BRACAnalysis® Technical Specifications
Myriad Genetic Laboratories, Updated: 29 August 2005

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

Description of Analysis

Comprehensive BRACAnalysis®:

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: 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).

Single Site BRACAnalysis®: DNA sequence analysis for a specified mutation in *BRCA1* and/or *BRCA2*. Analysis for one of the five *BRCA1* large genomic rearrangements described above includes analysis for all five rearrangements..

Multisite 3 BRACAnalysis®: 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:

Blood samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from white cells isolated from each sample. 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 genetic variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above. Genomic rearrangements are detected by recombination-specific PCR using primers specific for the normal gene as well as for the rearrangement.

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%).

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%).

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. Other than the five specific large genomic rearrangements specified above, this assay will not detect genomic rearrangements or some types of errors in RNA transcript processing. The proportion of clinically significant defects in *BRCA1* and *BRCA2* attributable to undetected genomic rearrangements is estimated to be 3- 4% (Myriad Genetic Laboratories unpublished data).

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:

"Positive for a deleterious mutation": Includes all nonsense and frameshift mutations that occur at or before amino acid 1853 and 3308 of *BRCA1* and *BRCA2,* respectively (based on documentation of deleterious mutations in *BRCA1* and *BRCA2*). 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 and/or demonstration of abnormal mRNA transcript processing.

"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. Includes *BRCA1* variants identified *in trans* with a deleterious mutation, and *BRCA2* variants identified *in trans* with a deleterious mutation if the individual has not been diagnosed with Fanconi anemia complementation group D1 (FANC D1). Also includes missense mutations in *BRCA2* that occur at or distal to amino acid 3326. 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 distal to amino acid position 1853 of *BRCA1* and between amino acid positions 3309 and 3325 of *BRCA2*.

"No deleterious mutation detected": Includes non-truncating genetic variants observed at an allele frequency of approximately 1% of a suitable control population (providing that no data suggest clinical significance), as well as all 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. 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 BRACAnalysis® (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. If one (or rarely two) specific deleterious mutations have been identified in a family member, a negative analysis for the specific mutation(s) indicates that the tested individual is at the general population risk of developing breast or ovarian cancer.

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.