MELARIS® Technical Specifications

Myriad Genetic Laboratories, Inc. Updated: November 2019

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

Description of Analysis

Comprehensive MELARIS®: The gene coding regions and portions of non-coding intronic regions of the p16 isoform (NM_000077.4) of *CDKN2A* is analyzed by sequence analysis and typically does not extend more than 20 base pairs (bp) before and 10 bp after each exon. This region may be adjusted based on the presence of either potentially significant variants or highly repetitive sequences.

Single Site MELARIS®: DNA sequence analysis for a specified variant in p16.

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. 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.

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). In addition, no false-positive results were seen in a sample set consisting of 75 DNA samples that were analyzed by the method described above.

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 method described above accurately identified 66 of 67 mutations in p16 mutation positive samples that had been analyzed previously by independent laboratories.

Limitations of method: There may be limited portions of p16 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 large chromosomal alterations such as deletions of complete exons or genes, or some types of errors in RNA transcript processing.

Description of Nomenclature:

All mutations and genetic variants are referenced to cDNA positions on their respective primary transcripts and named according to the HGVS convention (J Mol Diagn. 2007 Feb;9(1):1-6). The reference sequence used for variant naming is hg19/GRCh37. Nucleotide numbering starts at the first translated base of p16.

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.

Two mutations detected may be labelled "Positive for two mutations" or "Positive for two mutations, clinical significance uncertain" depending on whether test data can or cannot confirm that the mutations are on opposite alleles, respectively.

Clinical Variant Interpretations

A clinical interpretation is assigned to each variant identified. This interpretation reflects whether 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.

“Clinical Significance Unknown”: Includes genetic variants for which there is insufficient data to determine whether 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 or more genetic variants, which are associated with the potential to alter medical intervention, were 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.”

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. When 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.