COLARIS AP® Technical Specifications
Myriad Genetics, Inc.
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**Description of Analysis**

**COLARIS AP®**: COLARIS AP® testing can be performed using full sequence analysis of the APC and MUTYH genes, together with rearrangement testing of APC and MUTYH by microarray-CGH analysis. Other combinations of testing can be performed as ordered on the test request form.

**Full sequence analysis**: Full sequence determination of APC is performed in both forward and reverse directions of approximately 8532 base pairs comprising 15 exons and approximately 420 adjacent non-coding intronic base pairs. Full sequence determination of MUTYH 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. Gene coding regions and portions of non-coding intronic regions are analyzed by sequence analysis and typically do not extend more than 20 base pairs (bp) before and 10 bp after each exon and may be adjusted based on the presence of either potentially significant variants or highly repetitive sequences.

**Large rearrangement analysis**: All exons and promoter regions of APC and all exons of MUTYH are examined for evidence of deletions and duplications using microarray comparative genomic hybridization analysis (microarray-CGH).

**Single Site COLARIS AP®**: DNA sequencing analysis is performed for a targeted gene region containing the specified variant in APC or MUTYH. Microarray-CGH analysis is performed for all requests for single site mutation analysis of a large rearrangement in APC or MUTYH. In some cases, long-range PCR analysis and/or sequencing of the resulting PCR product is used to detect specific, previously reported insertions in APC or MUTYH.

**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 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.

**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 APC and MUTYH genes. Approximately 800 probes have been designed to interrogate all coding exons, limited flanking intron regions, and the respective promoters of APC. Approximately 220 probes have been designed to interrogate all coding exons, and limited flanking intron regions of MUTYH. 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.

**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). The incidence of a false report of a genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%). For APC large rearrangement analysis no false positive results were obtained through the large rearrangement testing process that uses microarray-CGH on a set of 307 individual samples. These included 7 samples that were previously examined for deletions and duplications in APC by Southern blot analysis and MLPA. For MUTYH 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.

**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 rate of such errors is estimated from validation studies to be less than one percent (<1%). The analytical sensitivity of DNA sequencing performed in both directions is estimated to be >99%. The large rearrangement testing process, using microarray-CGH correctly identified all 7 samples that were positive for an APC large rearrangement, previously detected by Southern blot analysis and MLPA. The large rearrangement testing process, using microarray-CGH correctly identified a homozygous MUTYH 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.
**Interpretive Criteria**

**Functional Variant Interpretations**

A functional interpretation is assigned to each variant identified. This interpretation reflects whether or not the variant is predicted to result in a significant change to normal protein production and/or function. It may not necessarily reflect cancer risk (see Clinical Variant Interpretations).

**Deleterious mutation**: Includes most nonsense and frameshift mutations that occur at/or before the last known deleterious amino acid position of the affected gene. 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, biochemical evidence, statistical evidence, and/or demonstration of abnormal mRNA transcript processing.

**Genetic variant, suspected deleterious**: Includes genetic variants for which the available evidence indicates a high likelihood, but not definitive proof, that the mutation is deleterious. The specific evidence supporting an interpretation will be summarized for individual variants on the Genetic Test Result.

**Genetic variant of uncertain significance**: Includes missense variants and variants that occur in analyzed intronic regions whose functional significance has not yet been determined, as well as nonsense and frameshift mutations that occur distal to the last known deleterious amino acid positions of the affected genes.

**Genetic variant, favor polymorphism** and **Genetic variant, polymorphism**: Includes genetic variants for which available evidence indicates that the variant is highly unlikely to alter protein production and/or function or contribute substantially to cancer risk. Variants of this type are not reported.

**Clinical Variant Interpretations**

A clinical interpretation is assigned to each variant identified. This interpretation reflects whether or not the variant is predicted to be associated with significantly increased risk for one or more cancer types.

**High Cancer Risk**: Includes genetic variants for which absolute cancer risk is predicted to be higher than ~5% with a ~3-fold or higher increased relative risk over that of the general population. Strong data is available to support gene-specific risk estimates, although actual variant-specific risks may differ.

**Elevated Cancer Risk**: Includes genetic variants for which there is sufficient data to indicate that the specific variant increases risk for one or more cancers over that of the general population. These risks may be lower than those conveyed by “High Cancer Risk” variants or may be supported by less solid, but still significant, data.

**Clinical Significance Unknown**: Includes genetic variants for which there is insufficient data to determine whether or not the variant is associated with increased cancer risk.

**Clinically Insignificant**: Includes genetic variants for which available evidence indicates that the variant is highly unlikely to significantly contribute to cancer risk. Variants of this type are not reported.

**Special Interpretation**: Includes genetic variants with more complex clinical interpretations. Specific interpretations will be provided for each variant on the Genetic Test Result.

**Summary Interpretations**

**Clinically significant mutation identified**: Includes Genetic Test Results in which one variant, which is associated with the potential to alter medical intervention, was identified.”

**No clinically significant mutation identified**: Includes Genetic Test Results in which either no genetic variants were identified or all identified variants were classified as “Clinical Significance Unknown” or “Clinically Insignificant.”

**Multiple Clinically Significant Mutations Identified**: Includes Genetic Test Results in which multiple genetic variants, which are associated with the potential to alter medical intervention, were identified.

Additionally, it includes observations of two MUTYH mutations that are on opposite alleles, or observations of two alleles of one mutation. The presence of two MUTYH mutations...
has been documented 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.

“Multiple MUTYH Clinically Significant Mutations Identified”: Includes observations of two MUTYH 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 may determine if these mutations are on opposite alleles, which can help delineate the colorectal polyposis and cancer risk in this patient. Patients with two mutations on opposite alleles have a higher colorectal polyposis and cancer risk than patients with two mutations on the same allele.

“Single MUTYH Clinically Significant Mutation Identified”: Includes observations of one allele of a causal mutation. Individuals who carry a single MUTYH pathogenic variant may have a small increased risk for colorectal cancer; the risk for colorectal polyposis in carriers of a single MUTYH pathogenic variant is currently unknown.

Change of interpretation and issuance of amended reports

The classification and interpretation of all variants identified in the assay reflect the current state of scientific understanding at the time the report is issued. In some instances, the classification and interpretation of such variants may change as new scientific information becomes available. Whenever there is a clinically significant change in the classification of a variant within a patient’s test result, an amended report will be provided by Myriad Genetic Laboratories.

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<th>Gene Name</th>
<th>Transcript ID</th>
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<tr>
<td>APC</td>
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<td>MUTYH (alpha3)</td>
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