited portions of *MLH1, MSH2, MSH6, PMS2,* or *MYH* for which sequence determination can be performed only in the forward or reverse direction. Unequal allele amplification may result from rare interfering polymorphisms. The large rearrangement analyses described above will detect deletion and duplication rearrangements involving the promoter and coding exons of *MLH1*, *MSH2, MSH6, PMS2, MYH* and the clinically relevant 3' region of *EPCAM*. These assays will not detect some types of errors in RNA transcript processing, regulatory mutations, or balanced rearrangements (i.e. inversions). Insertions that do not result in duplications will generally not be detected by microarray-CGH.

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, MSH6, PMS2,* and *MYH*.

Interpretive Criteria:

The classification and interpretation of all variants identified in the assay reflects the current state of scientific understanding at the time the report is issued. In some instances, the classification and interpretation of variants may change as scientific information becomes available.

"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 variants and variants 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*, *MSH6,* or *PMS2* 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. Data on polymorphic variants are available upon request.

There may be uncommon genetic abnormalities in *MLH1, MSH2, MSH6, PMS2,* and *MYH* that will not be detected (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) and *MYH*-associated polyposis (MAP). Data on polymorphic variants are available upon request.

"Positive for two *MYH* mutations": Includes observations of two *MYH* mutations, or observations of two alleles of one mutation. The presence of two *MYH* mutations has been documented in recent literature to be associated with colorectal polyposis and cancer.

Causal mutations include nonsense and frameshift mutations, as well as specific missense mutations and non-coding intervening sequence (IVS) mutations recognized as deleterious on the basis of data derived from functional assays, biochemical evidence, demonstration of abnormal mRNA transcript processing and/or segregation analysis in families.

Deletions and duplications of an entire exon(s) identified by microarray-CGH may also be interpreted to be causal mutations. 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.

"Positive for two *MYH* mutations, clinical significance uncertain": Includes observations of two *MYH* mutations but it cannot be determined from this analysis alone whether these two mutations are on opposite alleles. Testing one of this patient's parents or children will determine if these mutations are on opposite alleles, thereby indicating if this patient is at increased risk of colorectal polyposis and cancer. If these mutations are on the same allele, it is currently unknown if this patient is at some measure of increased risk for colorectal polyposis and cancer.

"One *MYH* mutation detected, colorectal polyposis and cancer risk unknown": Includes observations of one allele of a causal mutation. It is currently unknown whether individuals who carry a single *MYH* mutation are at some measure of increased risk for colorectal polyposis and cancer. Patients with one *MYH* mutation detected through the *MYH* mutation panel will automatically receive full sequence analysis of the *MYH* gene.

"Specific variant/mutation not identified": Indicates that specific and designated mutations or variants are not present in the individual being tested. If one (or rarely two) specific deleterious mutations have been identified in a family member, a negative analysis for the specific mutation(s) indicates that the tested individual is likely at the general population risk of developing Lynch Syndrome-associated cancers.

Change of mutation/variant classification and issuance of amended reports: Whenever there is a change in the classification of a mutation/variant within a patient's test result, an amended report will - be provided by Myriad Genetic Laboratories.