Myriad myRisk® Hereditary Cancer Technical Specifications
Myriad Genetic Laboratories
Effective: October 11, 2018

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 and large rearrangement (LR) testing for deletions and duplications of a panel of genes related to Hereditary Cancer. Sequence and LR analysis can be performed 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 *HOXB13, POLD1* and *POLE* only sequence analysis is performed. For *POLD1* and *POLE* only a portion of the genes are analyzed. 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 the *EPCAM* and *GREM1* genes, only large rearrangement analysis is performed for defined regions. In addition to this panel of genes, the comprehensive myRisk test also produces a combined riskScore™ result for breast cancer in eligible patients with no mutations associated with breast cancer. The riskScore™ result is generated by taking clinical and family history data, in the form of a Tyrer-Cuzick score, together with data generated from up to 86 weighted genetic markers throughout the genome.

Description of Method:

Patient samples are assigned a unique barcode 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 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 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. 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. Sequence variants identified by NGS with allele frequencies between 30% and 70% are called heterozygous and those above 90% are called homozygous. NGS variants with intermediate frequencies are tested with repeat NGS or orthogonal, site-specific Sanger sequencing, and putative NGS variants below 10% allele frequencies are not called. Where necessary due to *PMS2* gene conversion, non-specific NGS is performed and any potentially actionable variants are confirmed by site-specific Sanger sequencing nested from long-range PCR product.

Large Rearrangement 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. Additionally, samples are evaluated for an Alu insertion in *BRCA2* exon 3 (c.156_157insAlu) which is a Portuguese founder mutation, and a 10 Mb inversion mutation involving *MSH2* exons 1-7. Patient samples positive for large rearrangements 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. Microarray probe design was optimized to avoid known pseudogene regions and 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.

riskScore™ Analysis

Allele status at up to 86 genetic markers is collected during NGS sequencing. These data are weighted and combined with a Tyrer-Cuzick risk score based on personal and family history data[1]. This riskScore™ result has been validated solely in patients of White/Non-Hispanic ancestry, including Ashkenazi Jewish ancestry. riskScore™ is not valid, and may significantly over- or under-estimate risk, for women of other ancestries or who do not meet the other eligibility criteria listed below. Based on research at time of product launch, riskScore™ results are only calculated for women of solely White/Non-Hispanic[2,3,4] ancestry under the age of 85 and without a personal history of breast cancer, LCIS, hyperplasia, atypical hpyerplasia[5], or a breast biopsy of unknown results. riskScore™ results are not calculated if a woman or a blood relative is known to carry a mutation in a breast cancer risk gene (*BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, PALB2, CHEK2, ATM, NBN*, or *BARD1*). riskScore™ eligible patients will receive risk estimates based on riskScore™ analysis in addition to estimates based exclusively on the Tyrer-Cuzick model.

References:

1. Hughes *et al*. ASCO 2017
2. Mavaddat N, *et al*. J Natl Cancer Inst. 2015 Apr 8;107(5). PubMed PMID: 25855707
3. Michailidou K, *et al*. Nat Genet. 2013 Apr;45(4): 353-61. PMID: 23535729
4. Michailidou K, *et al*. Nat Genet. 2015 Apr;47(4):373-80. PubMed PMID: 25751625
5. Tyrer J, et al. Stat Med. 2004 23:1111-30. PMID: 15057881

Alternative Methodologies

In certain cases, testing for specific genes may be performed using alternative technologies to NGS, including Sanger sequencing, MLPA, or microarray-CGH analysis. The analysis of these genes will follow the technical specifications listed on www.MyriadPro.com/technical-specifications for BRAC*Analysis*®, Colaris®, and Colaris AP®.

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, GREM1, HOXB13, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, STK11* or *TP53*. 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, 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 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.I.) and the analytical specificity was 100% (>99.99%-100%, 95% C.I.) based on complete concordance in comparative studies to validated reference methods. These studies were performed on 703 anonymized DNA samples extracted from blood or saliva with 30,103 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 saliva samples. These samples included 205 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

Reproducibility and accuracy were assessed using a set of 5 anonymized samples with known sequencing and/or large rearrangement variants. 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 and large rearrangement 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 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 rare cases, testing blood-derived DNA may identify somatic sequence variants that display allele frequencies within the expected range for heterozygous germline variants (e.g., in the *TP53* gene). In the above 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). 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.

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.

Genes 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 LR variants within limited regions. Terminal *EPCAM* deletions that affect the adjacent *MSH2* gene expression are associated with Lynch syndrome; only LR analysis of the last two exons of *EPCAM* is performed. At least three unique duplications, which have been observed in the literature, leading to changes in expression of GREM1 have been reported in patients with Hereditary Mixed Polyposis Syndrome (HMPS). LR analysis of *GREM1* includes the upstream region overlapping the adjacent gene *SCG5*. Mutations in the exonuclease domains of *POLD1* and *POLE* are associated with increased risk of hereditary colorectal cancer and polyposis; only sequence analysis of the exons encompassing the exonuclease

domains of these genes is performed. In some cases, riskScore™ results may not be included with the test per healthcare provider or payer request. Regions included in the comprehensive test design but that are not part of the test ordered will be masked during processing and will not be reviewed for test interpretation or reporting.

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
GREM1	NM_013372.6
HOXB13	NM_006361.5
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
POLD1	NM_002691.3
POLE	NM_006231.3
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*. Development and analytical validation of a 25-gene next generation sequencing panel that includes the BRCA1 and BRCA2 genes to assess hereditary cancer risk. *BMC Cancer* (2015) 15:215 DOI 10.1186/s12885-015-1224-y

Hughes *et al.* ASCO 2017

Mavaddat N, *et al*. Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst. 2015 Apr 8;107(5). PubMed PMID: 25855707

Michailidou K, *et al*. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013 Apr;45(4): 353-61. PMID: 23535729

Michailidou K, *et al*. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet. 2015 Apr;47(4):373-80. PubMed PMID: 25751625

Tyrer J, *et al*. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med. 2004 23:1111-30. PMID: 15057881