**BRAC*Analysis*® Technical Specifications**
**Myriad Genetic Laboratories, Updated: April 2012**
**TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:**

## Description of Analysis

**Comprehensive BRAC*Analysis*®:**

***BRCA1***: Full sequence determination in both forward and reverse directions of approximately 5,400 base pairs comprising 22 coding exons and approximately 750 adjacent base pairs in the non-coding intervening sequences (introns). Exons 1 and 4, which are non-coding, are not analyzed. The wild-type *BRCA1* gene encodes a protein comprised of 1863 amino acids.

***BRCA2***: Full sequence determination in both forward and reverse directions of approximately 10,200 base pairs comprising 26 coding exons and approximately 900 adjacent base pairs in the non-coding intervening sequence (intron). Exon 1, which is non-coding, is not analyzed. The wild-type *BRCA2* gene encodes a protein comprised of 3418 amino acids.

The non-coding intronic regions of *BRCA1* and *BRCA2* that are analyzed 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.

This analysis also includes detection of the following five specific large genomic rearrangements of the *BRCA1* gene (5-site rearrangement panel): a 3.8-kb deletion of exon 13 and a 510-bp deletion of exon 22 described in individuals of Dutch ancestry (Petrij-Bosch, A et al., *BRCA1* genomic deletions are major founder mutations in Dutch breast cancer patients. *Nat Gen* 1997; 17:341-345), a 6-kb duplication of exon 13 described in individuals of European (particularly British) ancestry (The *BRCA1* Exon 13 Duplication Screening Group. The Exon 13 duplication in the *BRCA1* gene is a founder mutation present in geographically diverse population. *Am J Hum Gen* 2000; 67:207-212), a 7.1-kb deletion of exons 8 and 9 described in individuals of European ancestry (Rohlfs EM et al., An Alu-mediated 7.1 kb deletion of *BRCA1* exons 8 and 9 in breast and ovarian cancer families that results in alternative splicing of exon 10. *Genes Chr & Cancer* 2000; 28:300-307), and a 26-kb deletion of exons 14-20 (Myriad).

**BRAC*Analysis*® Rearrangement Test (BART):** All coding exons of *BRCA1/BRCA2*, limited flanking intron regions, and their respective promoters are examined for evidence of deletions and duplications by either quantitative endpoint PCR analysis or microarray comparative genomic hybridization analysis (microarray-CGH).

**Single Site BRAC*Analysis*®:** DNA sequence analysis for a specified variant in *BRCA1* and/or *BRCA2*. Analysis for one of the five *BRCA1* large genomic rearrangements described above includes analysis for all five rearrangements. When the single site mutation is a *BRCA1*/*BRCA2* deletion or duplication mutation other than the five common *BRCA1* large genomic rearrangements described, microarray comparative genomic hybridization analysis (i.e. BRAC*Analysis* Rearrangement Test) of all coding exons, limited flanking intron regions and the promoter regions of *BRCA1/BRCA2* is performed to assess large rearrangements. In some cases, long range PCR analysis and/or sequencing of the resulting PCR product is used to detect specific, previously reported insertions.

**Multisite 3 BRAC*Analysis*®:** DNA sequence analysis of specific portions of *BRCA1* exon 2, *BRCA1* exon 20 and *BRCA2* exon 11 designed to detect the mutations 187delAG and 5385insC in *BRCA1* and 6174delT in *BRCA2*.

## Description of Method:

Patient samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from peripheral blood samples or buccal mouthwash samples, submitted for molecular testing.

**Sequence analysis:** Aliquots of patient DNA are each subjected to polymerase chain reaction (PCR) amplification (35 reactions for *BRCA1*, 47 reactions for *BRCA2*). 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 clinically significant variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above.

**5-site Rearrangement Panel:** The five specific *BRCA1* rearrangements described above are detected by recombination-specific PCR using primers specific for the normal gene as well as for the rearrangement.

**Full Gene *BRCA1*/*BRCA2* Large Rearrangement Analysis (BRAC*Analysis* Rearrangement Test)**: Genomic DNA from patients is analyzed by either multiplexed quantitative PCR or microarray-CGH analysis to determine copy number abnormalities indicative of deletion or duplication mutations across the *BRCA1* and *BRCA2* genes. For multiplex quantitative PCR, twelve fluorescently labeled multiplex PCR reactions are designed to interrogate all exons and the respective promoters of *BRCA1* and *BRCA2*, with a minimum of two amplicons per target region. Proprietary software analysis is used to normalize the copy number of individual amplicons in the *BRCA1* gene against *BRCA2*, plus three control genes.

For microarray-CGH analysis, approximately 1700 probes have been designed to interrogate all coding exons, limited flanking intron regions, and the respective promoters of *BRCA1* and *BRCA2*. Each probe is analyzed using proprietary software that compares the ratio of bound patient DNA to that of a reference DNA to indicate regions of altered copy number. The microarray design includes probes to detect deletions and duplications in multiple genes tested by MGL; however, a data masking feature is used to limit the analysis only to specific genes for which testing has been requested.

Patient samples positive for deletions or duplications are confirmed by repeat multiplex quantitative PCR or microarray analysis of the *BRCA1*/*BRCA2*genes. For multiplex quantitative PCR, rearrangement positive samples are further assessed for sequence polymorphisms affecting the PCR primer binding sites, to minimize the possibility of false positive results.

## 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). The incidence of a false report of a genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%). No false positive results were obtained through the large rearrangement testing process using microarray-CGH on a set of 313 individual samples that were previously

examined for deletions and duplications in *BRCA1* and *BRCA2* by quantitative multiplex PCR.

**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 DNA sequencing performed in both directions is estimated to be >99%. In addition, all samples that were previously examined by alternative methods to be positive for deletions or duplications in *BRCA1*/*BRCA2* were correctly identified by the full gene large rearrangement analysis by multiplex quantitative PCR (BRAC*Analysis* Rearrangement Test).
The large rearrangement testing process using microarray-CGH correctly identified all 37 positives among 313 samples that were previously examined for deletions and duplications in *BRCA1* and *BRCA2* by quantitative multiplex PCR. Furthermore, these validation studies correctly identified two instances of an Alu insertion specific to the Portuguese population (156_157insAlu), among the 313 samples previously tested for large rearrangements.

**Overall test accuracy:** For a patient with at least a 10% probability of a positive test based on a personal or family history of cancer, the chance of an incorrect test result is less than 1%.

**Limitations of method:** There may be limited portions of either *BRCA1* or *BRCA2* 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. Comprehensive BRAC*Analysis* includes testing for only the five specific large genomic rearrangements specified above. The BRAC*Analysis* Rearrangement Test described above using either multiplex quantitative PCR or microarray-CGH will detect deletion and duplication rearrangements involving the promoter and coding exons of *BRCA1*/*BRCA2*, but will not detect some types of errors in RNA transcript processing, regulatory mutations, or balanced rearrangements (i.e. inversions). The Portuguese founder mutation in BRCA2, 156_157insAlu, can be detected by multiplex quantitative PCR and microarray-CGH; however, other insertions that do not result in duplications will generally not be detected by microarray CGH.

Among patients who underwent BRAC*Analysis* Rearrangement Testing, the proportion of clinically significant defects in *BRCA1* and *BRCA2* attributable to genomic rearrangements identified specifically by the BRAC*Analysis* Rearrangement Test is estimated to be 5-8% (Roa B et al. *BRCA1* and *BRCA2* large genomic rearrangement testing in a large cohort of hereditary breast/ovarian cancer patients: prevalence and mutation profiles in risk-stratified patient groups of different ethnicities. *ASHG annual meeting* 2011; 1231T).

## 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 transcribed base of *BRCA1* and *BRCA2* according to GenBank entries U14680 and U43746, respectively. (Under these conventions, the two mutations commonly referred to as "185delAG" and "5382insC" are named 187delAG and 5385insC, respectively.)

## Interpretive Criteria:

The classification and interpretation of all variants identified in the assay reflects the current state of scientific understanding at the time the report is issued. In some instances, the classification and interpretation of variants may change as scientific information becomes available.

**"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.

Deletions and duplications of an entire exon(s) identified by the BRAC*Analysis* Rearrangement Test may also be interpreted to be deleterious. Deleterious large genomic rearrangements include single exon and multi exonic deletions and duplications that are out of frame. 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 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 very close to the normal stop codon, unless otherwise documented (Mazoyer S et al., *Nature Genetics* 1996; 14:253-254).

**"No deleterious mutation detected":** Includes genetic variants for which published data demonstrate absence of substantial clinical significance. Includes truncating mutations in *BRCA2* that occur at and distal to amino acid 3326 (Mazoyer S et al., *Nature Genetics* 1996; 14:253-254). 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. Data on polymorphic variants are available upon request.

There may be uncommon genetic abnormalities in *BRCA1* and *BRCA2* that will not be detected by BRAC*Analysis*® (see **Limitations of method**, above). This analysis, however, is believed to rule out the majority of abnormalities in these genes which are believed to be responsible for most hereditary susceptibility to breast and ovarian cancer.

**"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**: Whenever there is a change in the interpretation of a patient's test result, an amended report will automatically be provided by Myriad Genetic Laboratories.

**(please see reverse side for Description of Analysis and Performance Characteristics)**