Myriad myRisk™ Hereditary Cancer Technical Specifications
Myriad Genetic Laboratories
Effective: 18 February 2016

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

Description of Analysis:

The Myriad myRisk™ Hereditary Cancer test includes germline DNA-based next generation sequencing (NGS) analysis of a panel of genes related to Hereditary Cancer. Large Rearrangement (LR) testing for deletions and duplications is performed primarily by NGS dosage analysis. Sequence analysis of the coding regions is performed using NGS for the following genes: *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A* (*p16* and *p14ARF*), *CHEK2, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53*. For the *EPCAM* gene, only large rearrangement analysis is performed (*EPCAM* deletions that affect adjacent *MSH2* gene expression are associated with Lynch syndrome). Portions of non-coding intronic regions are also analyzed by sequencing analysis and typically do not extend more than 20 base pairs (bp) proximal to the 5' end and 10 bp distal to the 3' end of each exon.

Description of Method:

Patient samples are assigned a unique bar-code for robotic-assisted continuous sample tracking. Genomic DNA is extracted and purified from either peripheral blood samples or buccal saliva samples submitted for molecular testing.

DNA sequence analysis by NGS
The samples are prepared through a PCR-based target-enrichment strategy for subsequent next generation sequencing. Aliquots of patient genomic DNA are sonicated. The fragmented DNA is dispersed in oil into picoliter- sized aqueous droplets that are merged with a dropletized target enrichment primer library. The resulting emulsion of microdroplets is subjected to PCR amplification. Emulsion PCR products are purified and subjected to secondary PCR to incorporate sequencing adaptors for NGS and indexing barcodes for individual sample tracking. Barcoded samples from up to 96 patients are pooled and loaded onto massively-parallel next generation sequencers for 2 x 150 base paired-end reads. Primer design and data analysis were optimized for NGS analysis of genes with known pseudogene regions. Supplementary workflows were developed to analyze the *PMS2* and *CHEK2* genes as described below.

Supplementary sample preparation and NGS of PMS2 and CHEK2
Long Range (LoRa) PCR is used for initial amplification of *PMS2* and *CHEK2* gene regions to avoid well-characterized pseudogenes. Aliquots of patient DNA are subjected to gene-specific LoRa amplification of: 1) *PMS2* exons 1-5; 2) *PMS2* exon 9; 3) *PMS2* exons 11-15; 4) *CHEK2* exons 10-14. The four separate LoRa amplicons are diluted and subjected to secondary PCR to incorporate sequencing adaptors for NGS and indexing barcodes for individual sample tracking. The barcoded samples are pooled and loaded onto massively-parallel next generation sequencers for 2 x 150 base paired-end reads.

NGS Data Analysis and Confirmation
A combination of commercial and laboratory-developed software is used for NGS data processing, which includes base-calling, alignment, variant identification, annotation, and quality metrics. Genetic variants are reviewed by computer software and human reviewers. The minimum depth of coverage used for sequence determination by NGS is 50x per base. All clinically significant variants identified by NGS and regions that do not meet NGS quality metrics are independently confirmed with orthogonal, site-specific Sanger sequencing.

Large Rearrangement Analysis
Genomic DNA from patients is analyzed by NGS dosage analysis to determine copy number abnormalities indicative of deletion or duplication mutations. Additionally, this method is used to evaluate samples for an Alu insertion in *BRCA2* exon 3, a Portuguese founder mutation, c.156_157insAlu. NGS dosage analysis uses normalized read counts from sequencing amplicons to determine gene copy number. Pseudogenes are avoided through primer design and alignment filters for NGS data analysis. For *PMS2* exons 12-15 and flanking regions, this approach is supplemented by dosage quantification involving previously defined paralogous sequence variants (PSVs) between *PMS2* and its highly homologous pseudogene *PMS2CL*. Approximately 2,000 amplicons for NGS are used to interrogate coding exons and limited flanking intron regions of tested genes. Functionally characterized promoter regions of certain genes are also analyzed for gross deletion or duplication (*APC, BMPR1A, BRCA1, BRCA2, MLH1, MSH2, MSH6, PTEN, SMAD4, STK11* and *TP53)*. For NGS dosage analysis, the normalized ratio of each amplicon is compared across patients to identify regions of altered copy number. Patient samples positive for deletions or duplications are confirmed by repeat testing using one or more methods, which can include NGS dosage analysis, Multiplex Ligation-dependent Probe Amplification (MLPA), or microarray comparative genomic hybridization (microarray-CGH) analysis. For microarray-CGH analysis, approximately 9,600 probes interrogate coding exons, limited flanking intron regions, and promoters of tested genes. Microarray probe design was optimized to avoid known pseudogene regions, which includes the use of flanking intron probes in certain genes. Probe signals are analyzed using laboratory developed software that compares the ratio of bound patient DNA to that of a differentially labeled reference DNA to identify regions of altered copy number. In addition to large rearrangements detected by NGS, a 10 Mb inversion mutation involving *MSH2* exons 1-7 is detected by targeted PCR and Sanger sequencing analysis across the 5' inversion breakpoint.

Single Site Analysis
DNA sequencing or large rearrangement analysis is performed for the specified variant in *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A* (*p16* and *p14ARF*), *CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11* or *TP53*. Single site testing for sequencing mutations is performed using Sanger sequencing. When the single site mutation is a deletion or duplication mutation, microarray–CGH analysis, 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 Validation Publication: Judkins et al. BMC Cancer (2015) 15:215 DOI 10.1186/s12885-015-1224-y

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 above). The incidence of a false report of a clinically significant genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%).

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 next-generation sequencing for genes in the myRisk test was 100% (99.96%-100%, 95% C.I.) and the analytical specificity was 100% (99.99%-100%, 95% C.I.) based on complete concordance in comparative studies to validated reference methods performed on 238 individual anonymized DNA samples extracted from blood or saliva with 9,303 identified sequence variants for genes in the myRisk test.

Large Rearrangement Validation
Validation studies for large rearrangement detection using NGS dosage analysis were performed using DNA samples extracted from blood and buccal saliva samples. These included 308 samples that had previously tested positive for large rearrangement mutations, which were all successfully detected by NGS dosage analysis of the genes in the myRisk panel. All reviewable results for large rearrangements were 100% concordant.

Test reproducibility
The 1st comparative analytical validation study included a reproducibility sample set comprised of 4 individual anonymized DNA samples extracted from blood, collectively carrying 199 sequence variants confirmed by Sanger sequencing. The 2nd study included a reproducibility sample set comprised of anonymized DNA extracted from 4 contributors who donated paired blood and saliva samples, collectively carrying 121 sequence variants which were found to be concordant between saliva and blood. In both studies, each of the 4 anonymized samples was sequenced by NGS in triplicate across three batches (i.e., 4 samples x 9 replicates each) which demonstrated 100% reproducibility.

Limitations of method
Unequal allele amplification may result from rare polymorphisms under PCR primer sites. The presence of pseudogenes may complicate the detection of rare sequencing and large rearrangement mutations in certain genes. 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 saliva samples may not reflect the germline genetic status of patients with a hematologic malignancy, or patients who underwent allogeneic bone marrow transplants. In such cases, please contact Medical Services to discuss re-submission of an appropriate sample type.

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). Transcript IDs are indicated on patient reports with their associated variants (Table 1).

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 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.

Table 1: Transcript IDs associated with myRisk genes

Gene Name	Transcript ID
APC	NM_000038.5
ATM	NM_000051.3
BARD1	NM_000465.3
BMPR1A	NM_004329.2
BRCA1	NM_007294.3
BRCA2	NM_000059.3
BRIP1	NM_032043.2
CDH1	NM_004360.3
CDK4	NM_000075.3
CHEK2	NM_007194.3
EPCAM	NM_002354.2
MLH1	NM_000249.3
MSH2	NM_000251.2
MSH6	NM_000179.2
MUTYH (alpha5) *MUTYH (alpha3)*	NM_001128425.1 NM_001048171.1
NBN	NM_002485.4
P14ARF	NM_058195.3
P16	NM_000077.4
PALB2	NM_024675.3
PMS2	NM_000535.5
PTEN	NM_000314.4
RAD51C	NM_058216.2
RAD51D	NM_002878.3
SMAD4	NM_005359.5
STK11	NM_000455.4
TP53	NM_000546.5

http://www.ncbi.nlm.nih.gov/refseq/

References:

Judkins T et al. *BMC Cancer* (2015) 15:215

https://www.myriadpro.com/