

MELARIS[®] Technical Specifications

Myriad Genetic Laboratories, Inc. Updated: February 2009

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

Description of Analysis

Comprehensive MELARIS[®]: Full DNA sequence determination in both forward and reverse directions of approximately 471 base pairs comprising the coding region of 3 exons of the p16 gene and approximately 95 base pairs of adjacent non-coding sequence. The non-coding intronic regions of *p16* are analyzed to identify consensus splice donor and acceptor sequences and include 20 base pairs proximal to the 5' end of exons 2 & 3 and 10 base pairs distal to the 3' end of exons 1 & 2. In addition 35 base pairs of 5'-untranslated region occurring upstream of the initiation codon are evaluated to specifically identify a known mutation ("5'UTR-34G>T") that creates an aberrant start codon (Liu L. et. al. Mutation of the CDKN2A 5' UTR creates an aberrant initiation codon and predisposes to melanoma. *Nat Genet* 1999; 21:128-32). Also, a known mutation ("IVS2-105A>G") which activates a cryptic splice donor is assessed (Harland M, et. al. A deep intronic mutation in CDKN2A is associated with disease in a subset of melanoma pedigrees. *Hum Mol Genet* 2001; 10:2679-86).

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 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 p16.

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.

"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 120.

A genetic variant of uncertain significance in *p16* 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 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.

There may be uncommon genetic abnormalities in *p16* that will not be detected by MELARIS[®] (see **Limitations of method**, above). 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 :

If and whenever there is a change in the clinical interpretation of a specific reported variant, an amended test report will automatically be provided by Myriad Genetic Laboratories.