BRCA 1/2 **Next Generation Sequencing** Assay Summary

Analyte

Mutation detection within the *BRCA1* and *BRCA2* genes

Test System

Methodology: Next Generation Sequencing
Instruments: Illumina MiSeq®

Assay Principle

PCR Amplification and Next Generation Sequencing

The entire gene coding region of *BRCA1/BRCA2* genes, as well as all flanking noncoding regions, is analyzed by next generation sequencing. The test uses Illumina's TruSeq® Custom Amplicon approach for target enrichment and MiSeq instrument for sequencing. It is a custom-designed pool comprised of 150 oligonucleotide probe pairs for the binding and amplification of products ranging from 220 bp to 280 bp.

Typical Applications of Assays

According to the National Comprehensive Cancer Network, testing is indicated if one of the features mentioned below is present in the family: Early-age-onset (age < 50 years) breast cancer, including both invasive and ductal carcinoma in situ (DCIS) breast cancers; two breast primaries or breast and ovarian/fallopian tube/primary peritoneal cancer in a single individual or two or more breast primaries or breast and ovarian/fallopian tube/primary peritoneal cancers in close (first-, second-, and third-degree) relatives(s) from the same side of the family; populations at risk (eg, Ashkenazi Jewish); member of a family with a known *BRCA1* or *BRCA2* mutation; any male breast cancer; ovarian/fallopian tube/primary peritoneal cancer at any age.

Biological Limitations

Samples must be whole blood

Preanalytical Considerations

Volume 7 mL
Minimum Volume 3 mL
Container Lavender-top (EDTA) tube or yellow-top (ACD) tube
Storage Instructions 2°–8°C (preferred), or room temperature (15°–25°C). Specimens should be shipped at ambient temperature.
Stability 48 hours at ambient temperature
Patient Preparation None

Assay Characteristics

Variant detection within the *BRCA1* and *BRCA2* genes is based on Integrated Genetics validation data and CMBP transfer validation data (June 2014).

Parameter	Description	Comments
Accuracy	• 72 Clinical and 30 Coriell DNAs used during transfer validation • 781 Het (SNV+indels1-40bp) • 445 Hom (all SNVs) • 71 Clinical and 31 Coriell DNAs used during validation • 1780 Het (SNV+indels1-40bp) • 984 Hom (all SNVs)	• Concordance to Sanger: 100% of mutations identified by NGS • Concordance between sites: 100%
Intraassay Precision	100% on all replicates of all identified variants on six samples	
Interassay Precision	100% on all replicates of all identified variants on six samples	Second tech and second lot verified
Sensitivity	99.9%	
Specificity	Test is 100% specific for the *BRCA1* and *BRCA2* genes	Manufacturer and SNPcheck verified
Minimum Coverage Threshold	30X	All samples except one passed the coverage metric.
Reference Interval	N/A; specific variant reported	
Reportable Range	N/A; variants reported from variants of unknown significance to causative variants	17,769 bp total

Assay Status

- Variant detection within the *BRCA1* and *BRCA2* genes has been validated at Integrated Genetics, a brand of Esoterix Genetic Laboratories, LLC, a wholly owned subsidiary of LabCorp, Westborough, Mass, and subsequently transferred and verified with additional validation at the Center for Molecular Biology and Pathology, Research Triangle Park, NC.
- The assay is analytically validated and is performed in a CLIA-compliant laboratory at the Center for Molecular Biology and Pathology (Research Triangle Park, NC).
- Validation summary is on file.
- Assay turnaround time is 7 to 12 days.

Limitations

Sequencing does not detect inversions or other rearrangements.

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