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

**Description of Analysis:**
The Myriad COLARIS® test includes germline DNA-based next generation sequencing (NGS) analysis and copy number variation (CNV) testing of a panel of genes related to hereditary cancer syndromes including colorectal and uterine. Sequence and CNV analysis is performed for the following genes: MLH1, MSH2, MSH6, MUTYH, PMS2. 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. For EPCAM, only copy number variation analysis is performed for defined regions.

**Description of Method:**
Patient samples are assigned a unique barcode for robotic-assisted continuous sample tracking. Genomic DNA is extracted and purified from peripheral blood samples, buccal saliva samples, or fibroblast samples submitted for molecular testing.

**DNA sequence analysis by NGS**
The samples are prepared through a hybridization capture based target-enrichment strategy for subsequent next generation sequencing. Aliquots of patient genomic DNA are fragmented. The fragmented DNA is built into a library by ligation of sequencing adaptors containing unique patient indices. This library is purified and then enriched for targets of interest through hybridization to a set of biotinylated probes which are then captured on streptavidin coated beads. Indexed samples are then pooled and loaded onto massively-parallel next generation sequencers for paired-end sequencing. Probe design and NGS data analysis were optimized for the analysis of genes with known pseudogene regions, with additional confirmatory testing as needed.

**NGS Data Analysis and Confirmation**
A combination of open source and laboratory-developed software is used for NGS data processing, which includes base-calling, alignment, variant identification, annotation, and generation of quality metrics. Genetic variants are reviewed by computer software and human reviewers. All clinically significant variants identified by NGS and regions that do not meet required NGS quality and coverage metrics are independently confirmed with repeat NGS or orthogonal, site-specific Sanger sequencing. Germline heterozygous sequence variants identified by NGS have allele frequencies between approximately 30% and 70%; homozygous sequence variants have allele frequencies above approximately 90%. NGS variants with intermediate frequencies are tested with repeat NGS or orthogonal, site-specific Sanger sequencing, and putative NGS variants below approximately 10% allele frequencies are not called. Where necessary due to PMS2 gene conversion, non-specific NGS is performed with modified thresholds and any potentially actionable variants are confirmed by site-specific Sanger sequencing nested from long-range PCR products.

**Copy Number Variation Analysis**
Patient samples undergo NGS dosage analysis to determine copy number abnormalities indicative of deletion or duplication mutations. NGS dosage analysis uses normalized read counts from sequencing data to determine gene copy number. Pseudogenes are avoided through assay design and alignment filters for NGS data analysis. Promoter regions of all genes undergoing full-gene sequence analysis are also analyzed for gross deletion or duplication. For NGS dosage analysis, the normalized ratio of each region of interest is compared across patients to identify regions of altered copy number. Limited analysis is also performed for additional structural variants (SV) and is included concurrently with the comprehensive CNV analysis for deletions and duplications. Samples are evaluated for a 10 Mb inversion mutation involving MSH2 exons 1-7. Additional transposon insertion detection is accomplished by a combination of direct analysis of NGS reads for inserted transposon sequence and by monitoring for the impacts of read misalignment due to large foreign sequence insertion. Due to the potential location and complexity of transposon insertions, not all events may be detected. Patient samples positive for CNVs or SVs are confirmed by repeat testing using one or more methods, which can include NGS dosage analysis, Multiplex Ligation-dependent Probe Amplification (MLPA), or PCR analysis.

**Alternative Methodologies**
In certain cases, testing for specific genes may be performed using alternative technologies to NGS, including Sanger sequencing or MLPA.

**Single Site Analysis**
DNA sequencing or copy number variation analysis is performed for the specified variant in MLH1, MSH2, MSH6, MUTYH, PMS2 or EPCAM. Single site testing for sequencing mutations may be performed using NGS or Sanger sequencing. When the single site mutation is a deletion or duplication mutation, NGS dosage analysis or MLPA is used. In some cases, long-range PCR analysis and/or sequencing of the resulting PCR product is used to detect specific, previously reported insertions.

**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 clinically significant genetic variants (see below). The incidence of a false report of a clinically significant genetic variant or mutation resulting from errors in specimen handling and tracking was assessed by performing a comparison of all eligible variants in 6,882 samples which underwent at least two independent DNA extractions and cycles of NGS testing. No evidence of sample switches was found during this comparison; therefore, the incidence of a false report of a clinically significant genetic variant or mutation is estimated to be <0.000033% (upper bound of the 95% CI).

**Analytical sensitivity**
Failure to detect a genetic variant 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 next-generation sequencing for genes in the myRisk test was 100% (>99.99%-100%, 95% C.L) and the analytical specificity was 100% (>99.99%-100%, 95% C.L) based on complete concordance of heterozygous and homozygous germline variant detection in comparative studies to validated reference methods. These studies were performed on 6,424 samples originating either from de-identified samples extracted from blood, saliva, or fibroblast, or well-characterized external reference samples (6 NIST Genome in a Bottle Consortium (GIAB), 2 Illumina platinum genomes (with one sample in common to GIAB samples), and 24 Broad Institute 1000 Genomes). A total of 306,649 heterozygous or homozygous sequence variants were successfully identified for genes in the myRisk test in these validation studies.

**Copy Number Variation Validation**
Validation studies for CNV detection using NGS dosage analysis were performed using DNA samples extracted from blood, saliva, and fibroblast samples. These samples included 545 that had previously tested positive for CNV mutations, which were all successfully detected by NGS dosage analysis for the genes in the myRisk panel. All reviewable results for CNVs were 100% concordant with the expected mutations.

Concurrent with comprehensive CNV validation, samples positive for a variety of previously identified transposon element insertions were also tested using NGS analysis. All 80/80 samples reviewed were concordant with the expected transposon insertion mutations.

**Test reproducibility**
Reproducibility and accuracy were assessed using a set of 4 well-characterized references samples from NIST Genome in a Bottle Consortium or Illumina platinum genomes. These samples were processed by NGS in triplicate within a batch and then repeated across three independent batches, to assess intra-batch and inter-batch assay reproducibility. All reviewable sequence results were 100% concordant.
Limitations of method
Unequal allele frequencies may result from certain DNA contexts, including repetitive or low complexity sequences. The presence of pseudogenes or gene conversion may complicate the detection of rare sequencing and copy number variation mutations in certain genes. Due to the potential location and complexity of transposon insertions, not all events may be detected. There may be uncommon genetic abnormalities such as specific insertions, inversions, and certain regulatory mutations that will not be detected by myRisk. This analysis, however, is believed to rule out the majority of abnormalities in the genes analyzed. Genetic testing results on blood or buccal salvia samples may not reflect the germline genetic status of patients with a hematologic malignancy, or patients who underwent allogeneic bone marrow transplants.

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. Transcript IDs are indicated on patient reports with their associated variants (Table 1). Allele differences have been documented at a limited number of nucleotide locations, based on the major/minor alleles observed upon testing and reference sequences used historically at Myriad Genetic Laboratories.

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.

In the case of genes with recessive risk transmission (MUTYH) these interpretations may be modified depending on the ability to determine whether the mutations are on opposite alleles. 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. If a single mutation is detected in one of these genes, an interpretation of “Carrier for a clinically significant mutation of a recessive condition” may be applied.

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 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. 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.

Gene features analyzed with myRisk Hereditary Cancer

Comprehensive myRisk testing includes the genes and transcripts listed in Table 1. Unless otherwise specified, all coding regions and portions of non-coding regions are analyzed for sequence variation. Analysis of flanking intronic regions typically do not extend more than 20 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. Coding regions and proximal promoter regions near the transcription start sites are analyzed for large deletions or duplications. Specific genes are tested only for sequence or CNVs within limited regions. Terminal EPCAM deletions that affect the adjacent MSH2 gene expression are associated with Lynch syndrome; only CNV analysis of the last two exons of EPCAM is performed.
Table 1: Transcript IDs associated with myRisk genes

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Transcript ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPCAM</td>
<td>NM_002354.2</td>
</tr>
<tr>
<td>MLH1</td>
<td>NM_000249.3</td>
</tr>
<tr>
<td>MSH2</td>
<td>NM_000251.2</td>
</tr>
<tr>
<td>MSH6</td>
<td>NM_000179.2</td>
</tr>
<tr>
<td>MUTYH (alpha5)</td>
<td>NM_001128425.1</td>
</tr>
<tr>
<td>MUTYH (alpha3)</td>
<td>NM_001048171.1</td>
</tr>
<tr>
<td>PMS2</td>
<td>NM_000535.5</td>
</tr>
</tbody>
</table>


References: