*BRCA*ssure® Technical Summary

Analyte

Detection of germline (inherited) pathogenic variants associated with hereditary breast and ovarian cancer (HBOC) syndrome, by DNA sequencing and exon-level deletion/duplication analysis of the *BRCA1* and *BRCA2* genes.

Methodology

Next generation DNA sequencing (NGS) with variant confirmation by Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA).

Assay Principle

The *BRCA*ssure NGS assay identifies germline DNA sequence variants and exon-level deletions and duplications in the *BRCA1* and *BRCA2* genes. Pathogenic variants in the *BRCA1* and *BRCA2* genes are associated with hereditary cancers, including breast, ovarian, pancreatic, prostate, and melanoma. They account for approximately 10% of all breast cancer cases. Women with *BRCA1* variants have a risk of up to 87% of developing breast cancer and up to 63% of developing ovarian cancer. Men with *BRCA1* variants have a risk of 1-2% of developing breast cancer. Women with *BRCA2* variants have a risk of up to 84% of developing breast cancer and a risk of 16-27% of developing ovarian cancer. Men with *BRCA2* variants have a risk of 9% of developing breast cancer. Individuals with *BRCA1* or *BRCA2* pathogenic variants have a lifetime increased risk of developing pancreatic and prostate cancers.

Testing includes NGS analysis of the entire coding region and flanking noncoding regions. Regions of *BRCA1* and *BRCA2* are captured using custom capture baits from Twist Bioscience. Final captured libraries are sequenced on the Illumina MiSeq Sequencer. Variants, including single nucleotide variants (SNVs) and copy number variants (CNVs, i.e. deletions and duplications), are identified using a custom bioinformatics pipeline. Pathogenic and likely pathogenic variants and variants of uncertain significance (VUS) are reported. Benign variants are not reported. Reported variants are confirmed by either Sanger sequencing (SNVs, small insertions and deletions) or multiplex ligation-dependent probe amplification (MLPA; deletions and duplications). All technical and performance characteristics have been internally validated following guidelines set forth by the College of American Pathologists (CAP).

Intended Applications

Breast carcinoma is the most common malignancy among women globally, and family history remains the strongest single predictor of breast cancer risk. Features characteristic of hereditary, versus sporadic, breast cancer are: younger age at diagnosis, frequent bilateral disease, and more frequent occurrence of disease among men.

Testing is intended for patients with a personal or family history of hereditary breast and ovarian cancer, or with a family history of prostate cancer, or patients at increased risk for prostate cancer, elevated Gleason scores, and metastatic prostate cancers. Positive results can prompt changes in patient screening protocols and patient care, and may enable early detection of malignancies, providing clinical benefit to the patient and, potentially, family members. Testing is not intended for individuals with sporadic cancers, no family history of cancer, individuals with a history of bone marrow transplant, or those with a family history of cancers not associated with hereditary breast and ovarian cancer (HBOC) syndrome.

Panel Content

BRCA1 and *BRCA2* genes are evaluated by DNA sequence and exon- level deletion/duplication NGS analysis. Available *BRCA*ssure tests are listed below.

Test No.	BRCAssure Test Name	Use
485030	*BRCA*ssure®: *BRCA1* and *BRCA2* Comprehensive Analysis	Full gene sequencing of *BRCA1* and *BRCA2* genes.
485050	*BRCA*ssure®: *BRCA1* and *BRCA2* Deletion/Duplication Analysis	Deletion and duplication analysis of *BRCA1* and *BRCA2* genes.
485097	*BRCA*ssure®: Ashkenazi Jewish Panel	Testing for founder variants prevalent in the Ashkenazi Jewish population: c.68_69delAG and c.5266dupC in *BRCA1* and c.5946delT in *BRCA2*.
485066	*BRCA*ssure®: *BRCA1* Targeted Analysis	Testing for known variant(s) in *BRCA1*.
485081	*BRCA*ssure®: *BRCA2* Targeted Analysis	Testing for known variant(s) in *BRCA2*.

Methods

For next-generation sequencing, genomic regions of interest are selected using a custom capture reagent for molecularly-barcoded patient library construction and target enrichment from genomic DNA. Regions of interest include all exons and intron/exon junctions (+/-20 nucleotides) of the *BRCA1* and *BRCA2* genes. Patient libraries undergo 2x300 paired-end sequencing using Illumina V3 chemistry. Any segment failing minimum read depth coverage is rescued by bi-directional Sanger sequencing.

Sequence reads are aligned to the hg19/GRCh37 reference human genome build. Variants, including SNVs, small insertions, small deletions, and exon-level CNVs, are identified and annotated using an internally validated custom analysis workflow on the CLCBio™ platform. All reportable NGS variants are confirmed: SNVs and small insertions and deletions by bi-directional Sanger sequencing, and exon-level deletions and duplications by MLPA. MLPA is performed with SALSA reagents from MRC Holland and MLPA analysis uses GeneMarker software.

Assay performance characteristics are summarized in the appendix.

Biological Limitations of Testing

Bone marrow transplantation, recent blood transfusion, and active hematological malignancies may affect results. Allele drop-out due to rare interfering sequence polymorphisms present in primer or probe binding sites and homopolymeric sequence regions may affect variant detection. The assay is not designed to detect mosaic variants, non-coding variants, deep intronic variants, somatic variants, translocations, inversions, or other complex gene rearrangements. The assay does not determine whether heterozygous variants in the same gene are present on the same or a different chromosome; to distinguish phase and determine clinical significance, rarely, parental testing may be required. Exact breakpoints of exon-level deletions/duplications are not determined. The presence of an inherited cancer syndrome due to a different genetic cause cannot be ruled out. Any interpretation should be clinically correlated with information about the patient's presentation and relevant family history.

Variant Classification

Variants are classified by an in-house variant classification protocol that is traceable, and in accord with guidelines from the American College of Medical Genetics (ACMG). Classification uses an algorithmically-weighted assessment of several components: predicted functional impact determined by in silico analysis; prevalence of the variant in the unaffected (general) population; segregation in affected individuals or families published in peer-reviewed literature; and co-occurrence with other deleterious variants. Variants are re-evaluated at defined intervals for relevant updates that could affect the final report interpretation. If a variant is reclassified and determined to be clinically actionable, patient reports are re-issued. For details regarding the variant classification algorithm, see LabCorp's variant classification summary in ClinVar at https://www.ncbi.nlm.nih.gov/clinvar/submitters/500026/.

Appendix 1 — Pre-analytical Considerations

Parameter	Description	Comments
Sample type	Whole blood; or saliva	
Volume	7-10 ml blood; or full saliva collection tube	
Containers	Lavender-top (EDTA) or yellow-top (ACD); or OraGeneDX 500 Saliva Collection Kit	
Storage and stability	When stored at 2-8ºC after sample collection, stability is up to 60 days for blood, and up to 30 days for saliva	Ship at ambient temperature.
Patient Preparation	None	
Clinical Questionnaire	Clinical Questionnaire for Hereditary Cancer	Submit with specimens

Appendix 2 — Assay Performance Characteristics

Parameter	Description	Comments
Accuracy	>99%	
Sensitivity (SNVs)	>99%	>99% sensitivity at 15X coverage
Sensitivity (CNVs)	>99%	>99% sensitivity at 40X coverage
Intra-assay Precision	>99%	
Inter-assay Precision	>99%	
NGS Coverage	Minimum: 40X	

References
1. NCCN Guidelines: Genetic/Familial High Risk Assessment: Breast, Ovarian, and Pancreatic. National Comprehensive Cancer Network. Version 1.2020, Dec. 4, 2019.
2. Peshkin BN, Isaacs C. Genetic testing and management of individuals at risk of hereditary breast and ovarian cancer syndromes. Wolters Kluwer; *UpToDate*; Mar 13, 2020.
3. Petrucelli N, Daly MB, Pal T. *BRCA1* - and *BRCA2* -Associated Hereditary Breast and Ovarian Cancer. *GeneReviews*. 1998 Sep 4 [updated 2016 Dec 15].

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