**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 (e.g., 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).
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td>• 72 Clinical and 30 Coriell DNAs used during transfer validation</td>
<td>• Concordance to Sanger: 100% of mutations identified by NGS</td>
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<tr>
<td></td>
<td>• 781 Het (SNV+indels-1-40bp)</td>
<td>• Concordance between sites: 100%</td>
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<td>• 445 Hom (all SNVs)</td>
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<tr>
<td></td>
<td>• 71 Clinical and 31 Coriell DNAs used during validation</td>
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</tr>
<tr>
<td></td>
<td>• 1780 Het (SNV+indels-1-40bp)</td>
<td></td>
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<tr>
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<td>• 984 Hom (all SNVs)</td>
<td></td>
</tr>
<tr>
<td><strong>Intraassay Precision</strong></td>
<td>100% on all replicates of all identified variants on six samples</td>
<td></td>
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<td>100% on all replicates of all identified variants on six samples</td>
<td>Second tech and second lot verified</td>
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<tr>
<td><strong>Sensitivity</strong></td>
<td>99.9%</td>
<td></td>
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<tr>
<td><strong>Specificity</strong></td>
<td>Test is 100% specific for the BRCA1 and BRCA2 genes</td>
<td>Manufacturer and SNPcheck verified</td>
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<tr>
<td><strong>Minimum Coverage Threshold</strong></td>
<td>30X</td>
<td>All samples except one passed the coverage metric.</td>
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<tr>
<td><strong>Reference Interval</strong></td>
<td>N/A; specific variant reported</td>
<td></td>
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<tr>
<td><strong>Reportable Range</strong></td>
<td>N/A; variants reported from variants of unknown significance to causative variants</td>
<td>17,769 bp total</td>
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</table>

**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.

**References**


www.LabCorp.com

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