

COLARIS AP[®] Technical Specifications

Myriad Genetic Laboratories, Inc. Updated: 29 Aug 2005

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

Description of Analysis

Comprehensive COLARIS AP[®]:

APC: Full sequence determination in both forward and reverse directions of approximately 8532 base pairs comprising 15 exons and approximately 420 adjacent non-coding intronic base pairs.

The non-coding intronic regions of *APC* that are analyzed 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: All exons of *APC* are examined for evidence of deletions and duplications by standard Southern blot methods.

MYH: DNA sequence analysis of specific portions of *MYH* exons 7 and 13 designed to detect the mutations Y165C and G382D. If just one copy of either of these mutations is detected, full sequencing of the *MYH* gene is performed automatically (see *MYH* Technical Specifications).

Single Site COLARIS AP[®]: DNA analysis, by direct sequence or Southern blot methods, for a specified mutation in *APC*. Southern blot analysis of all exons of *APC* is performed for all requests for single site mutation analysis of a large rearrangement.

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.

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 genetic variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above.

Southern blot analysis: Aliquots of genomic DNA are digested individually with three restriction enzymes or combinations of enzymes for *APC* analysis. Digested DNA is electrophoresed in an agarose gel, transferred to a membrane, and hybridized with a gene-specific probe labeled with ³²P. The probe binds to all fragments containing coding sequences of that gene. Autoradiographs and phosphorimages are produced and analyzed for the presence of novel bands and for fragment dosage, from which it is determined which, if any, exons have been deleted or duplicated. Positive and negative controls are run with each batch. 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 seen in a sample set consisting of thirty-three DNA samples obtained from individuals that were analyzed by the sequencing method described above. In addition, no false positive results were seen in Southern blot analysis of a set of twenty-two samples that were previously examined for deletions and duplications in *APC*.

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 twenty-four mutations in *APC* samples that had been analyzed previously by independent laboratories. In addition, seven samples that were previously examined by alternative methods for deletions and duplications in *APC* were correctly identified by the Southern blot method described above.

Limitations of method: There may be limited portions of *APC* for which sequence determination can be performed only in the

forward or reverse direction. Unequal allele amplification may result from rare polymorphisms under primer sites. This assay will not detect some types of errors in RNA transcript processing.

Analysis of *MYH* includes analysis for Y165C and G382D, and does not rule out the possibility of other mutations. Unequal allele amplification may result from rare polymorphisms under primer sites.

Additional information about performance characteristics of the *MYH* analysis is available in the *MYH* Technical Specifications.

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 *APC* and *MYH*.

Interpretive Criteria:

“Positive for a deleterious mutation”: Includes all nonsense and frameshift mutations in *APC* that occur at or before amino acid 2644 (based on documentation of deleterious mutations in *APC*).

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 and/or demonstration of abnormal mRNA transcript processing.

Deletions and duplications of an entire exon(s) identified by Southern blot analysis 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, as well as nonsense and frameshift mutations that occur distal to amino acid position 2644 in *APC*.

A genetic variant of uncertain significance in *APC* is considered less likely to be deleterious if it has been observed in one or more individuals with a known deleterious mutation in the same gene.

“No deleterious mutation detected”: Includes non-truncating genetic variants observed at an allele frequency of approximately 1% of a suitable control population (providing that no data suggest clinical significance), as well as all genetic variants for which published data demonstrate absence of substantial clinical significance. Also includes mutations 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 *APC* that will not be detected by Colaris APSM (see **Limitations of method**, above). This analysis, however, is believed to rule out the majority of abnormalities in this gene, which is believed to be responsible for most Familial Adenomatous Polyposis (FAP) and attenuated FAP (AFAP).

Y165C and G382D in *MYH* were not detected. There may be other mutations in *MYH* that were not detected because this test was designed to detect Y165C and G382D.

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

individual being tested. If a specific deleterious mutation has been identified in a family member, a negative analysis for the specific mutation indicates that the tested individual is at the general population risk of developing those cancers and benign findings associated with FAP and AFAP.

“Positive for two *MYH* mutations”: Includes observations of Y165C and G382D together, or observations of two alleles of Y165C or G382D. The presence of these two *MYH* mutations has been documented in recent literature to be associated with colorectal polyposis and cancer.

“One *MYH* mutation detected, colorectal polyposis and cancer risk unknown”: Includes observations of one allele of Y165C or G382D. 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 will automatically receive full sequence analysis of the *MYH* gene (see *MYH* Technical Specifications).

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.