

Myriad myChoice® HRD Technical Specifications

Effective Date: June 2017

Description of Analysis

Myriad myChoice® HRD, an *in vitro* diagnostic, laboratory developed test utilizing Illumina HiSeq next generation sequencing to assess tumor genomic instability in primary or metastatic ovarian cancer and breast cancer and simultaneously detects and classifies large rearrangements and sequencing variants in the *BRCA1* and *BRCA2* genes. This analysis is performed on genomic DNA isolated from formalin fixed, paraffin embedded (FFPE) tumor tissue. The Myriad myChoice HRD *in vitro* diagnostic laboratory developed test includes the following components:

Genomic Instability Status assay

The Genomic Instability Status Assay is a next generation sequencing (NGS) test designed to identify a comprehensive signature for Homologous Recombination Deficiency (HRD) by testing genome-wide single nucleotide variants in DNA extracted from FFPE tumor specimens. Genomic instability status is determined by measuring several elements including loss of heterozygosity, (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST).

BRCA1 and BRCA2 sequence analysis

BRCA1: Full sequence determination of approximately 5,400 base-pairs comprising 22 coding exons and approximately 750 adjacent base pairs in the non-coding intervening sequences (introns) is performed. Exons 1 and 4, which are non-coding, are not analyzed. The wild-type *BRCA1* gene encodes a protein composed of 1,863 amino acids.

BRCA2: Full sequence determination of approximately 10,200 base-pairs comprising 26 coding exons and approximately 900 adjacent base pairs in the non-coding intervening sequence (introns) is performed. Exon 1, which is non-coding, is not analyzed. The wild-type *BRCA2* gene encodes a protein composed of 3,418 amino acids.

The non-coding intronic regions of *BRCA1* and *BRCA2* that are analyzed typically do not extend more than 20 base pairs proximal to the 5' end and 10 base pairs distal to the 3' end of each exon. Sequence analysis is performed using NGS on DNA extracted from FFPE tumor specimens.

BRCA1 and BRCA2 Large rearrangement analysis

Genomic DNA derived from FFPE tumor tissue is analyzed by NGS dosage analysis to determine copy number abnormalities indicative of deletion or duplication mutations. Coding exons of *BRCA1/BRCA2* and limited flanking intron regions are examined for evidence of deletions and duplications (see Limitations of method section for any exceptions). Large rearrangement detection utilizes the number of reads that map to each nucleotide normalized to the run median depth of coverage of the same nucleotide.

Description of Method

Acceptable sample types are FFPE tumor tissue from blocks or slides of primary or metastatic ovarian cancer and breast cancer. The portion of the tumor should measure at least 5x5mm and contain 20% or greater tumor cellularity determined using the adjacent hematoxylin and eosin stained (H&E) section. In cases where blocks are not available, optimal myChoice HRD Assay testing requires 5-11 unstained slides sectioned in the following order: one 2-5 µm tissue section mounted on a charged slide for H&E staining followed by 4-10 consecutive 10µm unstained sections mounted on uncharged slides for macrodissection of tumor. Patient DNA is extracted and purified from the tumor specimen, assigned a unique bar-code for robotic-assisted continuous sample tracking, and submitted for molecular testing.

Genomic Instability Status analysis

The Genomic Instability Status assay is a custom hybridization capture panel that targets SNPs distributed across the genome. Whole genome next generation sequencing libraries are hybridized to this panel to enrich for sequence spanning these SNP locations. The enriched library is then sequenced using a HiSeq 2500 next generation sequencer (Illumina). The sequencing reads are used to generate allele specific copy number profiles, and these profiles are used for calculation of the Genomic Instability score. This score is calculated based upon an aggregate analysis of LOH, TAI, and LST. A validated cutoff is applied to the Genomic Instability score that provides a positive or negative result for the patient. For more information please refer to Telli et al. *Clinical Cancer Research* 2016.

Tumor DNA sequence analysis by next-generation sequencing

(NGS): The samples are prepared through a hybridization-based target-enrichment strategy for subsequent NGS. Fragmented patient tumor DNA is ligated to specific adaptors and hybridized to biotinylated RNA library 'baits' for selective capture of *BRCA1* and *BRCA2*. The captured DNA is amplified, purified and diluted for loading on the NGS instrument. Samples are loaded on the NGS Illumina instrument, and the criteria for both sequencing and large arrangement calls is 99% of the bases must have ≥ 100 reads. Average coverage exceeds 500X.

NGS data analysis and confirmation: A combination of commercial and laboratory-developed software is used for next-generation sequencing data processing, which includes base-calling, alignment, variant identification, annotation, and quality metrics. NGS dosage analysis for large rearrangement detection utilizes the number of reads that map to each nucleotide normalized to the run median depth of coverage of the same nucleotide. Genetic variants are reviewed by computer software and human reviewers. The minimum depth of coverage used for sequence and LR determination by NGS is 50x per base.

Performance Characteristics

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

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The validation studies demonstrated 100% concordance with the reference results, with a 95% lower confidence limit of 0.999997.

Analytical sensitivity: Failure to detect a genetic variant or mutation in the analyzed DNA regions may result from errors in specimen handling and tracking, hybridization, 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 *BRCA1* and *BRCA2* was demonstrated to be 100% with a 95% lower confidence limit of 0.993817, based on complete concordance of results from 54 individual anonymized breast and ovarian resection tumor DNA samples collectively carrying 483 sequence variants which were confirmed by a second laboratory (Myriad Genetic Laboratories in Europe). Comparisons were also made between the Genomic Instability scores generated by both laboratories for each of the 54 samples. These samples had a standard deviation of differences in Genomic Instability scores of 1.13.

The lower limit of detection was determined to require a minimum of 20% tumor content, based on a 'wild-type' cell line embedded in FFPE titrated with 50 known variants contained in six individual cell lines embedded in FFPE. All samples that were previously identified by alternative methods to be positive for deletions or duplications in *BRCA1/BRCA2* were correctly identified for large rearrangements using NGS dosage analysis.

Test reproducibility: Analytical validation studies included a reproducibility study for NGS. In a separate study, 4 samples (two breast and two ovarian primary re-section samples) were analyzed by the myChoice HRD assay across three batches. Each batch contained at least three replicates per sample. This study demonstrated 100% intra-run and inter-run reproducibility for sequencing analysis of *BRCA1* and *BRCA2* with a 95% lower confidence limit of 0.999957. Genomic Instability scores were also assessed for inter- and intra-run reproducibility across the same samples. The samples demonstrated a minimum standard deviation of differences in Genomic Instability scores between 0.54 (min) and 1.57 (max).

Limitations of method: Unequal allele amplification may result from rare sequence changes under hybridization sites. There may be uncommon genetic abnormalities such as specific insertions, inversions, and certain regulatory mutations that will not be detected by the myChoice HRD assay. The detection of large rearrangement deletions and duplications is dependent on the quality of the submitted specimen. Large rearrangements restricted to the non-coding exon 1 or exons 1 and 2 of the *BRCA1* and *BRCA2* genes are not assessed by the myChoice HRD assay. Other terminal duplications are reported as variants of uncertain significance. This analysis, however, is believed to rule out the majority of abnormalities in the genes analyzed.

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.

<i>BRCA1</i>	NM_007294.3
<i>BRCA2</i>	NM_000059.3

Interpretive Criteria:

Overall Interpretations

“myChoice® HRD Status: Positive”

The test results demonstrate homologous recombination deficiency. This overall status is based on the results of tumor genomic instability status of positive, and/or identification of a deleterious or suspected deleterious mutation in the *BRCA1* and *BRCA2* genes.

“myChoice® HRD Status: Negative”

The test results demonstrate homologous recombination proficiency. This overall status is based on the results of tumor genomic instability status of negative, and negative sequencing and large rearrangement analyses in the *BRCA1* and *BRCA2* genes.

“myChoice® HRD Status: Negative**”

This qualified negative report applies to a patient tumor sample with genomic instability status of negative, and negative results for tumor *BRCA1* and *BRCA2* sequencing. However, the test results for *BRCA1* and *BRCA2* large rearrangement analysis are not complete. It should be noted that in approximately 0.15% of cases (Myriad internal data), a false negative result may occur in tumor samples with a proficient Genomic Instability Status but carry a *BRCA1* or *BRCA2* large rearrangement associated with a homologous recombination defect.

“myChoice® HRD Status: Inconclusive”

This patient has a Genomic Instability Status indicative of proficient homologous recombination. However, a genetic variant in *BRCA1* or *BRCA2* was detected with a complex clinical interpretation. Specific interpretations are provided for each variant in the patient report.

myChoice® HRD Assay Components:

The overall assay is composed of two major components, namely Genomic Instability Status, and Tumor *BRCA1/BRCA2* Mutation Status. The combined results form the basis for an overall interpretation of the myChoice® HRD Status, as described above. Potential results for these two components are described below:

“Genomic Instability Status: Positive”

The test results demonstrate homologous recombination deficiency based on the Genomic Instability Status assay alone.

“Genomic Instability Status: Negative”

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The test results demonstrate homologous recombination proficiency based on the Genomic Instability Status assay alone.

“Tumor BRCA1/BRCA2 Mutation Status: Positive”

The test results demonstrate the presence of a deleterious or suspected deleterious sequencing mutation or large rearrangement.

“Tumor BRCA1/BRCA2 Mutation Status: Special Interpretation”

The test results demonstrate the presence of a genetic variant with a complex clinical interpretation. Specific interpretations are provided for each variant in the patient report.

“Tumor BRCA1/BRCA2 Mutation Status: Negative”

The test results do not demonstrate the presence of a deleterious or suspected deleterious sequencing mutation or large rearrangement. This category includes genetic variants for which published data demonstrate absence of substantial clinical significance and truncating mutations in *BRCA2* that occur at and distal to amino acid 3.326 (Mazoyer S et al., *Nature Genetics* 1996, 14:253-254). It also includes variants 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 pathogenic effect on the length or stability of the mRNA transcript. There may be uncommon genetic abnormalities in *BRCA1* and *BRCA2* that will not be detected by myChoice® HRD testing (see **Limitations of method**).

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. Deletions and duplications of an entire exon(s) identified by the myChoice HRD test may also be interpreted to be deleterious. Deleterious large genomic rearrangements include single exon and multi-exonic deletions that are out-of-frame. Out-of-frame single or multi-exonic duplications are classified as deleterious if the orientation is determined to be in tandem and head-to-tail. In-frame deletions/duplications are interpreted on an individual basis and the specific evidence supporting the classification of these mutations is included in the individual patient report.

“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 in the patient report.

“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. Variants of this type are not reported.

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.