TECHNICAL SUMMARY

VistaSeq® Hereditary Cancer Panels

Analyte
Detection of pathogenic variants in up to 60 genes associated with cancer susceptibility, by DNA sequence and exon-level deletion/duplication analysis.

Methodology
Next generation DNA sequencing (NGS), multiplex ligation-dependent probe amplification (MLPA), and array-based comparative genomic hybridization microarray analysis (aCGH).

Assay Principle
The VistaSeq hereditary cancer panels allow identification of germline DNA sequence variants and exon-level deletions/duplications of genes implicated in the development, progression, and/or susceptibility to hereditary cancers and cancer syndromes. Panels include genes with known risks and established clinical management guidelines from the National Comprehensive Cancer Network (NCCN) and other published sources, and may also include genes that confer a predisposition or susceptibility to familial cancer(s), thus providing clinically-driven panels as comprehensive as desired by the clinician. Testing includes analysis of the entire coding region and flanking noncoding regions by NGS and analysis of deletions and duplications by aCGH or MLPA. Reportable results include clinically significant variants and variants of uncertain significance (VUS). All reportable results are confirmed by either Sanger sequencing (SNVs, NGS deletions/duplications) or quantitative PCR (aCGH deletions/duplications). All technical and performance characteristics were internally validated following guidelines set forth by the College of American Pathologists (CAP).

Intended Applications
Testing is intended for patients with a personal or family history suggestive of a hereditary cancer syndrome, including multiple affected individuals in several generations, families with several cancer types associated with a single cancer syndrome (eg, Li-Fraumeni), and individuals with a family member diagnosed with a hereditary cancer syndrome. Testing is not intended for individuals with sporadic cancers, no family history of cancer, or a family history of cancers not associated with a hereditary cancer syndrome. The value of multigene panel testing is that a family member may have malignancies or deleterious variants that may not have been anticipated based on family history. Multiple studies to date have identified additional deleterious variants in genes that would not have been identified using a single-gene testing paradigm, with up to an additional 11% of individuals having a positive result.[1,2] Positive results can prompt changes in patient screening protocols and patient care, and enable early detection of malignancies, providing clinical benefit to the patient and, potentially, family members.

Panel Content
Available VistaSeq panels are listed below. Panel content is designed to increase the likelihood of detecting inherited mutations in cancer predisposition genes based on the patient's clinical features, including cancer type, family history and previous negative testing.

Each panel is derived from the following 60 genes: *ALK , APC, ATM, AXIN2, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, FAM175A, FANCC, FH, FLCN, GPC3, HOXB13, MAX, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, NF2, PALB2, PHOX2B, PMS2, POLD1, POLE, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RET, SDHB, SDHC, SDHD, SMAD4, SMARCB1, STK11, SUFU, TP53, TMEM127, TSC1, TSC2, VHL*, and *WT1*. Genes associated with each panel are listed at www.labcorp.com.

Test No.	VistaSeq Panel Name
481220	Hereditary Cancer Panel
481240	Hereditary Cancer Panel Without BRCA
481319	Breast Cancer Panel
481452	High/Moderate Risk Breast Cancer Panel
481341	Breast and GYN Cancer Panel
481330	GYN Cancer Panel
481386	Brain/CNS/PNS Cancer Panel

Test No.	VistaSeq Panel Name
481363	Colorectal Cancer Panel
481352	High Risk Colorectal Cancer Panel
481374	Endocrine Cancer Panel
481385	Pancreatic Cancer Panel
481407	Renal Cell Cancer Panel
483555	Prostate Cancer Panel
483543	Lynch Syndrome Panel

labcorp

Detailed Methods

Next-generation sequencing and deletion/duplication analysis

For most genes the entire coding region and flanking splice sites are analyzed by NGS and deletion/duplication analysis. For some genes, analysis is limited to specific variants (ie, *MITF* targeted c.952G>A variant), sequence analysis only (ie, *HOXB13, POLD1* and *POLE*), or deletion/duplication analysis only (ie, *EPCAM*), or expanded to included specific variants (ie, clinically relevant variants in the promoter regions of *APC* and *PTEN*). Exon flanking regions include ±20 bp for the *BRCA1* and *BRCA2* genes, and ±10 bp for all other genes. Exon-level deletions/ duplications are assessed by aCGH, and, for the *PMS2, CHEK2, NF1, SDH* genes, by MLPA. Assay performance characteristics are summarized in the appendix.

For NGS, a custom capture method is used for molecularly-barcoded patient library construction and target enrichment from genomic DNA. Patient libraries undergo 2x150 paired-end sequencing using Illumina V3 chemistry. Any segment failing minimum read depth coverage is rescued by bi-directional Sanger sequencing to complete sequence analysis. Sequence reads are aligned to the hg19/GRCh37 reference human genome build. Variant calling and annotation use an internally validated custom analysis workflow on the CLCBio™ platform. All reportable NGS variants are confirmed by bi-directional Sanger sequencing.

Exon-level deletion/duplication analysis is performed with a custom aCGH array that measures DNA copy number variation relative to a known reference sample. 60bp custom aCGH probes are tiled to cover all target exons. In exons smaller than 50bp, probes are added within the proximal introns. Y chromosome probes are included to provide additional quality control through gender verification. Analysis parameters are optimized, favoring sensitivity over specificity. Potential false positive calls are minimized and specificity ensured by using quantitative PCR for copy number confirmation. MLPA analysis uses SALSA MLPAs from MRC Holland and analyzed by GeneMarker software.

Highly homologous sequences and pseudogenes can interfere with variant detection. Several strategies included in the testing protocol reduce their potential negative effects. **[1]** *PMS2* exons 12 and 15 are highly homologous to the *PMS2CL* pseudogene. To increase sensitivity for these exons, a higher minimum coverage depth is required at each targeted base position (25X), and a lower variant allele fraction cutoff is used for variant detection (8.0%). **[2]** *PMS2* variants identified by NGS are confirmed using long-range PCR followed by Sanger sequencing to verify true variants. **[3]** *MLPA* is used in concert with aCGH to assess exon-level copy number changes in the *PMS2, CHEK2, NF1*, and *SDH* genes.

Biological Limitations of Testing

Bone marrow transplantation, recent blood transfusion and active hematological malignancies may affect results. Allele dropout 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 the American College of Medical Genetics (ACMG) guidelines. Classification utilizes 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/. For details regarding clinical experience, and initial VUS rates from the VistaSeq 27 gene panel (test number 481220), see Gardner *et al*. 2018 (reference 2).

Appendix 1: Preanalytical Considerations

Parameter	Requirement	Comments
Specimen Requirements	10 mL whole blood lavendar-top (EDTA) tube or 2 mL saliva Oragene® Dx saliva collection kit	
Storage	Ship specimens at ambient temperature	
Patient Preparation	None	
Clinical Questionnaire	Clinical Questionnaire for Hereditary Cancer	Submit with specimens

Appendix 2: Assay Performance Characteristics

Parameter	Requirement	Comments
Accuracy	>99.9%	
Sensitivity	>99.9%	
Intra-assay Precision	>99.9%	
Inter-assay Precision	>99.9%	
NGS coverage	Average: ~300X Minimum: 15X (25X for PMS2 exons 12-15)	>99.9% sensitivity is achieved at >15X coverage

References
1. Kurian AW. Hare EE, Mills MA, et al. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. *J. Clin. Oncol*. 2014 Jul 1;32(19):2001-2009.
2. Gardner SA, Weymouth KS, Kelly WS, et al. Evaluation of a 27-gene inherited cancer panel across 630 consecutive patients referred for testing in a clinical diagnostic laboratory. *Hered Cancer Clin Pract*. 2018 Jan 4;16:1.

labcorp

Oragene® is a registered trademark of DNA Genotek Inc.
©2022 Laboratory Corporation of America® Holdings All rights reserved. 28076-0122