**BRCA1 and BRCA2 Sequencing**  
Next Gen Sequencing Test Specifications

**Disease Overview**

*BRCA1* and *BRCA2* are associated with hereditary breast and ovarian cancer syndrome (HBOC). Germline genetic mutations in either of these two genes will lead to an increased risk for female and male breast cancer, ovarian cancer and other cancers such as prostate cancer, pancreatic cancer and melanoma. In the general population, women have a lifetime risk of 12% (1 in 8) of developing breast cancer, and a 1.4% (1 in 75) lifetime risk of developing ovarian cancer. Whereas women with a pathogenic variant in the *BRCA1* gene will have a significantly increased risk of 46%-87% of developing breast cancer, and 39%-63% of developing ovarian cancer, the risks for women with a pathogenic variant in the *BRCA2* gene will have increased risks of 38%-84% and 16.5%-27% for breast and ovarian cancer, respectively. HBOC-affected patients are also much more likely to have a second primary cancer.

About 5-10% of all breast cancer cases, and 15-20% of all ovarian cancer cases are hereditary. The diagnosis of HBOC is established by molecular genetic testing. Genetic testing is therefore useful for individuals with a personal and/or family history suggestive of hereditary breast and/or ovarian cancer. A positive test result will affect medical management of the patient. Physicians and clinicians can provide appropriate screening recommendations and risk-reduction options based on the test results. The results may provide information to other family members about their risks, and allow them to make appropriate decisions about preventive care.

According to the National Comprehensive Cancer Network (NCCN) guidelines, genetic testing of *BRCA1* and *BRCA2* is suggested for individuals with a personal or family history of any of the following:

- Breast cancer diagnosed at or before age 50 years
- Ovarian cancer at any age
- Multiple primary breast cancers either in one or both breasts
- Personal or family history of male breast cancer
- Triple-negative breast cancer, particularly when diagnosed before age 60 years
- The combination of pancreatic cancer and/or prostate cancer (Gleason score ≥7) with breast cancer, and/or ovarian cancer
- Of Ashkenazi Jewish ancestry and an associated cancer (breast, ovarian, pancreatic, or aggressive prostate)

**Indications for Testing**

1. Confirmation of diagnosis in patients with personal or familial history suggestive of Hereditary Breast and Ovarian Cancer (HBOC).
2. Assessment for at-risk family members
Requisition Form: Cancer Test Requisition Form or General Test Requisition Form (www.apollogen.com)

Genes (2): **BRCA1** and **BRCA2**

CPT Codes: 81211, 81213
Turnaround Time: 2 weeks
Specimen Requirement: 3-5 mL Blood (EDTA) – Lavender Top Tube (preferred);
Saliva samples are optional
Other Specimen Types: Contact ApolloGen Diagnostic Laboratory
Pricing: Please contact us at (949) 916-8886 or at inquiries@apollogen.com for current pricing

**Testing Methodology**

Genomic DNA is extracted from the patient’s specimen and fragmented via sonication. All of the exons, flanking intronic (at least 10 nucleotides into the introns), and untranslated regions (5’ and 3’) of the targeted genes are enriched using capture-based hybridization. Massively parallel sequencing is applied to the enriched target DNA regions to detect mutation. Variants with an allele frequency > 1% are considered likely benign polymorphisms, and are not included in the final report. Interpretation of rare alterations with allele frequency <1% is based on ACMG guidelines. All pathogenic and likely pathogenic variants are verified by Sanger Sequencing.

Massively parallel sequencing can reliably detect insertion/deletion mutations smaller than 10 base pairs. However, larger insertion, deletion, duplication due to rearrangement, and mutations in regulatory and deep intronic regions cannot be detected by this technology. Rare primer site variants may lead to erroneous results that may need further investigation.

**Analytical Sensitivity:**
This test can detect >95% of the small variants in the examined regions. Please contact us for detailed information regarding coverage for specific genes of interest.

**Related Test**

**Breast Cancer Panel**
Analyzes 19 established genes that are associated with an increased risk of breast cancer.

**iGene Cancer Panel**
Simultaneously analyzes 20 critical genes associated with an increased risk for breast, ovarian, endometrial, colorectal, pancreatic and other cancers.

**Variant Classification**

Sequencing results will be interpreted and reported following the recommendations of the American College of Medical Genetics (www.acmg.net). Sequence variations will be analyzed and classified into the following categories based on current scientific knowledge. Variants found in categories 1-3 (pathogenic, likely pathogenic, and variants of unknown clinical significance) will be reported.

1. **Pathogenic:** Pathogenic variants include nonsense mutations and frame shift mutations that are predicted to result in premature protein truncation, splice site mutations, and previously reported missense mutations that are recognized as disease-causing by databases and the scientific literature.

2. **Likely Pathogenic:** Likely pathogenic variants are those variants that are likely to adversely affect gene function, but for which there is no conclusive evidence to strongly support pathogenicity.
3. **Variant of Unknown Clinical Significance (VUS):** VUSs are sequence variations for which there is insufficient evidence to either confirm or exclude pathogenicity.

4. **Likely Benign:** Likely benign variants are sequence variations for which there is significant, but not conclusive evidence supporting that the variant is not disease-causing.

5. **Negative:** A negative classification is reported when no disease-causing variant is found, or a variant is classified as a benign variant based on the ACMG criteria, the population data, or if there is no clinical significance based on review of the literature and mutation databases.

**References**


